Abid Ali Ansari · Sarvajeet Singh Gill
Ritu Gill · Guy R. Lanza
Lee Newman *Editors*

Phytoremediation

Management of Environmental Contaminants, Volume 3

Springer

Phytoremediation

Abid Ali Ansari • Sarvajeet Singh Gill
Ritu Gill • Guy R. Lanza • Lee Newman
Editors

Phytoremediation

Management of Environmental Contaminants, Volume 3

Springer

Editors
Abid Ali Ansari
Department of Biology
Faculty of Science
University of Tabuk
Tabuk, Saudi Arabia

Ritu Gill
Centre for Biotechnology
MD University
Rohtak, Haryana, India

Lee Newman
College of Environmental Science
State University of New York
Syracuse, NY, USA

Sarvajeet Singh Gill
Centre for Biotechnology
MD University
Rohtak, Haryana, India

Guy R. Lanza
College of Environmental Science
State University of New York
Syracuse, NY, USA

ISBN 978-3-319-40146-1 ISBN 978-3-319-40148-5 (eBook)
DOI 10.1007/978-3-319-40148-5

Library of Congress Control Number: 2014952730

Printed on acid-free paper

This Springer imprint is published by Springer Nature
The registered company is Springer International Publishing AG Switzerland

Preface

> "*You must be the change you wish to see in the world*"
>
> ***Mahatma Gandhi***

Volume 3 of this 5 volume series adds some more examples on phytoremediation of heavy metal and metalloid contaminants from terrestrial and aquatic ecosystems. In this volume, various studies on phytoremediation of mining areas, agricultural soil, crude oil contaminated soil, shooting range soil and industrial areas have been included. The importance of fast growing trees, wild grasses, aquatic weeds, ferns, hyperaccumulator and some transgenic plants in removal, degradation or stabilization of heavy metals and metalloid has been described. Information on heavy metal uptake, tolerance mechanisms and the role of metal transporters in phytoremediation have also been provided. The role of phytochelatins, biochar and green sorbents in phytoremediation of heavy metal contaminated soils and water has been described in different chapters of this volume. The chapters in volume 3 also illustrate how phytoremediation applications can serve as one of several useful components in the overall management and control of environmental contaminants especially heavy metals and metalloids. Volume 3 of this book series provides additional accounts of some selected phytoremediation research projects and case histories from specific sites and/or laboratories. The editors and contributing authors hope that one result of publishing this book will be to provide a wide range of useful experimental data derived from global applications of phytoremediation. Hopefully, like the previous two volumes of this book series this volume can also provide new insights into the advantages and disadvantages of phytoremediation to manage the continuing threat of ecosystem degradation resulting from anthropogenic inputs of environmental contaminants.

Tabuk, Saudi Arabia	Abid Ali Ansari
Rohtak, India	Sarvajeet Singh Gill
Rohtak, India	Ritu Gill
Syracuse, NY, USA	Guy R. Lanza
Syracuse, NY, USA	Lee Newman

Contents

Part I Phytoremediation of Heavy Metal Contaminants

Part II Phytoremediation of Metalloid Contaminants

Contributors

Syed Shakeel Ahmad Department of Botany, University of Kashmir, Srinagar, India

Qasim Ali Department of Botany, Government College University Faisalabad, Faisalabad, Pakistan

Shafaqat Ali Department of Environmental Sciences and Engineering, Government College University Faisalabad, Faisalabad, Pakistan

Abid Ali Ansari Department of Biology, Faculty of Science, University of Tabuk, Tabuk, Saudi Arabia

Danijela D. Arsenov Faculty of Sciences, Department for Biology and Ecology, University of Novi Sad, Novi Sad, Republic of Serbia

Zubair Aslam Department of Agronomy, University of Agriculture Faisalabad, Faisalabad, Pakistan

Saule Atabayeva Department of Biology and Technology, Al-Farabi Kazakh National University, Almaty, Kazakhstan

Tharanga Bandara Chemical and Environmental Systems Modeling Research Group, National Institute of Fundamental Studies, Kandy, Sri Lanka

Safdar Bashir Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, Faisalabad, Pakistan

S.M. Bhatt Department of Biotechnology, Lovely Professional University, Jalandhar-Delhi, India

Irshad Bibi Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, Faisalabad, Pakistan

Southern Cross GeoScience, Southern Cross University, Lismore, NSW, Australia

MARUM and Department of Geosciences, University of Bremen, Bremen, Germany

Carlos Alberto Bonilla Department of Hydraulic and Environmental Engineering, Pontificia Universidad Católica de Chile, Santiago, Chile

Milan Borišev Faculty of Sciences, Department for Biology and Ecology, University of Novi Sad, Novi Sad, Republic of Serbia

Giovanna Cappai DICAAR, Department of Civil Environmental Engineering and Architecture, University of Cagliari, Cagliari, Italy

Alessandra Carucci DICAAR, Department of Civil Environmental Engineering and Architecture, University of Cagliari, Cagliari, Italy

Ritu Chaturvedi Department of Botany, St. John's College, Agra, India

Paulo J.C. Favas School of Life Sciences and the Environment, University of Trás-os-Montes e Alto Douro, Vila Real, Portugal

Manal Fawzy Environmental Sciences Department, Faculty of Science, Alexandria University, Alexandria, Egypt

Paulo Ademar Avelar Ferreira Federal University of Santa Maria, Santa Maria, Rio Grande do Sul, Brazil

Admir José Giachini Federal University of Santa Catarina, Florianópolis, Santa Catarina, Brazil

Madhuri Girdhar Department of Biotechnology, Lovely Professional University, Jalandhar-Delhi, India

Geetanshu Goyal Department of Biotechnology, Lovely Professional University, Jalandhar-Delhi, India

Amina Hayyat Department of Environmental Sciences and Engineering, Government College University, Faisalabad, Pakistan

Muhammad Tariq Javed Department of Botany, Government College University, Faisalabad, Pakistan

Maryam Javed Department of Environmental Science and Engineering, Government College University, Faisalabad, Pakistan

Tiziana Lai DICAAR, Department of Civil Environmental Engineering and Architecture, University of Cagliari, Cagliari, Italy

M.E. Lopes Chemistry Department of Science and Technology School, Évora University, Évora, Portugal

HERCULES Laboratory, Évora University, Évora, Portugal

Sumedha Madawala Department of Botany, University of Peradeniya, Peradeniya, Sri Lanka

Alaa El Din Mahmoud Environmental Sciences Department, Alexandria University, Alexandria, Egypt

Abdul Razaque Memon Department of Molecular Biology and Genetics, Usak University, Usak, Turkey

Virendra Kumar Mishra Department of Environmental Science, Indira Gandhi National Tribal University, Amarkantak, India

Anand Mohan Department of Biotechnology, Lovely Professional University, Jalandhar-Delhi, India

Fatima Maria de Souza Moreira Federal University of Lavras, Lavras, Minas Gerais, Brazil

Behzad Murtaza Department of Environmental Sciences, COMSATS Institute of Information Technology, Vehari, Pakistan

Ghulam Murtaza Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, Faisalabad, Pakistan

Muhammad Farrakh Nawaz Department of Forestry and Range Management, Faculty of Agriculture, University of Agriculture Faisalabad, Faisalabad, Pakistan

Nabeel Khan Niazi Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, Faisalabad, Pakistan

Southern Cross GeoScience, Southern Cross University, Lismore, NSW, Australia

MARUM and Department of Geosciences, University of Bremen, Bremen, Germany

Nataša Nikolić Faculty of Sciences, Department for Biology and Ecology, University of Novi Sad, Novi Sad, Republic of Serbia

Saša Orlović Institute of Lowland Forestry and Environment, Novi Sad, Republic of Serbia

Slobodanka Pajević Faculty of Sciences, Department for Biology and Ecology, University of Novi Sad, Novi Sad, Republic of Serbia

Manoj S. Paul Department of Botany, St. John's College Agra, Agra, India

A.P. Pinto Chemistry Department of Science and Technology School, Évora University, Évora, Portugal

Institute of Mediterranean Agricultural and Environmental Sciences (ICAAM), Évora University, Évora, Portugal

Gonzalo E. Pizarro Department of Hydraulic and Environmental Engineering, Pontificia Universidad Católica de Chile, Santiago, Chile

Prayad Pokethitiyook Department of Biology, Faculty of Science, Mahidol University, Bangkok, Thailand

Toemthip Poolpak Department of Biology, Faculty of Science, Mahidol University, Bangkok, Thailand

M.N.V. Prasad Department of Plant Sciences, University of Hyderabad, Hyderabad, Telangana, India

João Pratas MARE—Marine and Environmental Sciences Centre, Faculty of Sciences and Technology, University of Coimbra, Coimbra, Portugal

Department of Earth Sciences, Faculty of Sciences and Technology, University of Coimbra, Coimbra, Portugal

Instituto de Geologia e Petróleo de Timor Leste, Díli, Timor-Leste

Consuelo d.P. Rámila Department of Hydraulic and Environmental Engineering, Pontificia Universidad Católica de Chile, Santiago, Chile

Wesley de Melo Rangel Federal University of Lavras, Lavras, Minas Gerais, Brazil

Iqra Rasheed Department of Environmental Sciences and Engineering, Government College University, Faisalabad, Pakistan

Irfan Rashid Department of Botany, University of Kashmir, Srinagar, India

Hasibur Rehman Department of Biology, Faculty of Science, University of Tabuk, Tabuk, Saudi Arabia

Zafar A. Reshi Department of Botany, University of Kashmir, Srinagar, India

Muhammad Rizwan Department of Environmental Sciences and Engineering, Government College University Faisalabad, Faisalabad, Pakistan

Zulfiqar Ahmad Saqib Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, Faisalabad, Pakistan

Santosh Kumar Sarkar Department of Marine Science, University of Calcutta, West Bengal, Kolkata, India

Jerusa Schneider University of Campinas, Campinas, São Paulo, Brazil

Mihiri Seneviratne Department of Botany, Faculty of Natural Sciences, Open University of Sri Lanka, Nawala, Nugegoda

Manzoor A. Shah Department of Botany, University of Kashmir, Srinagar, India

Muhammad Shahid Department of Environmental Sciences, COMSATS Institute of Information Technology, Vehari, Pakistan

Munazzam Jawad Shahid Department of Environmental Science and Engineering, Government College University Faisalabad, Faisalabad, Pakistan

Muhammad Bilal Shakoor Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, Faisalabad, Pakistan

Reetika Shukla Department of Environmental Science, Indira Gandhi National Tribal University, Amarkantak, India

José Oswaldo Siqueira Vale Institute of Technology, Sustainable Development, Nazaré, Brazil

Cláudio Roberto Fonsêca Sousa Soares Federal University of Santa Catarina, Santa Catarina, Brazil

Neerja Srivastava Department of Biochemistry, IBSBT, CSJM University, Kanpur, India

D. Martins Teixeira Chemistry Department of Science and Technology School, Évora University, Évora, Portugal

HERCULES Laboratory, Évora University, Évora, Portugal

A. de Varennes Linking Landscape, Environment, Agriculture and Food, Instituto Superior de Agronomia, University of Lisbon (TU Lisbon), Lisbon, Portugal

Mayank Varun Department of Botany, St. John's College Agra, Agra, India

Meththika Vithanage Chemical and Environmental Systems Modeling Research Group, National Institute of Fundamental Studies, Kandy, Sri Lanka

Hailong Wang Key Laboratory of Soil Contamination Bioremediation of Zhejiang Province, Zhejiang A & F University, Zhejiang, China

Milan Župunski Faculty of Sciences, Department for Biology and Ecology, University of Novi Sad, Novi Sad, Serbia

Part I

Phytoremediation of Heavy Metal Contaminants

Chapter 1
Phytoremediation of Mining Areas: An Overview of Application in Lead- and Zinc-Contaminated Soils

Tiziana Lai, Giovanna Cappai, and Alessandra Carucci

1.1 Introduction

The metals concentration in soils is connected with natural and anthropogenic factors: metals are naturally present in soil in trace as a consequence of the decomposition of pedogenic substrate, while, anthropogenic activities such as emissions from the industrial areas, mine tailings, disposal of wastes, wastewater treatment, land fertilization and animal manures entail the release of metals into the environment, a large proportion of which are accumulated in soil [1–3]. On the basis of data reported by UNEP [4], mining is a significant contributor to the national economy in 158 countries worldwide.

Processing of lead and zinc metallic ores may involve a number of physical and chemical steps in order to separate the mineral resources from the less valuable material (gangue) [4]. Profitable recovery of lead and zinc ranges from about 3 % of metal in ore, for large and easily accessed mines, to more than 10 % in case of extremely costly and remote mines [5]. Minerals process, usually, produces several environmental impacts linked to each different stages of the process and generates large volumes of waste. Especially, waste rock and tailings represent a secondary source of pollutants that could contaminate soil, surface water and ground water even for hundreds of years after the mine closure. Moreover, the extent of contamination

---

T. Lai (✉)
DICAAR, Department of Civil-Environmental Engineering and Architecture,
University of Cagliari, Via Marengo 2, Piazza d'Armi, Cagliari 09123, Italy
e-mail: lai.tiziana@gmail.com

G. Cappai • A. Carucci
DICAAR, Department of Civil-Environmental Engineering and Architecture,
University of Cagliari, Via Marengo 2, Piazza d'Armi, Cagliari 09123, Italy

IGAG-CNR, Institute of Environmental Geology and Geoengineering, Research National
Council of Italy, Piazza d'Armi, Cagliari 09123, Italy
e-mail: gcappai@unica.it; carucci@unica.it

A.A. Ansari et al. (eds.), *Phytoremediation*, DOI 10.1007/978-3-319-40148-5_1

due to the mobilization of metals can interest areas of hundreds of kilometres away from historical mining sites depending on site characteristics [6, 7].

Metals are included in lists of priority pollutants of US Environmental Protection Agency (Ag, As, Be, Cd, Cr, Cu, Hg, Ni, Pb, Sb, Se, Tl and Zn) and of European Union with the Directive 2013/39/EU (Cd, Hg, Ni and Pb). These lists include both essential elements, toxic depending on the dose (e.g. Cr, Cu, Zn), and non-essential toxic elements, e.g. Hg and Pb [2, 8]. Different approaches can be considered for soil remediation: isolation, immobilization, toxicity reduction, physical separation and extraction. The selection of the most appropriate method depends on the site characteristics, nature of pollutants and their concentration. Physical and chemical technologies are well known and extensively applied [9], but can alter soil and landscape characteristics and entail high costs due to the wide areas involved [10–12]. Conversely, phytoremediation has been universally considered as a cost-effective technique that permits to restore biological activity and physical structure of soil (among others, [13–17]).

1.2 Lead and Zinc Mining Worldwide and Related Environmental Impacts

Mining activities produce several environmental impacts linked to each different stages of the mineral exploitation: starting from the exploration for the discovery of mineral deposits, the ore extraction and mineral processing until the mining closure and remediation of the site (Table 1.1).

The extent of impacts caused by mineral exploitation depends on site characteristics, amount of material handled, chemical composition of ore and surrounding rocks, extraction processes and technologies used to prevent or reduce the effects [4]. The excavation and the removal of vegetation related to exploration and operational phase are associated with metals contamination and erosion of soil [5]. The mineral processing includes physical and chemical methods. The physical methods present, generally, minor environmental impacts; chemical methods, due to the use of different reagents (sodium carbonate, sodium hydroxide, sulphuric acid, etc.) present instead a greater environmental impact [5].

Lead and zinc most often occur in association with the sulphide mineral group, in particular, galena (PbS) and sphalerite (ZnS). Other metals, such as copper, iron, mercury, arsenic, cadmium, silver and small quantities of gold are associated with sulphide ores [5]. Natural weathering process entails the oxidation of metal sulphide minerals in the host rock and the formation of sulphuric acid could occur prior to mining. However, the consequent release of acid and metal mobilization poses a limited threat to the environment. Conversely, extraction and mineral processing associated with mining activity expose larger volumes of sulphide rock material to weathering processes increasing the metal mobilization [18]. Especially after the mine closure, the runoff and leaching from waste rock and tailings increase the oxidation of remaining sulphides, through chemical, electrochemical and biological

Table 1.1 Stages of mineral processing and main related impacts [4, 5]

Stages	Process	Impacts	Emission/waste
Extraction	Removal of ore material from a deposit and activities prior to beneficiation	Destruction of natural habitats and landscape Erosion caused by removal of vegetation Influence on hydrology around the excavated area Soil, water, and air pollution	Waste rock piles containing minerals associated with sulphide ores (chalcopyrite, pyrite, calcite, and dolomite) Wastewater from excavation phase Sediment run-off from mining sites. Acid mine drainage Wind dispersion of dust and greenhouse gas emissions
Beneficiation	Crushing, grinding, physical and chemical separation	Soil, water, and air pollution	Waste rock and tailings containing high concentration of metals and minerals, and toxic chemicals Wastewater containing dissolved solids and reagents Wind dispersion of dust and greenhouse gas emissions
Processing	Smelting and refining of concentrates	Air pollution	Emission of sulphur dioxide, arsenic, lead, cadmium, and other metals, dusts
Closure	Residues disposal	Contamination of surface, ground water, and air due to re-entrainment and/or subsequent deposition of particulates	Waste rock and tailings Acid mine drainage Leaching of pollutants from tailings Wind dispersion of dust from tailings

reactions; furthermore, it could generate ferric hydroxides and sulphuric acid combined in acidic mine drainage that increases the leaching potential of metals and their transport into ground water, surface water and soil [18–20].

This phenomenon is site specific depending on many factors: climate conditions, neutralization capacity of local materials, etc. [18, 19, 21, 22]. The effects on the environment can be mitigated by both prevention and treatment options: minimization of oxygen diffusion, control of pH of mineral wastes, solidification of wastes, inhibition of iron and sulphur oxidizing bacteria [23]. Although modern mines are equipped and managed with technologies suitable to prevent or attenuate their impacts, countries with a long mining history may present, in most cases, significant environmental impacts due to a poor management after mine closure [4, 24]. In fact, in modern mine, concentrations of As, Cd, Cu, Mn, Pb and Zn in tailings are as low as 1 g kg^{-1} while in historic mine they can be greater than 50 g kg^{-1} [10].

Numerous authors have evaluated the environmental contamination in the surrounding area of mining sites in different countries (among others, [25–33]). Recently, the spatial variability of Pb, Zn and Cd pollution in the mining sites of Bama mine (Iran) and surrounding urban areas has been evaluated by Dayani and Mohammadi [34]. Candeias et al. [20] assessed the levels of soil contamination in the Aljustrel mine (SW Portugal), with the aim to understand the partitioning and availability of pollutants in soil. The results showed a severe contamination (maximum concentration of Pb and Zn of 20000 mg kg^{-1}). Pb and Zn contamination due to former mining and smelting carried out in Plombières and La Calamine (Belgium) was evaluated by Cappuyns et al. [35].

The effect of mining and metallurgical activities in the neighbourhood of the Bolesław Mine and Metallurgical Plant in Bukowno (Poland) was evaluated by Agnieszka et al. [3] by germination inhibition and luminescence inhibition test for the assessment of ecological risks in soil and water. Impact of Pb and Zn mining activity on superficial sediments of Lake Kalimanci (FYR Macedonia) related to the weathering of tailings dam material was studied by Vrhovnik et al. [36].

The metals accumulation in soil determines direct and indirect effect on biotic communities. Metal accumulation in plants alters seed germination, plant growth, absorption and transport of essential elements. In addition, it can cause chlorosis, photosynthesis inhibition and mortality. A study done on wild rodents and plants, reported negative effects, such as loss of diversity of the biotic communities, due to metals bioaccumulation [37]. Moreover, soil contaminated from a Pb and Zn mine showed a decrease on both the biomass and diversity of the bacterial community in soil [38].

The metal fraction that, within a given time span, is either available or can be made available for uptake by plant in addition to the total metals concentration in soil, must be evaluated and also the metal chemical speciation must be identified in order to define the most suitable remediation technology [9, 39]. The speciation of trace metals depends on the physical and chemical characteristics of the soil: pH, redox potential, organic, carbonate, clay and oxide contents [9].

With the aim to predict the mobility and availability of metal in soil, different extraction methods have been developed [39, 40].

1.3 Phytoremediation Technologies Applied in Pb/Zn Mining Areas

Phytoremediation is a technology based on the capacity of plants to accumulate both metals which are essential elements for their growth (i.e. Zn) and metals which have no known biological function (i.e. Pb) [8, 41]. Technologies applicable for cleanup of Pb- and Zn-contaminated soils include phytoextraction (metals removal from soil and their concentration in the harvestable parts of plants) and phytostabilization (reduction of the mobility and bioavailability of metals in the environment) [41–43]. Plants suitable in phytoextraction should be tolerant to high metal concentration in soil, have the capability to accumulate great levels of metal in the harvestable part, have high growth rate and biomass production and finally have an extended root system [44].

In case of phytostabilization plants should be tolerant to the soil conditions, have high growth rate, provide a dense ground cover and have an extended root system. Moreover, plants must concentrate contaminant in a greater extent in root in comparison to aerial part [16]. Plant species that are capable of colonizing soils highly polluted by metals are defined metallophyte and pseudometallophyte species. Metallophytes, including hyperaccumulators, are endemic plant of natural mineralized soils which have developed physiological mechanisms of resistance and tolerance to the high metal concentration in soil and are generally characterized by a reduced production of biomass. Pseudometallophytes are native species common also in non-metalliferous soil which, due to selective pressure, are capable of surviving in soils highly polluted by metals [17].

Over 400 hyperaccumulator plants have been identified, some of these species, belonging to the Aceraceae, Brassicaceae, Caryophyllaceae, Cistaceae, Dichapetalaceae, Plumbaginaceae, Poaceae, Polygonaceae and Violaceae, in particular, were demonstrated capable of accumulating Pb and/or Zn [45–48]. Plants species are considered hyperaccumulators if metal concentration in shoots is >1000 and >10000 mg kg^{-1} of dry weight for Pb and Zn, respectively, when grown in metal-rich soils [49]. *Thlaspi caerulescens*, common in Western and Central Europe, can accumulate a maximum of 4 % of Zn in its dry matter and a less extent of Cd and Pb [46]. *Thlaspi rotundifolium* ssp. c*epaeifolium*, from a Pb and Zn mining area in Northern Italy has accumulated Pb at about 0.8 % of dry weight [45]. Recently, van der Ent et al. [50] has proposed a critical review on criteria commonly used to delimit hyperaccumulation of some metals and indicated lower limit. For instance, a limit lowered to 3000 mg kg^{-1} of dry weight was proposed for Zn. On the other side, excluders are plant species able to accumulate metals in roots limiting their transport into aerial parts, these plants are ideal candidate for phytostabilization process. Indicators accumulate metals in their aerial parts generally in proportion to the metal concentration in soil [51].

Previous investigations have demonstrated the accumulation potential of tree species, such as *Salix* spp., *Populus* L. and *Betula* L., when growing on metal-contaminated soils [43, 52–55]. Potential to accumulate metals in harvestable parts of *Salix* spp. (*Salix purpurea* L., *Salix caprea* L. and *Salix eleagnos* Scop.) collected from abandoned sulphide mine dumps has been evaluated [56]. The metal accumulation capacity evaluated by translocation factor (TF), ratio between metal concentration in shoots and metal concentration in roots, has shown significant differences among the species studied: *S. purpurea* was able to uptake and translocate Pb from roots to shoots (TF = 3.42) while *S. caprea* demonstrated similar ability for Zn (TF = 3.48), considering a soil with a mean Pb and Zn concentration of about 9600 and 1250 mg kg^{-1}, respectively. The metal translocation ability, combined with high biomass production makes these species suitable for phytoremediation and phytoextraction, in particular [56, 57].

Even agricultural and ornamental species have the capability to concentrate metals together with a high biomass production. *Brassica napus*, *Brassica juncea*, *Helianthus annuus* and *Zea mays* have been considered among others; generally, these species can be applied in a multi-metal-contaminated soil [58–60]. In case of use of agricultural species, some factors have to be taken into account: adaptability at the local climate conditions and soil agronomic properties, and tolerance to metal concentration in soil of the species chosen. The ornamental species *Mirabilis jalapa* L. has demonstrated its capacity to accumulate 1500 mg kg^{-1} of Pb in roots and about 400 mg kg^{-1} in the aerial part of the plant, from a soil with a Pb concentration of about 5500 mg kg^{-1} [61]. In case of phytoremediation of mining areas, native plants are preferable in comparison to introduced or invasive species, in order to reduce possible impact on the ecosystem [10, 62, 63]. Moreover, native plant species growing on mine tailings demonstrated a better tolerance to local conditions (climate, contamination and nutrient deficiency, etc.) [17, 64, 65]. Recently, different studies, summarized in Table 1.2, have been conducted in Pb and Zn mining areas in order to identify native plant species potentially relevant in phytoremediation.

In natural or continuous phytoremediation, plants with a $TF > 1$ are considered suitable species for phytoextraction, while species with a $TF < 1$ are generally considered suitable for phytostabilization and revegetation process. In addition, with the aim to modify accumulation characteristics of plants, soil amendments can be applied either to increase metal availability in soil (e.g. chelating agents or acidifying amendments), in case of assisted phytoextraction, or improve soil agronomic proprieties (e.g. fertilization), in case of aided phytostabilization [41, 66]. A field experiment was conducted by Zhuang et al. [67] with the aim to evaluate the effect of EDTA (ethylenediaminetetraacetic acid) in phytoextraction. Three plants were tested: *Viola baoshanensis*, *Vertiveria zizanioides* and *Rumex K-1 (Rumex patientia* × *R. Timschmicus*). Among the species tested, *V. baoshanensis* showed high potential for phytoremediation, and the application of EDTA enhanced Pb and Zn phytoextraction rates from 0.01 to 0.19 %, and 0.17 to 0.26 %, respectively. However, in assisted phytoextraction the chemical treatments can become a secondary cause of pollution. In fact, chelating agents, such as EDTA, are slowly biodegradable and increase the leachable metal fraction into ground water [68, 69]. In order to overcome these effects, biodegradable chelating agents should be applied [70].

In an assisted phytoextraction experiment in pots, Cao et al. [71] compared Pb and Zn phytoextraction by *M. jalapa*, using EDDS ([*S*,*S*]-ethylenediaminedisuccinic acid) and MGDA (methylglycinediacetic acid) in two different dosages (4 and 8 mmol kg^{-1} of soil). Both chelating agents demonstrated to increase Pb accumulation in leaves as well as improve bacterial activity in the soil. In the case of Zn, metal accumulation was independent from chelating agents application. However,

Table 1.2 Native plants species in phytoremediation experiment in mine soil contaminated by Pb and Zn (TF = translocation factor)

Plant species	Mine	Location	Metal concentration in soil (mean) [mg kg^{-1}] Pb	Zn	TF Pb	Zn	Reference
Achyrocline alata (Kunth) DC.	Hualgayoc	Peru	16060	28058	1.5	2.0	Bech et al. [103][a]
Ageratina sp.	Hualgayoc	Peru	16060	28058	0.4	0.6	Bech et al. [103][a]
Aster gymnocephalus A. Gray	Santa Maria	Mexico	4183	4546	2.0	20.5	Sánchez-López et al. [104]
Betula celtiberica	Rubiales	Spain	3000	20000	0.2	0.8	Becerra-Castro et al. [95]
Bidens triplinervia L.	Hualgayoc	Peru	13105	28393	0.13	0.16	Bech et al. [105]
Brickelia veronicifolia (Kunth) A. Gray	San Francisco	Mexico	1923	4745	0.6	1.4	Sánchez-López et al. [104]
Brickelia veronicifolia (Kunth) A. Gray	Santa Maria	Mexico	4183	4546	1.3	4.2	Sánchez-López et al. [104]
Cistus populifolius L.	Caveira	Portugal	4245	494	0.1	5.0	Abreu et al. [106]
Cistus populifolius L.	Chança	Portugal	141	66	0.11	2.53	Abreu et al. [106]
Cistus salviifolius L.	Campo Pisano	Italy	3260	12000	2.0	2.2	Cao et al. [107]
Cistus salviifolius L.	Chança	Portugal	141	66	0.2	2.93	Abreu et al. [106]
Cistus salviifolius L.	São Domingos	Portugal	4853	605	0.54	2.14	Abreu et al. [108]
Cistus salviifolius L.	Caveira	Portugal	7416	357	0.1	2.72	Abreu et al. [108]
Cistus salviifolius L.	Caveira	Portugal	4245	494	0.1	2.17	Abreu et al. [106]
Cistus salviifolius L.	São Domingos	Portugal	5901	294	0.34	2.59	Abreu et al. [106]
Cistus × hybridus	Caveira	Portugal	4245	494	0.11	5.32	Abreu et al. [106]
Cistus × hybridus	Chança	Portugal	141	66	1.5	2.74	Abreu et al. [106]
Cortaderia hapalotricha Pilg.	Hualgayoc	Peru	16060	28058	1.7	1.2	Bech et al. [103][a]

(continued)

Table 1.2 (continued)

Plant species	Mine	Location	Metal concentration in soil (mean) [mg kg^{-1}]		TF		Reference
			Pb	Zn	Pb	Zn	
Crotalaria pumila Ortega	Santa Maria	Mexico	4183	4546	1.1	11.6	Sánchez-López et al. [104]
Cuphea lanceolata Aiton	San Francisco	Mexico	1923	4745	0.6	6.7	Sánchez-López et al. [104]
Cytisus scoparius	Rubiales	Spain	3000	20000	0.2	0.4	Becerra-Castro et al. [95]
Dalea bicolor Humb. & Bonpl. Ex Willd.	San Francisco	Mexico	1923	4745	0.6	2.3	Sánchez-López et al. [104]
Dalea bicolor Humb. & Bonpl. Ex Willd.	Santa Maria	Mexico	4183	4546	0.9	3.3	Sánchez-López et al. [104]
Debregeasia orientalis	Beiya	China	2217	240	0.93	0.83	Liu et al. [109]
Dichondra argentea Willd.	San Francisco	Mexico	1923	4745	0.6	3.4	Sánchez-López et al. [104]
Dichondra argentea Willd.	Santa Maria	Mexico	4183	4546	0.8	1.3	Sánchez-López et al. [104]
Epilobium denticulatum Ruiz & Pav.	Hualgayoc	Peru	10128	23678	1.1	1.5	Bech et al. [103][a]
Festuca rubra	Rubiales	Spain	3000	20000	0.10	0.2	Becerra-Castro et al. [95]
Flaveria trinervia	Santa Maria	Mexico	4183	4546	1.0	10.9	Sánchez-López et al. [104]
Gnaphalium sp.	Santa Maria	Mexico	4183	4546	1.6	20.8	Sánchez-López et al. [104]
Hyparrhenia hirta	Cartagena-La Union	Spain	4200	15000	0.8	0.3	Conesa et al. [110]
Juniperus sp.	San Francisco	Mexico	1923	4745	1.1	17	Sánchez-López et al. [104]
Pteridium sp.	San Francisco	Mexico	1923	4745	0.2	0.2	Sánchez-López et al. [104]
Ruta graveolens L.	San Francisco	Mexico	1923	4745	1.0	2.5	Sánchez-López et al. [104]
Scrophularia canina subsp. bicolor	Campo Pisano	Italy	3260	12000	0.8	1.1	Cao et al. [107]
Senecio sp.	Hualgayoc	Peru	13105	28393	9.4	4.7	Bech et al. [105]
Taraxacum officinale Weber	Hualgayoc	Peru	14197	25829	0.6	0.4	Bech et al. [103][a]

(continued)

Table 1.2 (continued)

Plant species	Mine	Location	Metal concentration in soil (mean) [mg kg^{-1}]		TF		Reference
			Pb	Zn	Pb	Zn	
Tephrosia candida	Beiya	China	2207	256	0.85	0.77	Liu et al. [109]
Teucrium flavum L. subsp. *glaucum*	Campo Pisano	Italy	3260	12000	1.6	0.7	Cao et al. [107]
Trifolium repens Walter	Hualgayoc	Peru	10128	23678	1.5	1.3	Bech et al. [103][a]
Viguiera dentata (Cav.) Spreng.	San Francisco	Mexico	1923	4745	0.5	0.9	Sánchez-López et al. [104]
Viguiera dentata (Cav.) Spreng.	Santa Maria	Mexico	4183	4546	0.6	2.0	Sánchez-López et al. [104]
Zygophyllum fabago	Cartagena-La Union	Spain	4800	13000	0.7	1.5	Conesa et al. [110]

[a] Data referring to the substrate having the higher metal concentrations

both EDDS and MGDA demonstrated to be toxic to the plant causing death at maximum dose. Response of treatment with chelating agents seems to be related to the dosages applied [72–75].

The application of complementary techniques such as additives application and fertilization could improve phytostabilization results [76, 77]. The organic amendments, as compost, increase the content of essential nutrients of soil (C, N, P, K), which improve plant growth and stimulate the microbial activities. The effectiveness of these treatments for the reduction of soil risks have been confirmed by ecotoxicological tests with bacteria *Vibrio fischeri,* crustaceans *Daphnia magna* and *Thamnocephalus platyurus* and earthworm *Eisenia fetida* tests [78, 79].

A greenhouse experiment was conducted by Lee et al. [80] to evaluate the effect of four different amendments (bone mill, bottom ash, furnace slag and red mud) as immobilizing agents and two Korean native plant species, *Miscanthus sinensis* and *Pteridium aquilinum,* in aided phytostabilization of Pb and Zn mine tailings. Results of the study suggest that *M. sinensis* is appropriate for phytostabilization, since it accumulated heavy metals mainly in the root, and had lower translocation factors compared with *P. aquilinum;* furthermore, amendments such as furnace slag and red mud are effective at reducing the availability and mobility of metals. Recently, phytostabilization experiments have been carried out in field with the use of native species, selected on the basis of their ability to survive and regenerate in the local environment.

The area of the experiments performed by de la Fuente et al. [81], was located downstream the Aznalcóllar mine (Spain) [82], previously object of different phytoremediation experiments [83]. Native species (*Retama sphaerocarpa, Tamarix gallica, Rosmarinus officinalis* and *Myrtus communis*) were grown under natural conditions, without any agricultural practice or irrigation system, in soil with a maximum metal concentration of about 839 and 1617 mg kg^{-1} for Pb and Zn, respectively. The results permitted to identify the *R. sphaerocarpa* as the most adequate plant species for soil restoration. At the end of the experiment, *R. sphaerocarpa* showed the highest percentage of plant survival (44 %), the ability to grow in soils with poor agronomic properties and acidic conditions, and the lower bioconcentration factor (i.e. metal concentration in shoot tissues versus total metal concentration in soil) equal to 0 and 0.19 for Pb and Zn, respectively.

Results from an application of P fertilizers (phosphate rock, calcium magnesium phosphate and single superphosphate) in field plots planted with *Brassica chinensis* L. *campestris* indicate that these amendments induced immobilization of metals such as Pb, Cd and Zn [84].

The phytostabilization experiment performed in the tailings dam of Campo Pisano (Sardinia, Italy), consisted in the use of different soil amendments, compost, chemical fertilizer and zeolites, used singly or in combination. In general, all amendments reduced the bioavailable metal fraction; in particular, compost proved to be the best amendment in the long-term for plant growth. Among the plant species tested (*Scrophularia canina* subsp. *bicolor Greuter* and *Pistacia lentiscus*) *P. lentiscus* appears to be the most suitable species for phytostabilization and revegetation, both for its resistance to metals and high phytomass production [85].

Galende et al. [86] evaluated the application of combined organic amendments (cow slurry, poultry manure and paper mill sludge mixed with poultry manure) in a phytostabilization experiment on an abandoned Pb and Zn mine located in the province of Biscay (Basque Country, Spain) with *Festuca rubra L.* species. Amendment application demonstrated to promote biomass production in *F. rubra* and caused a reduction in bioavailable Pb and Zn in soils. Further investigations focusing on phytoremediation of Pb and Zn mine areas have been conducted also by applying non-native species as reported in Table 1.3.

An additional aspect to be considered is that plants play an important role in reducing dispersion of soil-contaminated particles from mine tailings caused by atmospheric agents. Recently, the role of leaves of plants growing spontaneously on mine tailings acting as a barrier for the dispersion of particles containing potentially toxic elements has been evaluated [87]. Comprehensive reviews, summarizing the most important aspects of phytoremediation processes and physiological mechanisms of metal accumulation in plants are available (Table 1.4).

Table 1.3 Assisted phytoremediation experiments in mine soils contaminated by Pb and Zn

		Pb in soil	Zn in soil	Pb in soil	Zn in soil	Details on extraction agent		
Plant species	Location	Total (mg kg^{-1})	Total (mg kg^{-1})	Extractable (mg kg^{-1})	Extractable (mg kg^{-1})		Amendments	Reference
Triticum aestivum L.	Příbram (Czech Republic)	3035	4900	266	2925	Acetic acid	Digestate (biowaste anaerobic fermentation)	García-Sánchez et al. [111]
							Fly ash (wood chip combustion)	
							$(NH_4)_2SO_4$	
Mirabilis jalapa L.	Montevecchio-Ingurtosu (Italy)	5357	1767	3426	432	EDTA	Clinoptilolite	Lai et al. [61]
							Clinoptilolite NH_4^+-charged	
							Clinoptilolite CO_2-charged	
Zygophyllum fabago L.	Cartagena-La Unión (Spain)	–	–	277	495	DTPA	Pig manure + marble waste	Zornoza et al. [112]
Piptatherum miliaceum (L.) Coss.							Sewage sludge + marble waste	
Dittrichia viscosa (L.) Greuter								
Phragmites australis (Cav.) Trin. ex Steud								
Helichrysum decumbens DC.								
Sonchus tenerrimus L.								

(continued)

Table 1.3 (continued)

		Pb in soil	Zn in soil	Pb in soil	Zn in soil	Details on extraction agent		
Plant species	Location	Total (mg kg^{-1})	Total (mg kg^{-1})	Extractable (mg kg^{-1})	Extractable (mg kg^{-1})		Amendments	Reference
Vertiveria zizanioides	Lechang (China)	960	1050	119	93	DTPA	EDTA (ethylenediaminetetracetic acid)	Zhuang et al. [113]
Dianthus chinensis								
Rumex K-1 (Rumex upatientia × *R. timschmicus)*								
Rumex crispus								
Viola baoshanensis								
Sedum alfredii								
Brachiaria decumbens	Biscay (Spain)	4166	15277	–	–	–	EDTA (ethylenediaminetetracetic acid)	Santos et al. [114]
							EDDS (ethylenediaminedisuccinate)	
Vetiveria zizanioides	Lechang (China)	1155	1583	361	834	DTPA	Manure compost	Chiu et al. [115]
Phragmities australis							Sewage sludge	
Helianthus annuus	Bama (Iran)	1564	2739	29	182	DTPA	DTPA (diethylenetriaminepentaacetic acid)	Solhi et al. [116]
Brassica napus							Composted manure	
							Sulphuric acid	

Viola baoshanensis	Lechang (China)	2078	2472	177	161	DTPA	EDTA (ethylenediaminetetracetic acid)	Zhuang et al. [67]
Vertiveria zizanioides							$(NH_4)_2SO_4$	
Rumex K-1 (Rumex patientia · R. timschmicus)							NH_4NO_3	
Vetiveria zizanioides	Lechang (China)	4164	4377	331	187	DTPA	Domestic refuse	Yang et. [117]
Sesbania rostrata							Inorganic fertilizer	
Sesbania sesban								

Table 1.4 Selection of reviews related to phytoremediation of metals

Content	References < 2005	References > 2005
Source of contamination and its effects on the environment and human health	McLaughlin et al. [8]	Wuana and Okieimen [2]
		Bolan et al. [12]
		Su et al. [118]
Mining area specificity related to reclamation	Bradshaw [13]	Mendez and Maier [10]
	Tordoff et al. [14]	Li [119]
	Singh et al. [15]	
Availability of contaminants	Peijnenburg and Jager [39]	Leštan et al. [75]
	Kabata-Pendias [40]	Ali et al. [69]
		Bolan et al. [12]
Remediation technologies, including physical, chemical, and biological remediation	Mulligan et al. [9]	Marques et al. [16]
		Wuana and Okieimen [2]
		Yao et al. [11]
		Su et al. [118]
Phytoremediation techniques applicable in metal-contaminated soil including continuous and assisted phytoremediation	Salt et al. [41]	Li [119]
	Raskin et al. [58]	Evangelou et al. [73]
	Garbisu and Alkorta [44]	Padmavathiamma and Li [120]
	Singh et al. [15]	Mendez and Maier [10]
	McGrath and Zhao [121]	Marques et al. [16]
	Alkorta et al. [122]	Robinson et al. [51]
	Arthur et al. [123]	Vangronsveld et al. [66]
		Wu et al. [124]
		Vamerali et al. [60]
		Wuana and Okieimen [2]
		Cameselle et al. [125]
		Bolan et al. [12]
		Favas et al. [17]
		Gupta et al. [126]

Plants species suitable for remediation with particular focus on metallophytes (hyperaccumulators, excluders, indicators)	Singh et al. [15]	Padmavathiamma and Li [120]
	McGrath and Zhao [121]	Marques et al. [16]
	Yang et al. [127]	Robinson et al. [51]
		Sheoran et al. [128]
		Kavamura and Esposito [129]
		Rascio and Navari-Izzo [47]
		Bhargava et al. [130]
		van der Ent et al. [50]
		Ali et al. [69]
		Visioli and Marmiroli [131]
		Pollard et al. [48]
		Ullah et al. [132]
Plants species suitable for remediation (crops species, tree species, …)	Pulford and Watson [43]	Vamerali et al. [60]
		Bhargava et al. [130]
Parameters for evaluation of process efficiency	USEPA [133]	Robinson et al. [51]
		Wuana and Okieimen [2]
		Ali et al. [69]
		Favas et al. [17]
Genetic engineering plants	Garbisu and Alkorta [44]	Kotrba et al. [101]
	Pilon-Smits and Pilon [99]	Marques et al. [16]
	McGrath and Zhao [121]	Vangronsveld et al. [66]
	Eapen and D'Souza [134]	Wu et al. [124]
	Yang et al. [127]	Vamerali et al. [60]
		Bhargava et al. [130]

(continued)

Table 1.4 (continued)

Rhizosphere microbial community and its role in phytoremediation	Garbisu and Alkorta [44]	Lebeau et al. [135]
	Glick [136]	Marques et al. [16]
		Kavamura and Esposito [129]
		Glick [137]
		Vamerali et al. [60]
		Ma et al. [90]
		Bhargava et al. [130]
		Rajkumar et al. [138]
		Rajkumar et al. [139]
		Ullah et al. [132]
Economic aspects and costs evaluation	Glass [140]	Robinson et al. [51]
		Vangronsveld et al. [66]
		Wuana and Okieimen [2]

1.4 Synergistic Effects of Plants and Bacteria in Phytoremediation

Interactions between plant and rhizosphere microbial communities in Pb and Zn mine soil have been evaluated [88, 89]. Bacterial populations associated with plants growing in metalliferous soils should improve plant growth and nutrition by nitrogen fixation, production of phytohormones and siderophores, transformation of nutrient elements and by increasing metal tolerance and accumulation due to the capacity of microorganisms to increase bioavailable metals fraction, through the release of chelating agents, acidification, phosphate solubilization and redox changes [90–93].

The administration of selected plant growth-promoting bacteria can significantly speed up the phytostabilization process by improving plant establishment, growth and health as demonstrated in a bioaugmentation-assisted phytostabilization process based on autochthonous plant species and bacterial inocula from abandoned Sardinian mining areas (Italy) [94]. In a recent study, the plant-microorganism-soil system of three pseudometallophytes identified as metal excluders (*Betula celtiberica*, *Cytisus scoparius* and *Festuca rubra*) growing in a Pb and Zn mine was characterized. Becerra-Castro et al. [95] isolated metal-tolerant rhizobacteria from rhizosphere of selected plant species and verified, in a pot experiment, the effect of a re-inoculum of the rhizobacteria on growth and metal uptake of *Festuca pratensis* Huds. and *Salix viminalis* L., commonly used in phytoremediation. As a result, authors demonstrated that some of the plant-associated bacteria isolated from mine site could be exploited for improving plant growth, and performance, in metal-contaminated soil.

The effects of chelating agent's application in phytoextraction experiment have been, also, investigated. In an assisted phytoextraction process with *Cynara cardunculus*, the treatment with a single dose (1 g kg^{-1} of soil) of two chelating agents (EDTA and EDDS) on soil microorganisms has been evaluated through the determination of biological indicators of soil quality (i.e. enzyme activities, basal and substrate-induced respiration) [74]. EDTA was more efficient than EDDS in enhance root Pb uptake and root-to-shoot Pb translocation. However, EDDS was more rapidly degraded, and less toxic to the soil microbial community in control non-polluted soils. Pb-polluted soils treated with EDDS showed significantly higher values of basal and substrate-induced respiration than those treated with EDTA. In pots experiment with *Mirabilis jalapa*, the application of easily biodegradable chelating agents (EDDS and MGDA) seemed to have a positive influence on bacterial communities both in bulk soil and in the rhizosphere, whereas the endophytes were less affected by the treatments [71].

1.5 Genetically Engineered Plants for Phytoremediation

The genetically engineered plants (GEPs) have been considered in the last decades with the aim to evaluate their potential use in phytoremediation [96, 97]. In this frame, the goal of genetic engineering is to modify characteristics of plant species, such as metal uptake, transport and accumulation, and metal tolerance to enhance remediation efficiency (see related references in Table 1.4). Among different plant species growing on highly contaminated soils by metals in Eastern Spain, *Nicotiana glauca* R. Graham was selected for subsequent gene transfer (gene transferred: wheat gene-encoding phytochelatin synthase TaPCS1) because of its resistance to metals and physiological characteristics appropriate for phytoremediation [98]. Results demonstrated the increase of *N. glauca* tolerance to metals, such as Pb and Cd, and a higher accumulation Pb capacity in comparison of wild species in pot experiment with a Pb- and Zn-contaminated soil from a mine area.

When GEPs are used for applications in phytoremediation, the potential environmental risk needs to be considered. Possible risks include biological transformation of metals in more bioavailable forms, higher exposure of wildlife to metals in case of accumulation in palatable part of the plants, uncontrolled diffusion of transgenic plants [99–101]. Related to the latter, in order to control undesirable genetic spreading, Shim et al. [102] tested the non-flowering mutant poplar clone *Populus alba* × *P. tremula var. glandulosa,* transforming the plants with a metal resistance gene, ScYCF1 (yeast cadmium factor 1), and tested these transgenic plants in soil taken from a closed mine site contaminated with multiple toxic metals (As = 2171 mg kg^{-1}, Pb = 447 mg kg^{-1}, Zn = 2343 mg kg^{-1}) under greenhouse and field conditions. The results demonstrate that YCF1-expressing poplar plants have a higher tolerance to contaminated mining soil and higher metal accumulation capacity than control and are suitable for phytostabilization process of mine areas.

1.6 Conclusions

Phytoremediation is generally recognized as a cost-effective and environmental sustainable technology compared to physical and chemical remediation technologies. An extensive literature exists on the application of phytoremediation to soil contaminated by mining activity. Lead and zinc, extracted from sulphide ores, are among the most common contaminants in soils originated by mineral exploitation; both phytoextraction and phytostabilization can be applied in this case. However, it is well acknowledged that each mine site possesses specific physicochemical characteristics, and thus the identification of the ideal candidate for phytoremediation must be tailored on the individual mine site. Recently, greater attention is addressed to apply native plant species, which demonstrated a better tolerance to local conditions, and are preferable in comparison to introduced or invasive species in order to reduce possible impact on the ecosystem. On the basis of experimental results, phytoextraction of heavily polluted soils may be required decades to reduce the residual metal concentration to acceptable levels. In order to overcome this issue, assisted phytoextraction has been introduced, based on the use of chelating agents or acidifying amendments, aiming at lowering reclamation times and increasing process efficiency. However, the application of chemical treatments can become a secondary cause of pollution.

Taking into account the characteristics of mine areas, in particular size and level of contamination, phytostabilization seems the most preferable technique, while phytoextraction could be applied in areas surrounding mine sites when soil contamination is limited. Again, the use of amendments such as compost or fertilizers could enhance the process by improving the soil properties and assisting the plant growth. Finally, different studies are being conducted to evaluate potential use of genetically engineered plants for phytoremediation, but the environmental benefits and risks associated with GEPs should be carefully evaluated before field applications.

References

1. Wood M (1995) Environmental soil biology. Springer, New York
2. Wuana RA, Okieimen FE (2011) Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation. ISRN Ecol 2011:1–20. doi:10.5402/2011/402647
3. Agnieszka B, Tomasz C, Jerzy W (2014) Chemical properties and toxicity of soils contaminated by mining activity. Ecotoxicology 23(7):1234–1244
4. UNEP (2000) Industry and environment—mining and sustainable development II: challenges and perspectives, vol 23. http://www.uneptie.org/media/review/vol23si/unep23.pdf. Accessed 30 Nov 2015
5. USEPA (1994) Extraction and beneficiation of ores and minerals, vol 1: lead–zinc. http://www3.epa.gov/epawaste/nonhaz/industrial/special/mining/techdocs/leadzinc.pdf. Accessed 30 Nov 2015
6. Cao A, Cappai G, Carucci A et al (2008) Heavy metal bioavailability and chelate mobilization efficiency in an assisted phytoextraction process. Environ Geochem Health 30(2):115–119
7. Resongles E, Casiot C, Freydier R et al (2014) Persisting impact of historical mining activity to metal (Pb, Zn, Cd, Tl, Hg) and metalloid (As, Sb) enrichment in sediments of the Gardon River, Southern France. Sci Total Environ 481:509–521
8. McLaughlin MJ, Parker DR, Clarke JM (1999) Metals and micronutrients—food safety issues. Field Crop Res 60(1):143–163
9. Mulligan CN, Yong RN, Gibbs BF (2001) Remediation technologies for metal-contaminated soils and groundwater: an evaluation. Eng Geol 60(1):193–207
10. Mendez MO, Maier RM (2008) Phytoremediation of mine tailings in temperate and arid environments. Rev Environ Sci Biotechnol 7(1):47–59
11. Yao Z, Li J, Xie H et al (2012) Review on remediation technologies of soil contaminated by heavy metals. Procedia Environ Sci 16:722–729
12. Bolan N, Kunhikrishnan A, Thangarajan R et al (2014) Remediation of heavy metal(loid)s contaminated soils—to mobilize or to immobilize? J Hazard Mater 266:141–166
13. Bradshaw A (1997) Restoration of mined lands using natural processes. Ecol Eng 8(4):255–269
14. Tordoff GM, Baker AJM, Willis AJ (2000) Current approaches to the revegetation and reclamation of metalliferous mine wastes. Chemosphere 41(1):219–228
15. Singh AN, Raghubanshi AS, Singh JS (2002) Plantations as a tool for mine spoil restoration. Curr Sci 82(12):1436–1441
16. Marques AP, Rangel AO, Castro PM (2009) Remediation of heavy metal contaminated soils: phytoremediation as a potentially promising clean-up technology. Crit Rev Environ Sci Technol 39(8):622–654
17. Favas PJ, Pratas J, Varun M et al (2014) Phytoremediation of soils contaminated with metals and metalloids at mining areas: potential of native flora. In: Hernández-Soriano MC (ed) Environmental risk assessment of soil contamination. Intech, Rijeka, pp 485–517

18. USEPA (1994a) Acid mine drainage prediction. http://www3.epa.gov/epawaste/nonhaz/industrial/special/mining/techdocs/amd.pdf. Accessed 30 Nov 2015
19. Ardau C, Blowes DW, Ptacek CJ (2009) Comparison of laboratory testing protocols to field observations of the weathering of sulfide-bearing mine tailings. J Geochem Explor 100(2):182–191
20. Candeias C, da Silva EF, Salgueiro AR et al (2011) Assessment of soil contamination by potentially toxic elements in the Aljustrel mining area in order to implement soil reclamation strategies. Land Degrad Dev 22(6):565–585
21. Babbou-Abdelmalek C, Sebei A, Chaabani F (2011) Incurred environmental risks and potential contamination sources in an abandoned mine site. Afr J Environ Sci Technol 5(11):894–915
22. Iavazzo P, Adamo P, Boni M et al (2012) Mineralogy and chemical forms of lead and zinc in abandoned mine wastes and soils: an example from Morocco. J Geochem Explor 113:56–67
23. Pagnanelli F, De Michelis I, Di Tommaso M et al (2008) Treatment of acid mine drainage by a combined chemical/biological column apparatus: mechanisms of heavy metal removal. In: Sanchez ML (ed) Causes and effects of heavy metal pollution. Nova Science Publishers Inc., New York, pp 81–106
24. Concas S, Ardau C, Di Bonito M et al (2015) Field sampling of soil pore water to evaluate the mobile fraction of trace elements in the Iglesiente area (SW Sardinia, Italy). J Geochem Explor 158:82–94
25. Lee CG, Chon HT, Jung MC (2001) Heavy metal contamination in the vicinity of the Daduk Au–Ag–Pb–Zn mine in Korea. Appl Geochem 16(11):1377–1386
26. Li J, Xie ZM, Zhu YG et al (2005) Risk assessment of heavy metal contaminated soil in the vicinity of a lead/zinc mine. J Environ Sci (China) 17(6):881–885
27. Liu H, Probst A, Liao B (2005) Metal contamination of soils and crops affected by the Chenzhou lead/zinc mine spill (Hunan, China). Sci Total Environ 339(1):153–166
28. Navarro MC, Pérez-Sirvent C, Martínez-Sánchez MJ et al (2008) Abandoned mine sites as a source of contamination by heavy metals: a case study in a semi-arid zone. J Geochem Explor 96(2):183–193
29. Liao GL, Liao DX, Li QM (2008) Heavy metals contamination characteristics in soil of different mining activity zones. Trans Nonferr Metal Soc China 18(1):207–211
30. Ikenaka Y, Nakayama SM, Muzandu K et al (2010) Heavy metal contamination of soil and sediment in Zambia. Afr J Environ Sci Technol 4(11):729–739
31. Oyarzun R, Lillo J, López-García JA et al (2011) The Mazarrón Pb–(Ag)–Zn mining district (SE Spain) as a source of heavy metal contamination in a semiarid realm: geochemical data from mine wastes, soils, and stream sediments. J Geochem Explor 109(1):113–124
32. Onyeobi TUS, Imeokparia EG (2014) Heavy metal contamination and distribution in soils around Pb–Zn mines of Abakaliki District, South Eastern Nigeria. Front Geosci 2(2):30–40
33. Pourret O, Lange B, Bonhoure J et al (2016) Assessment of soil metal distribution and environmental impact of mining in Katanga (Democratic Republic of Congo). Appl Geochem 64:43–55
34. Dayani M, Mohammadi J (2010) Geostatistical assessment of Pb, Zn and Cd contamination in near-surface soils of the urban-mining transitional region of Isfahan, Iran. Pedosphere 20(5):568–577
35. Cappuyns V, Swennen R, Vandamme A et al (2006) Environmental impact of the former Pb–Zn mining and smelting in East Belgium. J Geochem Explor 88(1):6–9
36. Vrhovnik P, Šmuc NR, Dolenec T et al (2013) Impact of Pb-Zn mining activity on surficial sediments of Lake Kalimanci (FYR Macedonia). Turk J Earth Sci 22(6):996–1009
37. Espinosa-Reyes G, González-Mille DJ, Ilizaliturri-Hernández CA et al (2014) Effect of mining activities in biotic communities of Villa de la Paz, San Luis Potosi, Mexico. Biomed Res Int 1–13. http://dx.doi.org/10.1155/2014/165046
38. Qing H, Qi HY, Zeng HY et al (2007) Bacterial diversity in soils around a lead and zinc mine. J Environ Sci 19(1):74–79
39. Peijnenburg WJGM, Jager T (2003) Monitoring approaches to assess bioaccessibility and bioavailability of metals: matrix issues. Ecotoxicol Environ Saf 56(1):63–77

40. Kabata-Pendias A (2004) Soil–plant transfer of trace elements—an environmental issue. Geoderma 122(2):143–149
41. Salt DE, Blaylock M, Kumar NP et al (1995) Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants. Nat Biotechnol 13(5):468–474
42. Chaney RL, Malik M, Li YM et al (1997) Phytoremediation of soil metals. Curr Opin Biotechnol 8(3):279–284
43. Pulford ID, Watson C (2003) Phytoremediation of heavy metal-contaminated land by trees—a review. Environ Int 29(4):529–540
44. Garbisu C, Alkorta I (2001) Phytoextraction: a cost-effective plant-based technology for the removal of metals from the environment. Bioresour Technol 77(3):229–236
45. Baker AJM, McGrath SP, Reeves RD et al (1999) Metal hyperaccumulation plants: a review of the ecology and physiology of a biological resource for phytoremediation of metal-polluted soils. In: Terry N, Banuelos GS (eds) Phytoremediation of contaminated soil and water. CRC Press, Boca Raton, pp 85–107
46. Reeves RD, Baker AJM (2000) Metal-accumulating plants. In: Raskin I, Ensley B (eds) Phytoremediation of toxic metals. Using plants to clean up the environment. Wiley, New York, pp 193–229
47. Rascio N, Navari-Izzo F (2011) Heavy metal hyperaccumulating plants: how and why do they do it? And what makes them so interesting? Plant Sci 180(2):169–181
48. Pollard AJ, Reeves RD, Baker AJ (2014) Facultative hyperaccumulation of heavy metals and metalloids. Plant Sci 217:8–17
49. Blaylock MJ, Huang JW (2000) Phytoextraction of metals. In: Raskin I, Ensley B (eds) Phytoremediation of toxic metals. Using plants to clean up the environment. Wiley, New York, pp 53–70
50. van der Ent A, Baker AJ, Reeves RD et al (2013) Hyperaccumulators of metal and metalloid trace elements: facts and fiction. Plant Soil 362(1–2):319–334
51. Robinson BH, Banuelos G, Conesa HM et al (2009) The phytomanagement of trace elements in soil. Crit Rev Plant Sci 28(4):240–266
52. Ali MB, Vajpayee P, Tripathi RD et al (2003) Phytoremediation of lead, nickel, and copper by *Salix acmophylla* Boiss.: role of antioxidant enzymes and antioxidant substances. Bull Environ Contam Toxicol 70(3):462–469
53. Yanqun Z, Yuan L, Schvartz C et al (2004) Accumulation of Pb, Cd, Cu and Zn in plants and hyperaccumulator choice in Lanping lead–zinc mine area, China. Environ Int 30(4):567–576
54. Unterbrunner R, Puschenreiter M, Sommer P et al (2007) Heavy metal accumulation in trees growing on contaminated sites in Central Europe. Environ Pollut 148(1):107–114
55. Wang Y, Bai S, Wu J et al (2015) Plumbum/Zinc accumulation in seedlings of six afforestation species cultivated in mine spoil substrate. J Trop For Sci 27(2):166–175
56. Wahsha M, Bini C, Argese E et al (2012) Heavy metals accumulation in willows growing on Spolic Technosols from the abandoned Imperina Valley mine in Italy. J Geochem Explor 123:19–24
57. Wahsha M, Nadimi-Goki M, Bini C (2015) Land contamination by toxic elements in abandoned mine areas in Italy. J Soil Sediments 16(4):1300–1305. doi:10.1007/s11368-015-1151-1
58. Raskin I, Smith RD, Salt DE (1997) Phytoremediation of metals: using plants to remove pollutants from the environment. Curr Opin Biotechnol 8(2):221–226
59. Vara Prasad MN, de Oliveira Freitas HM (2003) Metal hyperaccumulation in plants: biodiversity prospecting for phytoremediation technology. Electron J Biotechnol 6(3):285–321
60. Vamerali T, Bandiera M, Mosca G (2010) Field crops for phytoremediation of metal-contaminated land. A review. Environ Chem Lett 8(1):1–17
61. Lai T, Cao A, Zucca A et al (2012) Use of natural zeolites charged with ammonium or carbon dioxide in phytoremediation of lead and zinc contaminated soils. J Chem Technol Biotechnol 87:1342–1348
62. González RC, González-Chávez MCA (2006) Metal accumulation in wild plants surrounding mining wastes. Environ Pollut 144(1):84–92

63. Pandey VC, Pandey DN, Singh N (2015) Sustainable phytoremediation based on naturally colonizing and economically valuable plants. J Cleaner Prod 86:37–39
64. Conesa HM, Faz Á, Arnaldos R (2006) Heavy metal accumulation and tolerance in plants from mine tailings of the semiarid Cartagena–La Unión mining district (SE Spain). Sci Total Environ 366(1):1–11
65. Wójcik M, Sugier P, Siebielec G (2014) Metal accumulation strategies in plants spontaneously inhabiting Zn-Pb waste deposits. Sci Total Environ 487:313–322
66. Vangronsveld J, Herzig R, Weyens N et al (2009) Phytoremediation of contaminated soils and groundwater: lessons from the field. Environ Sci Pollut Res 16(7):765–794
67. Zhuang P, Ye ZH, Lan CY et al (2005) Chemically assisted phytoextraction of heavy metal contaminated soils using three plant species. Plant Soil 276(1–2):153–162
68. Komárek M, Tlustoš P, Száková J et al (2008) The use of poplar during a two-year induced phytoextraction of metals from contaminated agricultural soils. Environ Pollut 151(1):27–38
69. Ali H, Khan E, Sajad MA (2013) Phytoremediation of heavy metals-concepts and applications. Chemosphere 91(7):869–881
70. Pinto IS, Neto IF, Soares HM (2014) Biodegradable chelating agents for industrial, domestic, and agricultural applications—a review. Environ Sci Pollut Res 21(20):11893–11906
71. Cao A, Carucci A, Lai T et al (2007) Effect of biodegradable chelating agents on heavy metals phytoextraction with *Mirabilis jalapa* and on its associated bacteria. Eur J Soil Biol 43(4):200–206
72. Piechalak A, Tomaszewska B, Barałkiewicz D (2003) Enhancing phytoremediative ability of *Pisum sativum* by EDTA application. Phytochemistry 64(7):1239–1251
73. Evangelou MW, Ebel M, Schaeffer A (2007) Chelate assisted phytoextraction of heavy metals from soil. Effect, mechanism, toxicity, and fate of chelating agents. Chemosphere 68(6):989–1003
74. Epelde L, Hernández-Allica J, Becerril J et al (2008) Effects of chelates on plants and soil microbial community: comparison of EDTA and EDDS for lead phytoextraction. Sci Total Environ 401(1):21–28
75. Leštan D, Luo CL, Li XD (2008) The use of chelating agents in the remediation of metal-contaminated soils: a review. Environ Pollut 153(1):3–13
76. Ye ZH, Shu WS, Zhang ZQ et al (2002) Evaluation of major constraints to revegetation of lead/zinc mine tailings using bioassay techniques. Chemosphere 47(10):1103–1111
77. Ernst WH (2005) Phytoextraction of mine wastes—options and impossibilities. Chem Erde-Geochem 65:29–42
78. Pardo T, Bernal MP, Clemente R (2014) Efficiency of soil organic and inorganic amendments on the remediation of a contaminated mine soil: I. Effects on trace elements and nutrients solubility and leaching risk. Chemosphere 107:121–128
79. Pardo T, Clemente R, Alvarenga P et al (2014) Efficiency of soil organic and inorganic amendments on the remediation of a contaminated mine soil: II. Biological and ecotoxicological evaluation. Chemosphere 107:101–108
80. Lee SH, Ji W, Lee WS et al (2014) Influence of amendments and aided phytostabilization on metal availability and mobility in Pb/Zn mine tailings. J Environ Manage 139:15–21
81. de la Fuente C, Pardo T, Alburquerque JA et al (2014) Assessment of native shrubs for stabilisation of a trace elements-polluted soil as the final phase of a restoration process. Agr Ecosyst Environ 196:103–111
82. Grimalt JO, Ferrer M, Macpherson E (1999) The mine tailing accident in Aznalcollar. Sci Total Environ 242(1):3–11
83. Clemente R, Almela C, Bernal MP (2006) A remediation strategy based on active phytoremediation followed by natural attenuation in a soil contaminated by pyrite waste. Environ Pollut 143(3):397–406
84. Wang B, Xie Z, Chen J et al (2008) Effects of field application of phosphate fertilizers on the availability and uptake of lead, zinc and cadmium by cabbage (*Brassica chinensis* L.) in a mining tailing contaminated soil. J Environ Sci 20(9):1109–1117

85. Bacchetta G, Cao A, Cappai G et al (2012) A field experiment on the use of *Pistacia lentiscus* L. and *Scrophularia canina* L. subsp. *bicolor* (Sibth. et Sm.) Greuter for the phytoremediation of abandoned mining areas. Plant Biosyst 146(4):1054–1063
86. Galende MA, Becerril JM, Barrutia O et al (2014) Field assessment of the effectiveness of organic amendments for aided phytostabilization of a Pb–Zn contaminated mine soil. J Geochem Explor 145:181–189
87. Sánchez-López AS, Carrillo-González R, González-Chávez MCA et al (2015) Phytobarriers: plants capture particles containing potentially toxic elements originating from mine tailings in semiarid regions. Environ Pollut 205:33–42
88. Epelde L, Becerril JM, Barrutia O et al (2010) Interactions between plant and rhizosphere microbial communities in a metalliferous soil. Environ Pollut 158(5):1576–1583
89. Jin Z, Li Z, Li Q et al (2014) Canonical correspondence analysis of soil heavy metal pollution, microflora and enzyme activities in the Pb–Zn mine tailing dam collapse area of Sidi village, SW China. Environ Earth Sci 73(1):267–274
90. Ma Y, Prasad MNV, Rajkumar M et al (2011) Plant growth promoting rhizobacteria and endophytes accelerate phytoremediation of metalliferous soils. Biotechnol Adv 29(2):248–258
91. Zhang WH, Huang Z, He LY et al (2012) Assessment of bacterial communities and characterization of lead-resistant bacteria in the rhizosphere soils of metal-tolerant *Chenopodium ambrosioides* grown on lead–zinc mine tailings. Chemosphere 87(10):1171–1178
92. Babu AG, Kim JD, Oh BT (2013) Enhancement of heavy metal phytoremediation by *Alnus* firma with endophytic *Bacillus thuringiensis* GDB-1. J Hazard Mater 250:477–483
93. Sessitsch A, Kuffner M, Kidd P et al (2013) The role of plant-associated bacteria in the mobilization and phytoextraction of trace elements in contaminated soils. Soil Biol Biochem 60:182–194
94. Tamburini E, Sergi S, Cau D et al (2015) Bioaugmentation-assisted phytostabilisation of abandoned mine sites in South West Sardinia. Bull Environ Contam Toxicol. doi:10.1007/s00128-016-1866-8.
95. Becerra-Castro C, Monterroso C, Prieto-Fernández A et al (2012) Pseudometallophytes colonising Pb/Zn mine tailings: a description of the plant–microorganism–rhizosphere soil system and isolation of metal-tolerant bacteria. J Hazard Mater 217:350–359
96. Raskin I (1996) Plant genetic engineering may help with environmental cleanup. Proc Natl Acad Sci USA 93(8):3164–3166
97. Kärenlampi S, Schat H, Vangronsveld J et al (2000) Genetic engineering in the improvement of plants for phytoremediation of metal polluted soils. Environ Pollut 107(2):225–231
98. Gisbert C, Ros R, De Haro A et al (2003) A plant genetically modified that accumulates Pb is especially promising for phytoremediation. Biochem Biophys Res Commun 303(2):440–445
99. Pilon-Smits E, Pilon M (2002) Phytoremediation of metals using transgenic plants. Crit Rev Plant Sci 21(5):439–456
100. Snow AA, Andow DA, Gepts P et al (2005) Genetically engineered organisms and the environment: current status and recommendations. Ecol Appl 15(2):377–404
101. Kotrba P, Najmanova J, Macek T et al (2009) Genetically modified plants in phytoremediation of heavy metal and metalloid soil and sediment pollution. Biotechnol Adv 27(6):799–810
102. Shim D, Kim S, Choi YI et al (2013) Transgenic poplar trees expressing yeast cadmium factor 1 exhibit the characteristics necessary for the phytoremediation of mine tailing soil. Chemosphere 90(4):1478–1486
103. Bech J, Roca N, Tume P et al (2016) Screening for new accumulator plants in potential hazards elements polluted soil surrounding Peruvian mine tailings. Catena 136:66–73
104. Sánchez-López AS, González-Chávez MCA, Carrillo-González R et al (2015) Wild flora of mine tailings: perspectives for use in phytoremediation of potentially toxic elements in a semi-arid region in Mexico. Int J Phytoremediat 17(5):476–484
105. Bech J, Duran P, Roca N et al (2012) Accumulation of Pb and Zn in *Bidens triplinervia* and *Senecio* sp. spontaneous species from mine spoils in Peru and their potential use in phytoremediation. J Geochem Explor 123:109–113

106. Abreu MM, Santos ES, Magalhães MCF et al (2012) Trace elements tolerance, accumulation and translocation in *Cistus populifolius*, *Cistus salviifolius* and their hybrid growing in polymetallic contaminated mine areas. J Geochem Explor 123:52–60
107. Cao A, Carucci A, Lai T et al (2009) Use of native species and biodegradable chelating agents in the phytoremediation of abandoned mining areas. J Chem Technol Biotechnol 84(6):884–889
108. Abreu MM, Santos ES, Ferreira M et al (2012) *Cistus salviifolius* a promising species for mine wastes remediation. J Geochem Explor 113:86–93
109. Liu X, Gao Y, Khan S et al (2008) Accumulation of Pb, Cu, and Zn in native plants growing on contaminated sites and their potential accumulation capacity in Heqing, Yunnan. J Environ Sci 20(12):1469–1474
110. Conesa HM, Faz Á, Arnaldos R (2007) Initial studies for the phytostabilization of a mine tailing from the Cartagena-La Union Mining District (SE Spain). Chemosphere 66(1):38–44
111. García-Sánchez M, García-Romera I, Száková J et al (2015) The effectiveness of various treatments in changing the nutrient status and bioavailability of risk elements in multi-element contaminated soil. Environ Sci Pollut Res 22(18):14325–14326. doi:10.1007/s11356-015-4678-1
112. Zornoza R, Faz A, Carmona DM et al (2012) Plant cover and soil biochemical properties in a mine tailing pond five years after application of marble wastes and organic amendments. Pedosphere 22(1):22–32
113. Zhuang P, Yang QW, Wang HB et al (2007) Phytoextraction of heavy metals by eight plant species in the field. Water Air Soil Pollut 184(1–4):235–242
114. Santos FS, Hernández-Allica J, Becerril JM et al (2006) Chelate-induced phytoextraction of metal polluted soils with *Brachiaria decumbens*. Chemosphere 65(1):43–50
115. Chiu KK, Ye ZH, Wong MH (2006) Growth of *Vetiveria zizanioides* and *Phragmities australis* on Pb/Zn and Cu mine tailings amended with manure compost and sewage sludge: a greenhouse study. Bioresour technol 97(1):158–170
116. Solhi M, Shareatmadari H, Hajabbasi MA (2005) Lead and zinc extraction potential of two common crop plants, *Helianthus annuus* and *Brassica napus*. Water Air Soil Pollut 167(1–4):59–71
117. Yang B, Shu WS, Ye ZH et al (2003) Growth and metal accumulation in vetiver and two *Sesbania* species on lead/zinc mine tailings. Chemosphere 52(9):1593–1600
118. Su C, Jiang L, Zhang W (2014) A review on heavy metal contamination in the soil worldwide: situation, impact and remediation techniques. Environ Skep Crit 3(2):24–38
119. Li MS (2006) Ecological restoration of mineland with particular reference to the metalliferous mine wasteland in China: a review of research and practice. Sci Total Environ 357(1):38–53
120. Padmavathiamma PK, Li LY (2007) Phytoremediation technology: hyper-accumulation metals in plants. Water Air Soil Poll 184(1–4):105–126
121. McGrath SP, Zhao FJ (2003) Phytoextraction of metals and metalloids from contaminated soils. Curr Opin Biotechnol 14(3):277–282
122. Alkorta I, Hernández-Allica J, Becerril JM et al (2004) Recent findings on the phytoremediation of soils contaminated with environmentally toxic heavy metals and metalloids such as zinc, cadmium, lead, and arsenic. Rev Environ Sci Biotechnol 3(1):71–90
123. Arthur EL, Rice PJ, Rice PJ et al (2005) Phytoremediation—an overview. Crit Rev Plant Sci 24(2):109–122
124. Wu G, Kang H, Zhang X et al (2010) A critical review on the bio-removal of hazardous heavy metals from contaminated soils: issues, progress, eco-environmental concerns and opportunities. J Hazard Mater 174(1):1–8
125. Cameselle C, Chirakkara RA, Reddy KR (2013) Electrokinetic-enhanced phytoremediation of soils: status and opportunities. Chemosphere 93(4):626–636

126. Gupta DK, Chatterjee S, Datta S et al (2014) Role of phosphate fertilizers in heavy metal uptake and detoxification of toxic metals. Chemosphere 108:134–144
127. Yang X, Feng Y, He Z et al (2005) Molecular mechanisms of heavy metal hyperaccumulation and phytoremediation. J Trace Elem Med Biol 18(4):339–353
128. Sheoran V, Sheoran AS, Poonia P (2009) Phytomining: a review. Miner Eng 22(12):1007–1019
129. Kavamura VN, Esposito E (2010) Biotechnological strategies applied to the decontamination of soils polluted with heavy metals. Biotechnol Adv 28(1):61–69
130. Bhargava A, Carmona FF, Bhargava M et al (2012) Approaches for enhanced phytoextraction of heavy metals. J Environ Manage 105:103–120
131. Visioli G, Marmiroli N (2013) The proteomics of heavy metal hyperaccumulation by plants. J Proteomics 79:133–145
132. Ullah A, Heng S, Munis MFH et al (2015) Phytoremediation of heavy metals assisted by plant growth promoting (PGP) bacteria: a review. Environ Exp Bot 117:28–40
133. USEPA (2000) Introduction to Phytoremediation. EPA/600/R-99/107. http://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=30003T7G.TXT. Accessed 30 Nov 2015
134. Eapen S, D'Souza SF (2005) Prospects of genetic engineering of plants for phytoremediation of toxic metals. Biotechnol Adv 23(2):97–114
135. Lebeau T, Braud A, Jézéquel K (2008) Performance of bioaugmentation-assisted phytoextraction applied to metal contaminated soils: a review. Environ Pollut 153(3):497–522
136. Glick BR (2003) Phytoremediation: synergistic use of plants and bacteria to clean up the environment. Biotechnol Adv 21(5):383–393
137. Glick BR (2010) Using soil bacteria to facilitate phytoremediation. Biotechnol Adv 28(3):367–374
138. Rajkumar M, Sandhya S, Prasad MNV et al (2012) Perspectives of plant-associated microbes in heavy metal phytoremediation. Biotechnol Adv 30(6):1562–1574
139. Rajkumar M, Prasad MNV, Swaminathan S et al (2013) Climate change driven plant–metal–microbe interactions. Environ Int 53:74–86
140. Glass DJ (2000) Economical potential of phytoremediation. In: Raskin I, Ensley B (eds) Phytoremediation of toxic metals. Using plants to clean up the environment. Wiley, New York, pp 15–31

Chapter 2
Phytoextraction of Heavy Metals by Fast-Growing Trees: A Review

Slobodanka Pajević, Milan Borišev, Nataša Nikolić, Danijela D. Arsenov, Saša Orlović, and Milan Župunski

2.1 Introduction

The accumulation of heavy metals in excess concentrations in the biosphere leads to environmental contamination. The impact of chemical degradation is a very serious danger and threat for the environment and this process would be irreparable and irreversible with unforeseeable negative consequences if the process of bioremediation does not occur. Bioremediation employs most often microorganisms and plants which degrade, detoxify, or sequester toxic chemicals present in natural waters and soils [1].

Excess concentrations of many metals in the environment could be easily absorbed by plants and animals and therefore affect humans by entering the food chain. The risk assessment for human health, therefore, is assuming an active effort of researchers to develop effective and inexpensive methods for the extraction of contaminants from polluted soils, sediments, and waters. The removal of pollutants from soil by traditional technologies could be more or less successful depending on specific circumstances and costs. During the last few decades, attention has been focused on innovative and cost-effective biological technologies such as phytoremediation, which is based on the use of plants to extract (absorb), destroy, or sequester hazardous contaminants from contaminated growing media [2]. Using plants to clean up the environment is an effective in situ technology which is applicable in the restoration of contaminated soils and waters [1, 3–5].

S. Pajević • M. Borišev • N. Nikolić • D.D. Arsenov • M. Župunski (✉)
Faculty of Sciences, Department for Biology and Ecology, University of Novi Sad,
Trg Dositeja Obradovića 3, Novi Sad 21000, Serbia
e-mail: slobodanka.pajevic@dbe.uns.ac.rs; milan.borisev@dbe.uns.ac.rs;
natasa.nikolic@dbe.uns.ac.rs; danijela.arsenov@dbe.uns.ac.rs; milan.zupunski@dbe.uns.ac.rs

S. Orlović
Institute of Lowland Forestry and Environmental Protection, University of Novi Sad,
Antona Čehova 13, Novi Sad 21000, Serbia
e-mail: sasao@uns.ac.rs

A.A. Ansari et al. (eds.), *Phytoremediation*, DOI 10.1007/978-3-319-40148-5_2

Phytoremediation is a complex technology which comprises several techniques with respect to the specificity in physiological (morpho-anatomical, biochemical, and molecular) responses of plants to excessive concentrations of different contaminants. The main criteria for the selection of plants with good remediation potential are their potential to bioaccumulate pollutants and their ability to transform/translocate them in above ground (harvestable) organs as well as high organic production. Many researchers have suggested that the use of trees (rather than smaller plants) in environmental bio-cleaning purposes provides good results because of their long roots which penetrate deep into the ground [6]. According to this, plants used in phytoremediation technologies in order to revitalize contaminated sites have to be hyperaccumulators with deep root systems, and they must possess good potential for using (polluted) groundwaters. All desirable properties are achieved by a specific metabolism defined by enzyme activity, particularly enzymes that are involved in a plant detoxification strategy [7, 8].

According to their ability to accumulate heavy metals, plant species are classified into two groups, i.e., "excluders," characterized by preferential accumulation of heavy metals in roots and low translocation into aerial organs, and "hyperaccumulators," species capable of accumulating and tolerating considerable levels of heavy metals in their shoots [9]. The latter group is able to accumulate above 100 mg kg^{-1} dry weight of Cd, more than 1000 mg kg^{-1} dry weight of Ni, Cu, Co, Pb, and over 10,000 mg kg^{-1} dry weight of Zn and Mn [10] in aerial organs. Besides having a high production of biomass, the accumulation of target metals in harvestable (aboveground) plant organs is pivotal for efficient phytoextraction.

There are several techniques involved in phytoremediation strategies, according to different metabolic processes in mobilization and uptake of metal ions from soil, efficiency of metal translocation to shoots via symplast and apoplast (xylem), sequestration of metals within cells and tissues, transformation of accumulated metals into metabolically less harmful forms. *Phytoextraction* is the most commonly used technique of phytoremediation which involves the utilization of plant-hyperaccumulators for the absorption of pollutants from the soil, their transport, and accumulation (concentration) in the biomass of harvestable organs [11, 12]. High biomass production has been more than welcome in plants used in phytoremediation/phytoextraction projects. Therefore, fresh and/or dry biomass determination has been often included in investigations related to their (bio) concentration potential because it is a significant indicator of specific tolerance to elevated metals in the environment. Plants suitable for successful phytoextraction of heavy metals should be tolerant to high concentrations of metals, and, at the same time, be able to accumulate high amounts of essential and unessential metals in harvestable plant organs [13, 14].

Therefore, the efficiency of phytoextraction is determined by two main factors which should have high values: biomass production and pollutant bioconcentration degree [15]. *Phytostabilization* involves the remediation of polluted soils (and waters) by cultivation of plants whose excluded metabolites in the reaction with metal (metalloids) ions reduce the solubility and mobility of contaminants within the rhizosphere. In this process, plants reduce the bioavailability of contaminants by their absorption, adsorption onto the root surface, or by formation of insoluble compounds, therefore neutralizing their harmful effect on the environment [16]. Accumulation and precipitation of contaminants in the rhizosphere prevent their migration, reduce solubility, and minimize their bioavailability. Growing plants for phytostabilization is useful in preventing soil erosion and reduces the spreading of pollutants from contaminated localities. Phytostabilization can be enhanced by using soil amendments that are effective in the immobilization of metal or metalloid ions [17].

Phytovolatilization involves a process in which plants take up contaminants from the soil and release them in a volatile form into the atmosphere through transpiration. The removal of contaminants, especially organic matters and mercury (Hg), by phytovolatilization could be achieved by the implementation of enzymes which promote plants' capacity to convert metals into volatile chemical forms [18]. *Phytodegradation* is a metabolic strategy of plants in detoxification which involves the uptake and degradation of different xenobiotics within the plant tissues or in soil (water) by enzymes. Rhizodegradation involves the use of plants associated microorganisms in the rhizosphere which carries out the degradation of contaminants in soils [19]. The main prerequisite for successful implementation of phytoremediation is to identify native plants and develop strategies for making hybrids and genetically modified plants which are good candidates for phytoremediation [20]. Significant effort has been made with the aim to identify species suitable for decontamination of heavy metal-polluted environments, primarily soil.

2.2 Phytoremediation/Phytoextraction by Trees

Although herbaceous plants can accumulate higher concentrations of metals and have higher bioconcentration factors compared to woody plants [12, 21], a very useful ecological solution for cleaning contaminated (forest) areas is growing woody plants which are characterized, primarily, by good accumulation capacities for pollutants and high biomass production [22]. Generally, trees have been considered appropriate plants for the utilization of phytoremediation because of their fast and large biomass production with significant economic value, genetic variability, established cultivation practices, high degree of public acceptability, and their contribution to site stability prevention of downward migration of heavy metals by leaching, wind dispersion, or erosion by water [23]. Although the efficiency of phytoremediation could be limited to sites with lower contaminant concentrations and might depend on soil properties and variation from year to year, growing woody plants for phytoremediation/phytoextraction purposes enables the recovery of contaminated sites to their natural conditions and also provides economic returns in obtaining woody biomass which can eventually be used in producing energy [24]. Compared to some other techniques, such as physical excavation and landfill, phytoextraction can significantly reduce the costs of decontaminating and revitalizing chemically degraded lands [25–27].

Phytoextraction by fast-growing, high biomass-producing Salicaceae species, poplar (*Populus* sp.) and willow (*Salix* sp.) have been recognized as a promising approach for the decontamination of polluted soils. However, new and additional data is necessary to improve its large-scale application in the field [23, 28–31]. Poplar and willow forest populations are good vegetation options for phytoextraction and phytostabilization techniques applicable for in situ decontamination of heavy metal-contaminated soils [32, 33]. However, there are several limitations in applying phytoextraction techniques for the remediation of polluted sites, such as a relatively long duration of the process, better absorption of pollutants in shallow layers within the root area, and also the possibility of dissemination of contaminated plant organs (leaves) and the risk of transmission of contaminants to other sites [34].

2.2.1 Heavy Metals in the Environment

The distribution and mobilization of heavy metals into the biosphere are involved in many forms of environmental contamination. Soil is contaminated when concentrations of nutrients, different chemicals, and trace metals deviate from naturally occurring sources. Widespread accumulation of cadmium, copper, and zinc in soils has been the result of human activities, such as mining, fossil fuel production, irrigation with metal-containing wastewater, agricultural utilization of municipal sewage sludge, the application of herbicides and pesticides, and the application of organic and phosphorus fertilizers. Some heavy metals, especially Pb, enter soil and surface/ground waters directly, by deposition, precipitation, or drainage of atmospheric polluted particles emitted from vehicles which use gasoline with lead as an additive [35].

Pollution is evident when contaminants are present in significantly greater than natural concentrations and when nutrient content, organic matter decomposition, soil microflora, acidic and alkaline buffer capacity, etc. are not capable of enabling normal bioproduction and diversity of plants and animals [36]. Accumulated chemicals and particularly heavy metals in excess concentrations disturb life and self-regulation processes in soil. There are many forest and agricultural localities polluted by heavy metals as the result of atmospheric deposition of industrial and traffic emissions [37]. Regarding the applicability and effectiveness in cleaning the environment, attention must be paid to the research of numerous environmental physicochemical parameters and biological parameters related to plant metabolism.

2.2.2 Physicochemical Properties of Soil and Bioavailability of Metals

Successful phytoextraction can be achieved by the selection of the most suitable genotypes for growing on soils with specific physicochemical properties. The availability of metal ions for root uptake and their toxicity depends on many abiotic factors such as their concentrations (total element content), chemical forms, types of binding, mobility, solubility, etc. According to Free Ion Activity Model (FIAM), the activity of particular metal species in the soil solution is a major determinant in bioavailability [38]. The phytoavailability of heavy metals in soil can be significantly limited by neutral to alkaline pH values, texture, cation exchange capacity, mineral composition, and the concentration of organic matter [39–44]. Cadmium (Cd) is a highly toxic trace element and a soil pollutant which could be easily taken up by roots via metal transporters and translocated to the shoots [45, 46]. Borišev et al. [47] reported that the quantity of essential nutrients present in the soil can have a significant influence on the uptake and accumulation of Cd in plants. They observed increased Cd accumulation in the leaves, stems, and roots of a *Salix viminalis* clone under conditions of Mg and Fe deficiency.

These results indicated that Cd, Mg, and Fe ions use the same transport pathways for their uptake in roots and translocation to aerial plant parts. Thus, competitive interactions can occur between these elements, in both the apoplasm and symplasm regions of the plant. If Cd^{2+} is present in soluble form in the root area, it can reach the root apoplast. In order to reach aboveground plant parts, it has to pass through the root cells' plasma membranes, which requires mobility in both apoplastic and simplastic regions of the plant [20]. Cd ion solubility, and thus mobility, is reduced at pH values above 6–7 and is also dependent on other soil properties [48–50]. An increase in the concentration of other nutrients or heavy metals can also create antagonistic competition between different ions [51]. The capacity for metal ion adsorption in the apparent free space of the plant root can be reduced by competition for membrane transporters. Elevated concentrations of both essential and non-essential metals can result in growth inhibition and toxicity symptoms which could be the result of competition with nutrient ions for the same membrane transporters; however, displacing of essential elements results in deficiency symptoms [46].

Plants phytoextraction capacity (uptake, accumulation, and translocation of metal ions from roots to shoots) could be significantly increased by the addition of chelating substances, which are present in soil. Therefore, different agronomical practices have been developed with the aim to enhance phytoextraction (pH adjustment, addition of fertilizer, or chelating agents). Induced phytoextraction is achieved through the addition of chelating agents (and strong acids) to the soil in order to increase the metals' bioavailability and their translocation from root to shoot [16]. Hammer et al. [32] observed that the use of chelating agents EDTA (Ethylene-diamine-tetra-acetic acid) in soil a few weeks before harvesting improves phytoextraction via better translocation of Cu, Pb, and Zn from roots to shoots of *Salix viminalis*. The results of Hernandez-Allica et al. [52] indicated that proper management of EDTA application can reduce metal phytotoxicity and increase the uptake of metals with low phytoavailability. Although the use of soil amendments such as EDTA should increase metal ion activity, the success of its application is not guaranteed. One of the problems is the depletion of ions around roots, so rhizosphere conditions may not reflect bulk soil conditions [53, 54].

The use of EDTA-metal complexes in induced phytoextraction must be carried out with caution because they are highly stable and can easily reach the groundwater together with other heavy metals, consequently inducing environmental damage [52, 55]. Robinson et al. [29] reported that compared to control plants treated with EDTA accumulated higher concentrations of Cd which caused necrosis and abscission in most of the leaves. During this time, leaves were examined and a significantly lower biomass and no significantly higher Cd concentrations were observed. Meers et al. [56] suggested that EDDS (Ethylene-diamine-*N*,*N*′-disuccinic acid) chelating agents might enhance the removal of Cd, Cu, and Zn by *Salix dasyclados*. Although authors concluded that these results are limited to certain soil types, the efficiency of chelating agents has been evident and these preliminary results should be implemented into strategies of field trial organizing. In addition, authors suggested that before applying the method of phytoextraction in field conditions, pot experiments should be performed by screening suitable clones and evaluating the efficiency of chelating agents.

2.2.3 Physiological and Molecular Responses to Excessive Heavy Metals-Plants' Detoxification Strategy

Heavy metals are potentially toxic because they cause many morphological and physiological disorders in excessive concentrations in plant tissue. Phytotoxicity is caused directly or indirectly by the disturbance of cell membranes and the inactivation of many enzymes by the replacement of essential ions in enzymes, such as Fe, Mn, Cu, and Zn. Binding of metals with disulfide groups (–SH) in proteins leads to an inhibition of activity or disruption of their structure [46]. Excessive Pb concentrations in plant cells impair the uptake of essential elements such as N, P, and Mg, which consequently damages metabolism—mineral nutrition, photosynthesis, and transpiration. The inhibition of nutrient cycling and the displacement of essential cations (Ca, Mg, K) by Cu and Ni cations result in a decrease of base cation concentrations in the organic layer [57]. Exposure to excessive Cd concentrations decreases plant growth rate by affecting the water regime and transpiration, photosynthesis, enzyme activity, absorption, and translocation of many macro- and micronutrients [58].

Under stress caused by heavy metals, plants have developed defense mechanisms in order to preserve metabolic rate and stable organic production. They can reduce the uptake of heavy metals into cells, sequestrate them into vacuoles by formation of complexes, bind heavy metal ions by phytochelatins, synthesize osmolytes, such as proline. [59]. Mechanisms of absorption, translocation from roots to shoots and accumulation of metals, the rate of chemical transformation into less toxic compounds in plant cells and tissues, as well as metal redistribution in plant cells are implicated in plant metal tolerance and homeostasis and define the level of tolerance and adaptability [60]. Plants cope with deleterious effects of heavy metal exposure and accumulation, such as oxidative stress and disturbance of cellular ionic homeostasis, by engaging physiological and biochemical detoxification mechanisms such as the activation of enzymes involved in chelation, subcellular compartmentalization and exclusion of pollutants [46]. The activity of enzymes involved in antioxidative protection, catalase, and superoxide dismutase levels increased in leaves and roots of plants grown in heavy metal-polluted soil compared to control plants. Also, the remarkable induction of glutathione *S*-transferase activity in poplar plants grown under Cd excess in soil was recorded [61].

The results of many studies suggest the occurrence of different detoxification strategies in poplar species (clones, cultivars) exposed to heavy metals. For instance, the exclusion strategy has been employed by young poplar plants (*Populus* × *canescens, P. tremula* × *P. alba*) exposed to different Cd concentrations, according to specific accumulation and distribution of the metal in plant organs. Treatments resulted in significant differences in Cd concentrations in the following order: roots > stem > leaves [62]. Phytochelatins (PCs) are reported to play a significant role in metal sequestration in vacuoles; however, relationships between PC content and metal tolerance in hyperaccumulators are still under consideration [63].

Considering the fact that low molecular mass cysteine-rich proteins, metallothioneins, can neutralize the toxic effect of heavy metals, and also take part in the regulation of gene expression and cell metabolism, Castiglione et al. [64] analyzed the expression of genes belonging to class II metallothioneins (including all those from plants and fungi). The expression profiles of certain genes in stems, leaves, and roots of poplar plants exposed to Zn treatments were differentially affected by Zn in an organ-specific manner, and the relationship between Zn concentration and exposure time was rarely linear. According to the lack of a strict dependency of gene expression and zinc concentration and/or exposure time, authors concluded that tolerance to metals in Villafranca poplar is not based on the "exclusion" mechanism (i.e., restricted uptake and/or limited root-to-shoot translocation). Obtained results indicated the participation of other mechanisms of plants' detoxification strategies (e.g., other low-molecular-weight chelators, vacuolar sequestration).

Results from the in vitro experiment of Macovei et al. [65] with cell suspension cultures have been used as a model system to investigate molecular mechanisms responsible for Cd, Cu, and Zn tolerance of *Populus alba* L. cv. Villafranca. The authors concluded that the *VFMT2* gene, encoding a type 2 metallothionein, was differently regulated in response to the type of metal and its concentration. Phytochelatins, cysteine-rich peptides capable for efficient chelation of heavy metals, are synthesized inductively from reduced glutathione by phytochelatin synthase activity, following plant exposure to various heavy metals, such as Cd, Hg, Cu, Zn, Pb, and Ni [60, 66]. The significance of glutathione in the tolerance of Zn stress in *Populus nigra*, *Populus canescens*, and two transformed *Populus canescens* clones over-expressing a bacterial gene encoding γ-glutamylcysteine synthetase has been investigated by Bittsánszki et al. [67]. Considering an elevated level of glutathione recorded in transgenic poplars along with higher heavy metal uptake than in non-transformed clones, authors concluded that the transgenic poplars were more suitable for the phytoremediation of soils contaminated with Zn than wild-type plants. Complexation of heavy metals with these peptides results in the sequestration of Cd in the vacuole, protecting plant cells from its toxic effects [68].

Besides experiments under controlled/semicontrolled conditions, the performance of the wild-type poplar hybrid *Populus tremula* × *Populus alba* and a transgenic mutant over-expressing bacterial genes encoding the enzymes of glutathione biosynthesis has been investigated via their cultivation under field conditions for 3 years on a relatively clean (control) site and a site contaminated with heavy metals [69]. Although considerable changes in aboveground biomass accumulation were not recorded, changes in chloroplast structure, as a consequence of over-expression of bacterial gene gsh1 in poplar plants, were evident due to an exchange of the excess glutathione produced between the cytosol and the chloroplasts. The sequestration of heavy metals by phytochelatin complexes in the vacuole may partially prevent changes of chloroplast structure in plants from the contaminated site.

Investigating physiological processes related to the uptake and accumulation of heavy metals by woody plants is of great practical importance for a better understanding of phytoextraction and enlarges the possibilities of the exploitation of trees for the remediation of polluted sites. According to this, the determination of reliable physiological/biochemical indicators for plants successful survival and remediation potential in unfavorable ecological conditions is of crucial importance for distinguishing genotypes with high adaptive potential in contaminated environments. Plant selection criteria for high phytoextraction capacities are: photosynthetic and transpiration potential, produced enzymes involved in detoxification and their activity, biomass production, which is related to growth and survival rate, root system, and other criteria which affect the adaptive ability to tolerate different contaminants [7, 70]. In this sense, the aim of breeding programs is to produce genotypes (cultivars, clones) characterized by superior growth and resistance to high levels of pollutants which have to be extracted from the soil to aboveground plant parts.

2.2.4 Phytoextraction, Photosynthesis, and Water Management

Preserved photosynthetic activity under heavy metal stress conditions benefit plant survival and growth potential in unfavorable (polluted) ecological conditions and enables high biomass production, resulting in successful and efficient phytoextraction. Defining CO_2 photosynthetic assimilation of different woody species is important in order to choose genotypes which are suitable for phytoremediation breeding programs [71, 72]. The high toxicity of divalent heavy metal ions for overall plant metabolism is the most evident in the inhibition of photosynthesis. Multiple inhibitory effects were detected such as leaf chlorosis, decreasing of leaf area, and biomass production. The most depressive effect on the photosynthetic CO_2 assimilation rate according to investigated poplar and willow genotypes was evident in plants grown on diesel fuel and metal mixtures in soil [5]. Küpper et al. [73] observed that during excessive Cd-induced stress, a few mesophyll cells became more inhibited and accumulated more Cd than the majority of cells and this heterogeneity disappeared during acclimation in plants with good bioconcentration potential. Chlorophyll fluorescence parameters related to photochemistry were more strongly affected by Cd stress than nonphotochemical parameters indicating that Cd inhibits photosynthetic light reactions more than the Calvin-Benson cycle.

The substitution of Mg ion in chlorophyll by heavy metals leads to a breakdown in photosynthesis. By growing white poplar clones on excessive Pb concentrations in cell culture media, Katanić et al. [74] found that, depending on Pb levels, plant height was decreased, as was multiplication, and chlorophyll concentration in shoots. Excessive heavy metal presence in plant tissue can affect photosynthesis by reducing photosynthetic CO_2 fixation as a result of the partial closure of stomata in leaves. Heavy metal uptake and translocation from the root zone to the stems and leaves of plants are driven by transpiration, and because of that the water status of the plant tissues and soil moisture are of crucial importance for photosynthesis and organic assimilation [11].

According to Klang-Westin and Perttu [75], water availability is a critical factor for the growth of *Salix* and during some periods of the growing season, water availability will probably be the most limiting growth factor. Transpiration rate is affected by heavy metal stress, and plants which are more adapted to polluted environments need lower water amounts for nutrient absorption. Plant populations, which suffer a strong selective pressure in contaminated conditions, perform lower WUE and higher N, suggesting that plants may be "wasting water" to increase N delivery for photosynthetic apparatuses via the transpiration stream [76]. Becerril et al. [77] found that different metals may have different effects on transpiration and growth in the same plant. Pb caused a drastic reduction of water use efficiency, while Cd inhibited transpiration and carbon assimilation to a similar degree and thus did not change WUE.

Extensive physiological characterizations of the genotypes under a variety of conditions, including heavy metal soil pollution, is likely to reveal more about the specific suitability of each hybrid for site-specific remediation.

2.3 Salicaceae Trees in Phytoremediation/Phytoextraction Technologies

Plants from two genera: *Populus* and *Salix* (family: Salicaceae, order Salicales, class: Magnoliopsida-dicots) are recognized as the most commercially exploited forest trees with great economic importance. Although taxonomic subdivisions have been under continuous revision because of intra/interspecific differentiations [78, 79], these 2 genera, together, consist of 480 species [80, 81] and form a monophyletic group [82]. *Salix* L. (willows) is the largest genus of the family Salicaceae with about 450 species [8, 80]. The genus *Populus* L. (poplars) consists of 30 species with 6 taxonomic sections [80, 83]. It comprises fast-growing deciduous trees commonly named as poplars, cottonwoods, and aspens [84]. The wide distribution of these species over the northern hemisphere and potential to adapt to contrasting environmental conditions rely upon their wide natural variability [85]. Because of their rapid growth rate and a high biomass yield, adaptability to different ecological conditions, genetic intraspecific variability, as a result of a large number of intraspecific hybridizations and differentiations, willows and poplars are valuable resources for bioremediation/phytoextraction uses [27, 86].

*2.3.1 Willows (***Salix** *spp.)*

The efficiency of phytoextraction using *Salix* spp. depends on soil type, contamination level, the accumulation of metals in harvestable parts, as well as on the yield of aboveground biomass [87–89]. Next to the many favorable characteristics for phytoextraction such as rapid growth, high biomass production, deep root systems, as well as the ability to uptake large amounts of heavy metals, willow species have great potential for vegetative propagation having the ability to form roots from stem cuttings [90]. Easy vegetative propagation by cuttings and the production of a large number of new shoots leads to high biomass production in a short period of time. Therefore, willows are commonly used as an energy source [91, 92]. Vegetative propagation, i.e., clonal plantations, reduces variability between plants in comparison with plants produced from seeds [93]. Different species of this genus show significant differences in the accumulation of heavy metals [28, 94]. Heavy metal accumulation and biomass production in *Salix* species showed a complex relationship as a result of different developments of individual taxa [23, 95]. In general, *Salix* species are not hyperaccumulators, but some clones could be grown in heavily contaminated soils, accumulating large amounts of heavy metals due to their fast growing and high biomass production [96, 97].

In recent years, a large number of studies have been conducted in order to identify the most efficient willow clones which could be used in cleaning ecosystems which have been contaminated by heavy metals. Their tremendous genetic variability could be used in creating genotypes with high biomass production in contaminated environments [28, 93]. Various literature data have been obtained from investigations conducted in different experimental conditions and the results are often difficult to compare. The in vitro studies performed with cell cultures and shoots (cuttings) in many controlled experimental conditions could reveal possible uses of selected plants in phytoextraction methods [98]. This technique is often used in short-term experiments with the aim of testing the effect of excessive heavy metals in growth media on their uptake, accumulation, and translocation in plants [99, 100]. Several studies indicated that growing plants as tissue cultures enables the selection of genetic materials for use in genetic engineering [101]. In addition, numerous works highlighted this approach as suitable in the determination of relevant *Salix* genotypes for phytoextraction [64, 102].

Willows have a long life cycle, therefore short screening technologies such as growing plants in water culture—hydropones, are suitable for the selection of genotypes for phytoremediation/phytoextraction utilization [100, 103, 104]. There are numerous advantages of applying this growing method, such as precise definitions of the substance concentrations taken up by roots, the possibility for controlling the temperature and aeration, visual monitoring of root growth and aboveground parts, etc. In addition, this technique reduces the period of growth, duration of treatments, and also variability due to environmental factors. The analyses of morphological parameters of *Salix* species exposed to excessive heavy metals in nutrient solution showed significant variability. In general, disturbed plant growth as a result of elevated concentrations of heavy metals in nutrient solution was observed, regardless of the applied metals and their concentrations [14, 97].

Generally, cadmium (Cd) in plants suppresses root growth—length and biomass production [105]. The root length reduction and decrease in shoot biomass, as a primary toxic effect of heavy metals, are confirmed on several *Salix* species and genotypes [54, 87, 94, 106]. Furthermore, Luković et al. [107] reported a higher reduction in biomass of roots, leaves, and stems in willows than in poplars, with the same applied Cd concentrations. Zacchini et al. [14] have found that total root length was significantly reduced by 50 % in analyzed *Salix* clones treated with 50 μM of Cd. Contrary to this, Cocozza et al. [108] found no reduction in root length of *Populus nigra* and *Salix alba* in the same experimental conditions regarding applied Cd treatment and duration of the experiment. Root length and active absorption area play a predominant role in the absorption of water and nutrients; therefore, metal uptake is more strongly related to root length than root weight [28]. In addition to that, root elongation is an important parameter in screening tests for highlighting different plant sensitivity to Cd [14, 109].

Analyses of growth parameters of six fast-growing trees (four willows and two poplars) showed that roots are more sensitive to the presence of Cd than shoots [110]. According to the tolerance index (Ti), a significant negative correlation was estimated between plant biomass and Cd concentration in plants [72]. In this context, Dickinson and Riddell-Black [93] reported that productivity represented the most important trait in the uptake of heavy metals. Beside the reduction of biomass, visual symptoms such as chlorosis and necrosis are common occurrences of heavy metal toxic effects in leaves. Cosio et al. [111] observed the occurrence of chlorosis in all plants with applied Cd treatments (5, 10, 50, and 200 μM Cd) while necrosis was obtained at concentrations of 10 and 50 μM Cd. At the same time, they recorded severe root and shoot biomass reductions which were more than 90 % in plants exposed to the highest applied Cd concentration. This indicated that the root growth, elongation, and absorption zones were restricted by exposure to Cd causing a decrease in root capacity for nutrient and metal uptake, resulting in the inhibition of plant growth.

Contrary to this, Borišev et al. [54] did not detect chlorosis and a decrease in shoot biomass production with Pb-EDTA-treated willow plants. Since the accumulated Pb was retained mostly in roots, the photosynthetic plant parts were protected from the toxic effects of metals. The results also indicated an increase in root length of plants grown on media with excessive Cd. Literature data showed variation in biomass production as a response to different Cu and Zn concentrations. In a comparison between treated and untreated plants, excess concentrations of heavy metals may cause a decrease, an increase, or may have no significant effect on plant growth and biomass production [105, 111]. The results of numerous studies illustrated high genetic variation associated with the tolerance of the *Salix* genus toward heavy metals [14, 112–114].

The variation in accumulation of heavy metals between *Salix* clones is confirmed in several studies, particularly pointing to the efficiency of the use of willows in Cd phytoextraction [5, 115, 116]. Yang et al. [104] compared 39 willow clones in order to establish the best clone for the application of phytoextraction. The authors observed that the shoot Cd contents varied up to 91-fold among the clones, ranging from 29.8 (a hybrid *S. babylonica* × *S. alba*) to 2726.52 kg plant^{-1} DW (*S. babylonica*), respectively. The accumulation and allocation of heavy metals in plant tissues determine various remediation goals and relevance. Therefore, three principal patterns of Cd distribution can be distinguished [104, 117, 118]. Stem Cd accumulators are plant genotypes with a capacity of cadmium accumulation and retention mainly in the stem, while the leaves and roots have smaller Cd concentrations.

According to the same researchers, leaf Cd accumulators are characterized by high Cd content in leaves and clones with this type of Cd tissue (organ) allocation are suitable for phytoextraction. Root Cd accumulators are clones with high Cd content in roots with low transport to aboveground plant parts. Species/clones with such characteristics are good candidates for phytostabilization. Cadmium allocation is clearly species specific, but its visualization is important for understanding patterns of Cd accumulation and translocation [119]. Different patterns were observed by mapping Cd distribution in roots between species and clones within the *Salicaceae* family in the accumulation of Cd [108]. Observations suggested that the allocation of Cd within the root profile could not be used as a single parameter for the translocation of metals; other parameters must also be taken into count. Furthermore, the localization of Cd is dependent on the age of the leaves. It is smaller in young in comparison to old leaves, while in old leaves it is also dependent on the treatment duration and its concentration in nutrient solution. The superior ability of willows to translocate and concentrate Cd in leaves with respect to poplar clones is confirmed in several studies [14, 107, 118].

A hydroponic survey of metal resistance and accumulation of Cd and Zn in 20 clones of willows and poplar species revealed that *S. dasyclados* (315 mg Cd kg^{-1}) and *S. smithiana* (3180 mg Zn kg^{-1} dry weight) had the largest metal content in leaves, while *S. matsudana*, *S. fragilis*, and *S. purpurea* have been shown as the best metal-tolerant species [113]. A very important characteristic for phytoextraction is metal tolerance which is manifested as a combination of high metal accumulation with a reduction of its damaging effects. Moreover, metal tolerance seems to be associated with low metal transport, thus protecting aboveground plant parts which are involved in photosynthesis [97]. Cosio et al. [111] indicated that *S. viminalis* grown in hydroponics with 20 μM Cd performed as a highly tolerant plant species, with no reduction in biomass. The concentration of heavy metals which trigger the injury of leaves is still not established for tree species, due to the lack of consistent methods which are needed to characterize metal toxicity. According to Kabata-Pendias and Pendias [120], heavy metal toxicity in crop plants occurs when the heavy metal concentration in foliage exceeds 5–10 ppm for Cd, 150–500 ppm for Zn, or 15–20 ppm for Cu. The adaptation of willows to toxic metals could be achieved by gradually enlarging concentrations of heavy metals in the nutrient solution [121].

The genes for metal resistance may become expressed if the clones were gradually adapted to high metal concentrations, therefore this could improve metal resistance properties to elevated metal concentrations in media [113]. Furthermore, the allocation of heavy metals in aerial plant parts is the most important feature in the effective utilization of plants for phytoextraction. Zn distribution in *Salix* demonstrated that high amounts of Zn are concentrated in the leaves and the stem due to high mobility and easy transport to aerial parts [106, 122]. *S. matsudana* showed high Zn content in shoots (4497.7 μg plant^{-1} dry weight) and in combination with high biomass production, showed the highest phytoextraction potential in 12 analyzed clones [112]. On the other hand, Cu noticeably accumulated in the roots with low transport to aerial parts. Short-term exposure in hydroponics may not be sufficient for metal accumulation to occur in aerial parts of willows, causing higher metal concentrations in the roots [123]. Therefore, Zacchini et al. [14] pointed out fundamental aspects such as uptake, tolerance, and translocation to the aerial parts as the selecting criteria in screening tests for phytoremediation.

The phytoextraction capacity of plants could be changed if they were exposed to metal mixtures with respect to one metal which is present in excessive concentrations [124]. Dos Santos et al. [113] observed the accumulation of metals with a cocktail treatment of Cd and Zn. They found a significant reduction of Zn and Cd accumulation in mixed treatments in most cases. The possible reason for that is ion competition between metals. Consequently, the clones with a high uptake of combination heavy metals in their mixture are still not defined due to the effect of antagonism between metals [125].

In order to investigate antagonism between Cd and Ni ions in plant absorption, two willow genotypes (*Salix alba*—clone 68/53/1 and *Salix nigra*—clone 0408) were analyzed in the presence of elevated concentrations of Cd, and Ni. They were subjected to a combined treatment of both Cd and Ni, in two concentrations (10^{-4} and 10^{-5} M L^{-1}) in water culture solutions. Some symptoms of toxicity were evident at 10^{-4} M L^{-1} of applied heavy metals.

Both heavy metals accumulated mostly in roots, but translocation to aboveground plant parts was sufficient enough to confirm a good phytoextraction potential of analyzed genotypes, especially regarding Cd. The combined treatment of both Cd and Ni significantly reduced the metals' accumulation indicating a strong antagonistic relationship between these two elements. The determined antagonism between Cd and Ni probably occurs due to competition for the same metal transporters and carriers which enable the transport of metal ions to the stems and leaves of investigated willow genotypes [126]. Pajevic et al. [127] found that the content of Pb in plant tissue was higher in the treatment where only Pb was applied, compared to the plants grown on soil contaminated with metal mixtures, which indicated competition between other ions in Pb uptake [127].

The results obtained by investigations of phytoextraction capacity of willow genotypes grown under hydroponic experimental conditions should be confirmed by experiments performed in soil/field-growing conditions. Willows as pioneer trees are a common species grown on different soils, often severely contaminated, and therefore are widely used in screening strategies for identifying trees suitable in phytoextraction techniques [128]. Greenhouse experiments are conducted with the aim to separate different clones for their possible use in field environmental conditions. Willows showed considerable differences in metal uptake, translocation, and accumulation. Their adaptability and resistance to excess metals in tissues depend on clone characteristics [129]. Although the capacity of willows for heavy metal accumulation varies due to experimental conditions, Watson et al. [130] have found a significant correlation between Cu and Ni accumulation in the *Salix* clones grown in hydroponics and the accumulation of the same metals in *Salix* clones grown in the field.

A large number of studies highlighted that among the fast-growing trees, *Salix* species are leading candidates in the removal of Cd [131, 132]. In addition, genotypic variation in metal transport and allocation among the organs plays an important role in remediation. Cd and Zn are mainly translocated to aboveground plant parts, whereas As, Cr, Ni, Cu, and Pb are dominantly retained in roots [30, 88, 94]. Several studies confirmed that Cd and Zn are mostly concentrated in young leaves [23, 131, 132]. In addition, Vysloužilová et al. [87] showed that the amount of Cd and Zn removed by willow leaves were up to 83 % for Cd and 71 % for Zn with respect to total absorbed metal concentrations. The high Zn-transfer factor to aboveground plant parts might be the mechanism which enables the elimination of excess metals from plant via defoliation [33]. In many plants grown in chemically contaminated soils, the lead (Pb) mainly accumulates in roots, rather than in leaves. Jensen et al. [133] reported that in aboveground plant parts, Pb was more concentrated in leaves than in twigs, which is in disagreement with results obtained by Evangelou et al. [132] who found higher Pb content in the stem than in the leaves. In general, willows showed a low uptake of arsenic (As).

The removal of As from soil to aboveground parts was less than 1 %, which indicated that willows are not suitable for cleaning sites contaminated with arsenic [88]. Many obtained results indicated that the highest removal of heavy metals could be found in moderately contaminated soils, while phytoextraction potential is significantly decreased in extremely contaminated soils [88]. Heavy metal uptake and translocation are reduced in extremely polluted soils as a consequence of biomass reduction—leaves undergo chlorosis, necrosis, and partial defoliation [87]. Pot experiments have limitations that are reflected in relatively short growing periods which alter heavy metal uptake by plants; therefore, long-term field experiments are needed for the evaluation of results from hydroponic and pot experiments.

Although methods of growing plants under field conditions are still not widespread, these types of experiments provide more realistic data which indicate the ability of plants to carry out phytoextraction [134]. The influence of soil properties on metal uptake and biomass production on moderately contaminated soils was specified in different studies. It has been proven that willows are not suitable for the remediation of heavily contaminated soils, but they could be very effective in the remediation of moderately contaminated soils [133]. Their ability to remove heavy metals under field conditions is particularly limited in calcareous soils because of strong metal-ions bonding at alkaline pH levels [55, 135]. The comparison of Cd and Zn uptake by *Salix viminalis* grown in acidic and alkaline (calcified) soils showed that *Salix* produced more biomass and showed higher metal uptake and translocation in shoots in the acidic soils, with lower pH values [56]. Hammer et al. [32] found that during the 5 year-long experiments conducted on polluted soil, the biomass of willows increased every year, whereas metal concentrations decreased linearly causing an increase in annual metal uptake with time. The total extraction of heavy metals was at a maximum of about 60 g Cd ha^{-1} per year and about 5 kg Zn ha^{-1}. In addition, they confirmed the existence of Cd storage in leaves as an important physiological trait for remediation and pointed out the necessity of collecting leaves as well as shoots in order to clean up contaminated soils.

Many field experiments confirmed a dissimilarity incapacity for biomass production and metal accumulation within the *Salix* genus [31, 136]. Mleczek et al. [92] revealed the significant differences among the eight *S. viminalis* clones and one *S. alba* clone. The maximum rate of difference between the highest and lowest heavy metal content in the shoots of investigated *Salix* clones were 84 % for Cd, 90 % for Cu, 167 % for Hg, 190 % for Pb, and 36 % for Zn. Phytoextraction potential for Cd, Zn, Pb, Cu, Cr, and Ni using willows (*Salix* sp.) and poplars (*Populus* sp.) has been tested by Algreen et al. [137]. The results obtained after 10 years of field experiments, indicated very low phytoextraction efficiency for investigated heavy metals: the highest was registered in willows for Cd, but still not very high, below 0.5 %. Despite this, benefits from using willows in the decontamination of soils by the process of phytoextraction techniques are significant.

Rosselli et al. [33] found that the results of phytoextraction potential of fast-growing trees conducted in field conditions are in correlation with those grown in pots, under controlled conditions. However, bio-concentration factors for Cu, Zn, and Cd were higher in pot experiments. Authors explained the obtained results by the restricted volume of soil prospected by the roots and thus better ion uptake. In contrast to this study, Jensen et al. [133] revealed a two to tenfold higher metal uptake (Cd, Zn, Pb, Cu) in field trials in comparison to metal extraction by willow plants in growth-chamber experiments. Regarding Cd and Zn absorption by plants, authors obtained high percentages of their removal, up to 0.13 % of total soil Cd and 0.29 % of total Zn. These percentages are small, but represent the most soluble fraction. The risk, therefore, of groundwater and subsurface water leaching, is reduced.

*2.3.2 Poplars (***Populus** *spp.)*

In order to investigate the efficiency of poplar species, hybrids, and cultivars in the removal of heavy metals from contaminated sites, numerous experiments have been set differing in the type of nutrient medium, heavy metal concentration and application (single metal or combined contamination using several metals), and the level of control of cultivation conditions. The results obtained from these experiments which were performed under controlled or semi-controlled conditions provide reliable guidelines for the selection of highly efficient genotypes. The accomplishment of these experiments under laboratory instead of field conditions enables precise control of growth conditions, as well as of heavy metal concentration and their bioavailability. The phytoremediation/phytoextraction potential of poplars has been tested in vitro (cell culture experiments), in hydroponic systems, using pot experiments (sand or soil), as well as in field experiments.

In vitro experiments have been successfully used to test the specificity of poplar species' (hybrids, cultivars) potential to withstand excesses of heavy metals in cultivation media. Many authors marked in vitro screening as a useful tool in studies aimed to test the ability of poplar clones to take up, tolerate, and survive heavy metal stress [64, 102, 138]. However, considering the lower availability of heavy metals in soil, the higher juvenility of the in vitro material, and the complexity of interactions between plants and their habitat, reliable evaluation of the particular genotype performance under conditions of a contaminated environment becomes necessary [139].

Recently, Di Lonardo at al. [102] investigated As, Cd, Cu, and Zn phytoremediation potential using in vitro multiplied microshoots of a commercial and two autochthonous *Populus alba* clones. Obtained data showed that plants might be able to accumulate high levels of heavy metals with no unfavorable effects on their biomass production. Higher concentrations of applied metals in roots than in shoots of poplar plants suggested a metal exclusion strategy of tested clones. Metal content was generally higher in the shoots than in the roots in all the clones. The highest content of all metals in shoots was recorded in the fast-growing commercial clone, suggesting biomass production as the key factor in evaluating the phytoextraction capacity of *P. alba* clones.

With the aim to evaluate the potential of four white poplar (*Populus alba* L.*)* clones (Villafranca, L-12, L-80, and LBM) for nickel (Ni) phytoextraction, Katanić et al. [138] carried out experiments using the shoot tips for cultivation on a solid growth medium with the addition of different nickel concentrations. Higher concentrations of nickel in the growth medium had significant inhibitory effects on plant fresh mass and especially on the photosynthetic pigments content, while the presence of Ni in the concentration of 10^{-3} M caused a serious disturbance of growth and decay in investigated clones. Authors singled out genotypes L-80 and L-12 as convenient candidates for phytoextraction and phytostabilization, as well as for the reforestation of areas moderately contaminated with Ni.

White poplar (*Populus alba* L.) genotypes have also been tested in vitro for Pb tolerance and accumulation. These tests were aimed to evaluate genotype performance in phytoremediation projects and landscaping in areas endangered by Pb contamination [139]. Some white poplar genotypes considered to be interesting for biomass production, landscaping, and horticulture were cultivated on media supplemented with different concentrations of Pb. The obtained results distinguish investigated genotypes according to the ability of Pb accumulation and two of them achieved a significantly higher lead shoot content compared to the widespread control genotype (almost 200 % and 125 % higher, respectively). The investigation of several poplar genotypes by Pajević et al. [127] showed a very high capacity for metal accumulation, especially for Pb (average content in plant tissue was 300 $\mu g \cdot g^{-1}$ dry mass) with the highest translocation factor. Compared to the control group, bioaccumulation factors for Cd were also high, but the translocation factor was lower, depending on the genotype investigated.

A commercial clone Villafranca of *Populus alba* L. has been used in the experiment conducted by Castiglione et al. [64] which was aimed to investigate tolerance to high concentrations of zinc (Zn) using an in vitro model system with shoot cultures. Applied Zn concentrations (0.5–4 mM) negatively affected chlorophyll content and the rate of adventitious root formation although to different extents. With the aim to explore the role of aluminum (Al) in the tolerance of poplars to heavy metals, Bojarczuk [140] carried out in vitro experiments using adventitious bud cultures of *P. tremula* L. × *P. alba* L. Plants originating from cultures grown in the presence of Al showed greater tolerance to the presence of Al and Cu in the medium than plants derived from cultures grown on media without Al. Although high concentrations of Cu and Pb inhibited shoot and root development, the author recommended in vitro selection of tolerant plants in order to obtain valuable material for research on mechanisms of plant sensitivity to metal toxicity.

Nikolić et al. [141] calculated the tolerance index (TI) on the basis of shoot weight in both treated and control plants (shoot fresh weight in plants from polluted soils × 100/shoot fresh weight in control plants) in order to define the tolerance of *P. deltoides* to soil contamination. The pot culture experiment was established using soil contaminated with Cd, Pb, and Ni. The metals were applied separately, or in combination in lower and higher concentrations. The lowest tolerance of *P. deltoides* was found after Ni treatment, probably due to the highest ability of translocation of this metal from roots to shoots (with respect to cadmium and lead). The obtained results of a very high bioconcentration factor for Cd and moderate tolerance, indicated that some poplar genotypes might be considered for evaluation of phytoextraction potential in outdoor/field experiments. In hydroponic experiments with plants concurrently exposed to several metals, Migeon and coworkers [142] identified clones of *P. nigra* and *P. maximowiczii* × *P. nigra* as highly tolerant to the heavy metals applied, with the TI value above 100.

The reliable evaluation of tolerance and performance in poplar plants exposed to excessive concentrations of heavy metals in the growth medium can be evaluated by other parameters, besides plant biomass, such as morpho-anatomical parameters, photosynthetic parameters, and water regime parameters [107]. The importance of different parameters in an indication and evaluation of tolerance to soil contamination was confirmed by the results of Pilipović et al. [143]. They investigated the influence of excessive Cd, Ni, and Zn concentrations in soil on pigment concentrations, photosynthesis, and activity of the nitrate reductase enzyme in *P. deltoides* clones and *Populus* × *euramericana*.

The obtained results for heavy metal tolerance of poplars indicated a significant correlation between investigated parameters: variations in aboveground and root biomass production, depending on heavy metal treatment, were in correlation with variations in obtained results for physiological parameters. Pietrini et al. [118] investigated the sensitivity of photosynthesis in *Populus* × *canadensis* and *P. nigra* L. plants to cadmium (50 μM $CdSO_4$) under hydroponic conditions. Both net photosynthesis and transpiration were considerably lower in treated plants, but high concentrations of phytochelatins were recorded in the leaves of both species. Also, the same authors showed that the confinement of Cd accumulation accompanied with the absence of phytochelatins in necrotic tissues of *Salix alba* L. leaves represents an efficient strategy for maintaining high photosynthetic activity in the willow genotypes.

Bioindication and phytoextraction potentials of poplars (*Populus nigra* × *maximovitzii* × *P. nigra var. Italica*; *Populus* × *euramericana*; *P. deltoides*) for Cd and Ni were investigated using hydroponic cultures under glasshouse conditions by Nikolić et al. [144]. Although the highest accumulation of both heavy metals was found in roots, their distribution in shoots in the same experimental treatment was metal specific: poplars preferentially accumulated Ni in leaves while Cd in stems. The obtained results also elucidated the potential of tested poplars as bioindicators of environmental pollution, even in the absence of other toxicity symptoms, such as growth reduction or chlorosis. There are implications in literature data which suggest that poplars might engage not only one, but several mechanisms related to accumulation patterns and tolerance to increased levels of heavy metals in the growth substrate. Sebastiani et al. [145] studied the effects of organic waste enriched with nonhazardous levels of Zn, Cu, Cr, and Cd on biomass partitioning and heavy metal accumulation in plant organs in two poplar clones.

Authors observed the presence of both phytoextraction and phytostabilization physiological strategies in studied plants, considering active ion transport and accumulation of Zn in leaves of both clones, Cu retention in the roots, and nonspecific intermediate transport of Cr. Similar conclusions have been drawn for a poplar clone cultivated in a sand-vermiculite substrate under glasshouse conditions treated with different Zn concentrations [146]. The higher bioaccumulation coefficient of the control group than of Zn-treated plants, the continuous Zn uptake during the growing season, and accumulation of Zn in old leaves, suggest that investigated clones employ both excluding and compartmentation mechanisms, confirming the potential of poplars to be used for plantations in Zn-contaminated soils.

Results related to heavy metal resistance and accumulation in various plant species obtained by using in vitro experimental systems always need to be confirmed by field performance trials [102, 130]. The phytoextraction potential of many plant species has been tested in hydroponic systems. These methods of cultivation are useful means for the selection of appropriate plants for the removal of heavy metals from contaminated substrates, due to short periods of plant growth and the treatment duration, as well as reduced variability of environmental factors [14]. Migeon et al. [142] employed the nutrient film technique to screen poplar clones for the tolerance and accumulation of trace elements. After 4 weeks of exposure to multipollution solutions containing 10 μM Cd, Cu, Ni, and Pb, and 200 μM Zn, the highest Cd, Zn, and Ni concentrations in leaves were measured in *Populus trichocarpa* and its hybrids. Among studied clones, the highest concentration of Cu was measured in *Populus deltoides* hybrids.

In recent decades, researchers were faced with the necessity to find alternatives for fossil fuel consumption in heat and electric power systems. With this aim, the short-rotation coppice (SRC) systems of growing plants were studied and developed with the aim to select appropriate bioenergy crops. Short rotation coppice cultures (SRC) are intensively managed, high-density plantations of multi-shoot trees, and its cultivation regime allows higher biomass yields per unit of land area [147]. The establishment of SRC on soils contaminated with heavy metals might fulfill several objectives. First of all, considerable amounts of heavy metals might be removed through repeated coppicing of the aerial biomass of plants [6]. Also, this renewable energy source is both economically and ecologically very attractive [148]. In this sense, extensive studies have been related to poplar cultivation in SRC systems [149].

These investigations were based on the ability of poplars to accumulate relatively high concentrations of certain metals, along with high biomass production exploitable for energy production. Laureysens et al. [24] studied the variation in heavy metal accumulation and biomass production among 13 poplar clones cultivated under SRC systems, which were established on a site which was moderately polluted by heavy metals. Clones with very high concentrations of all metals measured were not found, but significant clonal differences in accumulation were evident for most metals. Among the heavy metals measured, Cd, Zn, and Al were the most efficiently taken up by plants. Results presented by Laureysens and coworkers [147] related to an SRC field trial with 17 different poplar clones established on a former waste disposal site suggested that the selection and improvement of poplar clones for phytoextraction should be focused on biomass production, shoot survival, and metal concentration in the biomass.

2.4 Other Commonly Used Fast-Growing Trees in Phytoremediation/Phytoextraction Technologies

In the last 20 years, there have been numerous studies on heavy metal tolerance and uptake by plants, mostly examining willows and poplars from the Salicaceae family. Though they have great potential in the phytoextraction of pollutants and high biomass production, species like eucalypts, black locust, birch, and paulownia are better adapted to nutrient-poor, acidic soils, and harsh environments. In temperate regions, poplars, willows, and black locusts (*Robinia pseudoacacia* L.) predominate as short rotation woody crops [150]. They are characterized as excellent coppicing species with very intensive and fast growth [151]. They are able to survive droughts and severe winters, tolerate infertile and acidic soils in contrast to other tree species, and are widely used for erosion control and reforestation [150]. Concerning abovementioned characteristics and adaptations, they represent suitable candidates for phytoextraction purposes, which were confirmed in numerous researches [152–154]. Besides the aforementioned, it is noteworthy that the black locust is able to form a symbiotic relationship with nitrogen-fixing bacteria, which represents a well-adapted trait for survival in soils rich with heavy metals.

Potentive biomass species include over 700 Eucalyptus species (*Eucaliptus* spp.), commonly known as eucalypts, which are native plants to the Australian region. Eucalypts are the most promising energy crops in semitropical and tropical areas [150]. They have been successfully planted as exotics due to their fast growth and tolerance of harsh, disturbed environments involving many effective adaptations: indefinite growth, coppicing, lignotubers, drought, fire, insect resistance, and tolerance of soil acidity and low fertility [155]. The potential of eucalypts in the phytoextraction of different trace elements has been reported in several researches conducted in hydroponic trials [156–159], pot experiments [160–162], and field trials [163, 164] for the reclamation of sewage effluents, municipal wastewater, and heavy metal-contaminated substrates.

Another promising species, paulownia (*Paulownia* spp.), which was introduced into North America and Europe, has been recently used for phytoremediation due to its ability to tolerate high concentrations of metals, strong transpiration rates, rapid growth, and high biomass production [165–167]. The effects of heavy metals on plant biomass production may vary, from stimulating to deleterious effects, mostly dependent on the applied concentration and the duration of metal exposure. The effect of cadmium on plant biomass, metal accumulation, and distribution within plants tissue was the main object of research in several studies. Pietrini et al. [159] examined morpho-physiological and biochemical responses of two eucalypt genotypes (hybrid clones of *Eucalyptus camaldulensis* × *Eucalyptus globulus* spp.), by exposing 1-year-old rooted cuttings to Cd in solution during 1 month. The presence of Cd in nutrient solution reduced the root growth of selected clones up to 30 and 50 %. The reduction of biomass was less pronounced in the aboveground parts, and this was followed with a similar decrease in root/shoot biomass between eucalypt clones. Noteworthy is the fact that the accumulation of Cd in roots of tested clones has been very high (up to 14778 mg kg^{-1}), while the accumulation in stems reached the values of approximately 600 mg kg^{-1} of dry weight. Accumulation in the leaves of selected clones was 20-fold smaller compared to the values in the stem. These findings unlock notable perspectives for the future utilization of these species in phytoremediation purposes. Fine et al. [158] reported that the biomass of *Eucalyptus camaldulensis* saplings was not adversely affected during a 1-month exposure to Cd and organic ligands (EDTA and EDDS) in hydroponic solution. Older and bigger plants were used for the second experiment where plants were treated with significantly higher concentrations of Cd (89 mM Cd).

The obtained results indicated significantly reduced growth of saplings after Cd treatments (with the absence of ligands), up to 40 % in comparison to the control group. Treatments with ligands added showed concentration-dependant influence on sapling growth, which had stimulating or deleterious effects. Also, EDTA has been proven as a more effective ligand than EDDS, possibly due to the higher stability constant of its complex with Cd. In the author's opinion, *E. camaldulensis* may successfully be used for the phytoextraction of cadmium from soils that are contaminated at environmental concentrations, with chelating agents' assistance. Gomes et al. [168] had reported deleterious effects on *E. camaldulensis* plants grown in the presence of 90 μM of Cd in solution, with obvious symptoms of Cd toxicity, like wilted growth and leaf chlorosis, as well as blackened and thickened roots. Increased root/shoot translocation rate led to high Cd concentrations in shoots. Such adverse effects of heavy metals may be reduced by associating plants with arbuscular mycorrhizal fungi. The roots of *E. globulus* were inoculated with arbuscular mycorrhizal and saprobe fungi in order to prevent harmful effects of excessive concentration of Cd in nutrient solution [169]. According to the authors, inoculation with fungi led to the redistribution of absorbed Cd mostly in the stem of plants where the harmful effects during the development of the plant were minor, explaining why arbuscular mycorrhizal fungi conferred resistance of eucalyptus to the toxic impact of Cd in spite of high accumulation of this metal in the plant. Contrary to the various responses in the presence of Cd, certain eucalypt species may have remarkable tolerance to aluminum (Al).

Silva et al. [156] have subjected six eucalypt species and clones to different concentrations of Al^{3+} in solution. Root growth and elongation was either stimulated or unaffected by low to intermediate Al concentrations and Al mostly accumulated in the roots, and differed among investigated species. The restriction of Al translocation from roots to shoots may provide a mechanism of protecting the shoots from the harmful effects of the metal. Such results indicated the potential of selected eucalypts in the phytostabilization of aluminum-contaminated soils. It is suggested that aluminum phytoextraction potential of these species should be investigated further, with prolonged exposure to the contaminant. Furthermore, such trials would be very useful if we keep in mind that the exploitation of short-rotation eucalypts may lead to a notable reduction in exchangeable Ca and Mg and enhance exchangeable Al in the soil over time [170]. Assareh et al. [157] have evaluated the bioconcentration (enrichment) coefficient, metal uptake, and translocation among three eucalypt species (*E. camaldulensis*, *E. microtheca,* and *E. occidentalis*).

Enrichment coefficients varied between species and depended on metals applied: *E. occidentalis* had a greater ability to accumulate Zn, *E. camaldulensis* had a higher ability for Cu uptake, in the stem. Another promising species with a high affinity to accumulate Zn in aboveground biomass are paulownia species. Their very high annual increment in biomass, up to 150 t ha^{-1} [171], with extensive deep-digging root systems and high transpiration rates makes these plants an effective natural pump capable of absorbing large quantities of water and pollutants from the soil [172]. The significant removal of trace elements from substrates and a rather low rate of metal absorption [165] are useful parameters in specifying the future strategy for the utilization of these species in phytoremediation/phytoextraction. At rather high Zn concentrations (above 2000 μM in the nutrient solution), Azzarello et al. [172] have determined typical plant stress symptoms in exposed *Paulownia tomentosa* plants, such as growth inhibition and the loss of leaf area. However, when plants were exposed to lower Zn concentrations in the nutrient solution, the accumulation of zinc in aboveground biomass had exceeded 1500 μg g^{-1} with insignificant effects on plant growth parameters, confirming the fact that paulownia species may be suitable candidates for phytoextraction processes.

Authors of this study have proposed a tolerance mechanism to high Zn levels in *P. tomentosa* plants, throughout the use of advanced mechanisms that are able to sequester the heavy metals in specific cell structures, such as the petiole cell walls and the vacuoles in the root hairs, or they may be capable of extruding a percentage of the Zn in exudates located on the surface of the petiole. To our knowledge, there is a lack of data when it comes to hydroponic screening of black locusts (*Robinia pseudoacacia* L.) and their phytoextraction ability. Župunski et al. [154] have determined the importance of genotype (half-sib) selection in accumulation and tolerance of Cd, Ni, and Pb. Specific half-sib families of black locusts have showed promising perspectives for phytoextraction of Cd and Ni.

The effects of different heavy metals on growth, uptake, and tolerance of fast-growing species like eucalypts, birch, and paulownia have been reported in several soil phytoextraction studies with the assistance of synthetic chelators [165, 167], and without chelator assistance [132, 160–162, 173, 174]. Doumett et al. [165] have grown paulownia plantlets in soil rich with Cd, Cu, Pb, and Zn in order to determine whether complexing agents (EDTA, tartarate, and glutamate) have influenced metal uptake by plants and mobilization in soil. The visual assessment did not show any signs of phytotoxicity, and neither did it affect the whole plant dry weight. Tartarate and glutamate have shown good potential in complexing heavy metals, very similar to those of EDTA, followed by the absence of a significant increment of metal leaching probability.

A comparison between plant metal accumulation and the bioavailable metal fraction in soil has shown that uptake and translocation were not mainly dependent on the bioavailable fraction and that the predominant mechanism for metal accumulation was not the concentration gradient between the soil and plant tissues. The phytoremediation potential of paulownia species is mostly assigned to its high biomass production rather than its uptake and accumulation potential. The same authors [167] investigated the influence of repeated applications of tartarate and glutamate (50 mmol kg^{-1} of soil) on Cd, Cu, Pb, and Zn distribution between a contaminated soil sample and *Paulownia tomentosa*. Cu, Pb, and Zn uptake was stimulated by repeated glutamate applications. Cd and Pb were mostly isolated in the roots of paulownia plants and were excluded from aboveground biomass. Such an exclusion mechanism can explain the observed tolerance of *Paulownia tomentosa* to Cd and Pb, but with evident limits in phytoextraction potential for these elements. Furthermore, no significant effect was found in soil on heavy metal bioavailability and concentration, thus decreasing the potential risk of leaching into groundwater [165].

The potential of 13 eucalypt clones for both heavy metal uptake and biomass production from natural and polluted soils was assessed by Mughini et al. [161]. All tested clones have survived and grown well under pollution, which indicated tolerance to the contaminant levels set during the early stress-sensitive growth period. The authors have reported that As, Cu, Pb, and Zn accumulated more in the leaves than in the stems and branches, suggesting that the removal of the entire aboveground biomass, including the leaves, would enhance the phytoextraction potential for these contaminants using *Eucalyptus* spp., especially having in mind that eucalypts are evergreens. Similarly, Coupe et al. [160] investigated the potential of *E. camadulensis* for the phytoextraction of Pb and Zn due to the high bioconcentration factors for these elements and great ability for root/shoot allocation. The uptake and distribution to the aboveground parts in eucalypts was higher compared to the other two species, one of which was *Brassica juncea*, a hyperaccumulator plant with a great ability for Pb and Zn uptake [175, 176].

Further, Mughini et al. [161] pointed out important positive pair-wise correlations between heavy metal average contents (Cd and Pb, Cd and Cu, Cd and Zn, Pb and Cu, Pb and Zn, and Cu and Zn) detected in leaves, stems and branches suggesting that clone selections based on the uptake of these contaminants may notably improve the potential for the remediation of abovementioned pairs. Characteristics such as high yield, tolerance to the presence of metals in soil, reallocating and partitioning of metals in aboveground tissues classify eucalypts and paulownia species side by side with poplars and willows, which were confirmed in numerous researches as great phytoextractors for various trace elements. Wang et al. [162] conducted a pot experiment with six different species, including *Betula alnoides, Alnus nepalensis,* and *Eucalyptus globulus*, for the phytoremediation of mining-spoiled substrate. The obtained differences in growth and lead/zinc uptake differed within plant species, their parts and the kind of metals. For all three species, it was confirmed that they were fast growing, highlighting *A. nepalensis* as a species with the most intensive growth and biomass yield, together with the highest obtained accumulations of Pb and Zn in aboveground tissues. As a conclusion, the authors stated that *A. nepalensis* and *B. alnoides* could serve as appropriate species for the reforestation of mine tailing areas with high levels of Pb and Zn.

The possibility for heavy metal uptake from contaminated soils was assessed in three researches with *B. pendula* plants. Bojarczuk et al. [177] have tested the effect of aluminum-polluted soil and fertilization on the growth and physiology of silver birch (*Betula pendula* Roth.) seedlings. Treatments with fertilizers have a beneficial effect on plant growth, not only in unpolluted soil, but also in soils with high Al content. The authors have suggested that lower Ca/Al ratio in polluted soils may contribute to reduced membrane permeability and to the leakage of some cations from the cytosol. The obtained accumulations for Al and other trace elements lead to possible implications in the phytostabilization of heavy metals, due to the reduced allocation of elements to aboveground parts. In other studies regarding the same species, Bojarczuk et al. [174] have indicated that young seedlings of *B. pendula* are suitable plant material for the recultivation of soils highly contaminated with Cu and Pb, especially in pretreatments with ectomycorrhiza and fertilizers. Cu and Pb were mostly isolated in the roots of the silver birch, with lower translocation to them stem and leaves. Furthermore, the efficiency of the ectomycorrhiza/plant community in the amelioration of the Cu and Pb toxic effects on birch seedlings may be enhanced by providing propagules of heavy metals tolerant ectomycorrhiza fungi, able to restrict allocation of the metals from the roots to the aboveground parts. Similar to these observations, Evangelou et al. [132] found that birch is most suitable for biomass production combined with phytostabilization of soils with high Cd and Zn, but low Pb concentrations.

The only limitation is the fact that birch cannot be coppiced, so it is not useful for short rotation, in contrast to the eucalypts and black locusts which are suitable for methods of biomass production. It is very often that the results of field experiments are confirmed by pot experiments under controlled conditions. Obtained accumulations of trace elements might be the same in both pot and field experiments during short periods of growth, while metal concentrations in the vegetative tissues of plants grown in pots are always higher than those from the field after a longer growth period. This can be explained by the limited volume of soil available to the roots and thus their better efficiency. *Betula* and *Salix* species grown in (pot) field conditions may be useful for phytoextraction as they are able to mobilize reasonably high concentrations of metals to their aboveground parts. Theriault et al. [178] suggested that *Betula papyrifera* (white birch) might play a key role in the phytoextraction of Zn and Ni in a mining-reclaimed region.

This was a dominant species in Northern Ontario after land reclamation, with high bioaccumulation and translocation factor for Zn and Ni, particularly due to the lower bioavailability of these metals in the soil. *Betula pendula* plants were also used for the monitoring of uranium mining dumps [179] on the basis of a significant correlation between heavy metal content of foliage and soil. Promising results were found for the accumulation of Mn in the foliage (284–1724 mg kg^{-1}) with a high accumulation ratio, which indicated the following heavy metal absorption capability of *B. pendula* in order: Cd > Mn > Zn > Pb > Cu > Ni > Fe. The evaluation of heavy metal uptake and application of *Paulownia fortunei* for revegetation on heavy metal-polluted sites was carried out in two Chinese provinces with intense mining activities, which generate a significant quantity of dust, slag, and tailings every year, and contaminate the surrounding areas over several square kilometers [153, 166]. The effect of the paulownia plant rhizosphere on soil properties was studied by Wang et al. [166], who found that immobility and bioavailability of heavy metals were enhanced, with an evident change in the microenvironment of the rhizosphere. Paulownia plants exhibited the threshold limit for lead uptake, by accumulating up to 2700 mg kg^{-1} in leaves during revegetation.

These results are very similar to those obtained from the research of Zhao et al. [153], who have also reported that Pb concentrations in leaves of *P. fortunei* exceeded the hyperaccumulation threshold limit (>1000 mg kg^{-1}). Along with a high uptake of Pb, a significant uptake of Zn has been found in leaves (over 1300 mg kg^{-1}), pointing to prospective features of this species for phytoextraction purposes. Higher metal concentration in the leaves than in the branches and trunks imply that metal pollution might be removed by combining pulping management and harvesting. Short rotation coppicing has been proven as a useful and desirable operation for the dendroremediation of contaminated soils [180].

Such a method of harvesting plant biomass is cheap and efficient in the removal of pollutants, but highly dependent on a time scale for the removal of significant amounts of metals. Since the time needed for remediation of contaminated localities may be very long, even up to 150 years for the remediation to environmentally acceptable levels of pollutants, different methods and approaches must be reconsidered in order to decrease the duration of soil rehabilitation. According to the results obtained by Luo et al. [164], who subjected *Eucaliptus globulus* plants to different coppice systems in order to verify its viability as an alternative to the Cd phytoremediation practice in field scale, the replanting treatment should be recommended as a suitable method which may shorten the phytoremediation time and its efficiency. It is even more expensive to establish and implement replanting systems into practice, but in the long term it would be much faster to remediate soil for agriculture production.

2.5 Treatment After Phytoextraction

Trees grown on degraded and underused lands can bring both aesthetic and economic improvements. Phytoextraction could improve soil quality of mostly moderately contaminated lands within realistic time scales. However, a biomass with a higher content of heavy metals, grown during phytoextraction, is potentially hazardous, and regarded as a "waste mass." The disposal of such material must be carried out with special care in order to minimize heavy metal reentry into the environment. At the same time, crop biomass can bring some economic return [30]. The first step after successful phytoextraction is to reduce crop volume and weight of harvested biomass for easier and safer disposal, and if possible, to utilize obtained energy during this process. One of the most important benefits of dendroremediation is that tree biomass, after phytoextraction, can be used for different purposes. Although a number of crop disposal methods have been proposed, such as composting, compaction, incineration, ashing, pyrolysis, direct disposal, and liquid extraction, it seems that the energetic utilization of biomass by incineration or gasification is the most feasible [181].

Šyc et al. [182] investigated composting pretreatment of wood biomass, followed by incineration and fractional ash disposal. The success of this process depends on which heavy metal is present in the ash, but nevertheless the process can significantly reduce biomass weight and heavy metal leach ability compared to usual landfill biomass disposal. After combustion, most heavy metals remain in the bottom ash, but on the negative side, some heavy metals such as Cd can partly be volatilized by stack emissions, thus reentering into the atmosphere [183, 184]. It seems that high volatility of heavy metals in the flue gas presents a serious restrain of the biomass combustion process after phytoextraction. For example, Delpanque et al. [185] concluded that the combustion of *Salix* wood after the phytoextraction of Cd and Zn should occur only if combustion boilers are equipped with suitable filters, in order to reduce air pollution to an acceptable level. On the other hand, higher volatilization and gasification of heavy metals during thermal biomass treatments provide a possibility that bottom ash could be recycled and used as a fertilizer. All these technologies must be assessed based on each individual site characteristics. Thus, sustainability and economic valorization of phytoextraction are greatly correlated with the further development of appropriate methods for the efficient treatment of biomass yield.

2.6 Feasibility and Duration

The general phytoextraction efficiency on each specific site depends on biomass production and the bioconcentration factor (ratio of metal concentration in the aboveground plant parts to metal concentration in the soil). It is widely accepted that, for a reasonable duration of successful phytoextraction, the bioconcentration factor should be higher than 1, or in many cases much higher. It is very difficult to give precise predictions of phytoextraction durations on each specific site. For example, Dickinson and Pulford [30] state that the number of years needed to reduce soil Cd concentrations by 5 mg kg^{-1} using *Salix viminalis* could range from 3 to 33, 7 to 67, and 14 to 133 years in different soil depths (10, 20, and 40 cm, respectively), depending on the rate of metal uptake into aboveground tissues. Delpanque et al. [185] determined that *Salix* could reduce Cd in a contaminated dredged sediment landfill site from 2.39 to 2 mg kg^{-1} in 19 years. Many different variables have significant impact on the duration of phytoextraction, assessed on the basis of different hydroponic or soil tests and experiments. Pollution is often characterized by a heterogeneous spatial dispersion of heavy metals in soil [186]. Heavy metal uptake in trees is mostly confined to the roots [28, 130, 187], so the duration of the process could be significantly reduced if root bole could be periodically removed along with aboveground parts. Microbiological activity in contaminated soil is often seriously depressed, so bacterial activity is not as supportive to plant growth as a result of Glick [188].

Macronutrient content in contaminated soil has a significant impact on heavy metal availability and uptake [47], and it can change during the years, thus affecting heavy metal uptake. Specific physicochemical properties of soil highly affect the success of phytoextraction depending on soil pH values, texture, cation exchange capacity, mineral composition, and the composition of organic matter [38–43]. It is possible that the uptake ratio can change during different years in relation to many abiotic and biotic factors. For example, climate change will be one of the main driving forces in future yield performance and stability [189]. Low water availability is one of the main environmental factors affecting plant growth and yield in different regions of the world [190]. We can hypothesize that, in long-scale phytoextraction trials, climate shift will significantly change their duration. All mentioned parameters have a significant impact on both metal uptake and plant biomass productivity, thus affecting the duration of successful site remediation. In general, on heavily contaminated sites, with high concentrations of heavy metals, phytoextraction with fast-growing trees would last unrealistically long. Nevertheless, it is widely accepted by scientific community that fast-growing trees had shown high potential for the phytoextraction of low- to moderately contaminated sites, especially if economic valorization of produced biomass is possible.

References

1. Crawford RL (2006) Bioremediation. In: Dworkin M, Falkow S, Rosenberg E, Schleifer KH, Stackebrandt E (eds) Prokaryotes, vol 1. Springer, Singapore, pp 850–863
2. Pilon-Smiths E (2005) Phytoremediation. Annu Rev Plant Biol 56:15–39
3. Susarla S, Medina VF, McCutcheon SC (2002) Phytoremediation: an ecological solution to organic chemical contamination. Ecol Eng 18:647–658
4. Ghosh M, Singh SP (2005) A review on phytoremediation of heavy metals and utilization of its byproducts. Appl Ecol Environ Res 3:1–18

5. Pajević S, Borišev M, Nikolić N, Krstić B, Pilipović A, Orlović S (2009) Phytoremediation capacity of poplar (*Populus* spp.) and willow (*Salix* spp.) clones in relation to photosynthesis. Arch Biol Sci 61:239–247
6. González-Oreja JA, Rozas MA, Alkorta I, Garbisu C (2008) Dendroremediation of heavy metal polluted soils. Rev Environ Health 23:1–12
7. Prasad MNV (2003) Phytoremediation of metal-polluted ecosystems: hype for commercialization. Russ J Plant Physiol 50:686–700
8. Marmiroli M, Pietrini F, Maestri E, Zacchini M, Marmiroli N, Massacci A (2011) Growth, physiological and molecular traits in *Salicaceae* trees investigated for phytoremediation of heavy metals and organics. Tree Physiol 31(12):1319–1334
9. Baker AJM (1981) Accumulators and excluders—strategies in the response of plants to heavy metals. J Plant Nutr 3:643–654
10. Baker AJM, Brooks RR (1989) Terrestrial higher plants which hyperaccumulate metallic elements—a review of their distribution, ecology and phytochemistry. Biorecovery 1: 81–126
11. Marchiol L, Sacco P, Assolari S, Zerbi G (2004) Reclamation of polluted soil: phytoremediation potential of crop-related Brassica species. Water Air Soil Pollut 158:345–356
12. Dickinson NM, Baker AJM, Doronila A, Laidlaw S, Reeves RD (2009) Phytoremediation of inorganics: realism and synergies. Int J Phytoremediation 11:97–114
13. Garbisu C, Alkorta I (2001) Phytoextraction: a cost-effective plant-based technology for the removal of metals from the environment. Bioresour Technol 77:229–236
14. Zacchini M, Pietrini F, Scarascia Mugnozza G, Iori V, Pietrosanti L, Massacci A (2009) Metal tolerance, accumulation and translocation in poplar and willow clones treated with cadmium in hydroponics. Water Air Soil Pollut [Internet] 197(1–4):23–34. http://link.springer.com/10.1007/s11270-008-9788-7
15. McGrath SP, Zhao FJ (2003) Phytoextraction of metals and metalloids from contaminated soils. Curr Opin Biotechnol 14:277–282
16. Singh NP, Santal AR (2015) Phytoremediation of heavy metals: the use of green approaches to clean the environment. In: Ansari A, Singh S, Gill R, Lanza GR, Newman L (eds) Phytoremediation: management of environmental contaminants, vol 2. Springer International Publishing, Cham, pp 115–129
17. Bolan NS, Park JH, Robinson B, Naidu R, Huh KY (2011) Phytostabilization: a green approach to contaminant. Adv Agron 112:145–204
18. Kotrba P (2013) Transgenic approaches to enhance phytoremediation of heavy metal-polluted soils. In: Gupta DK (ed) Plant-based remediation processes. Springer, Berlin, pp 239–272
19. Singh OV, Jain RK (2003) Phytoremediation of toxic aromatic pollutants from soil. Appl Microbiol Biotechnol 63:128–135
20. Maestri M, Marmiroli M, Visioli G, Marmiroli N (2010) Metal tolerance and hyperaccumulation: costs and trade-offs between traits and environment. Environ Exp Bot 68:1–13
21. Lai HY, Chen ZS (2009) In-situ selection of suitable plants for phytoremediation of multi-metals contaminated sites in central Taiwan. Int J Phytoremediation 11:235–250
22. Komives T, Gullner G (2006) Dendroremediation: the use of trees in cleaning up polluted soils. In: Mackova M et al (eds) Phytoremediation rhizoremediation, theoretical background. Focus on biotechnology. Springer, Dordrecht, pp 23–31
23. Pulford I, Watson S (2003) Phytoremediation of heavy metal-contaminated land by trees—a review. Environ Int [Internet] 29(4):529–540. http://linkinghub.elsevier.com/retrieve/pii/S0160412002001526
24. Laureysens I, Blust R, Temmerman L, Lemmens C, Ceulemans R (2004) Clonal variation in heavy metal accumulation and biomass production in a poplar coppice culture: I. Seasonal variation in leaf, wood and bark concentrations. Environ Pollut 131:485–494
25. Turnau K, Henriques FS, Anielska T, Renker C, Buscot F (2007) Metal uptake and detoxification mechanisms in Erica andevalensis growing in a pyrite mine tailing. Environ Exp Bot 61:117–123

26. Chen J, Chen Y, Shi ZQ, Su Y, Han FX (2015) Phytoremediation to remove metals/metalloids from soils. In: Ansari AA, Gill SS, Gill R, Lanza GR, Newman L (eds) Phytoremediation: management of environmental contaminants, vol 2. Springer, Cham, pp 297–304
27. Pahdey VC, Bajpai O, Singh N (2016) Energy crops in sustainable phytoremediation. Renew Sust Energy Rev 54:58–73
28. Greger M, Landberg T (1999) Use of willow in phytoextraction. Int J Phytoremediation 1:115–123
29. Robinson BH, Mills TM, Petit D, Fung L, Green S, Clothier B (2000) Natural and induced cadmium accumulation in poplar and willow: implications for phytoremediation. Plant Soil 227:301–306
30. Dickinson NM, Pulford ID (2005) Cadmium phytoextraction using short-rotation coppice *Salix*: the evidence trail. Environ Int 31(4):609–613
31. Unterbrunner R, Puschenreiter M, Sommer P, Wieshammer G, Tlustos P, Zupan M et al (2007) Heavy metal accumulation in trees growing on contaminated sites in Central Europe. Environ Pollut [Internet] 148(1):107–114. http://www.ncbi.nlm.nih.gov/pubmed/17224228
32. Hammer D, Kayser A, Keller C (2003) Phytoextraction of Cd and Zn with *Salix viminalis* in field trials. Soil Use Manag 19(3):187–192
33. Rosselli W, Keller C, Boschi K (2003) Phytoextraction capacity of trees growing on a metal contaminated soil. Plant Soil 256:265–272
34. Migeon A, Richaud P, Guinet F, Chalot M, Blaudez D (2009) Metal accumulation by woody species on contaminated sites in the North of France. Water Air Pollut 204(1–4):89–101
35. Guerra F, Gainza F, Perez M, Zamudio F (2011) Phytoremediation of heavy metals using poplars (*Populus* spp.): a glimpse of the plant responses to copper, cadmium and zinc stress. In: Golubev IA (ed) Handbook of phytoremediation. Nova Science, New York, pp 387–413
36. Logan TJ (1990) Chemical degradation of soil. In: Lal R, Stewart BA (eds) Advances in soil science. Soil degradation. Springer, New York, pp 187–223
37. Borišev M, Pajević S, Nikolić N, Krstić B, Župunski M, Kebert M et al (2012) Response of *Salix alba* to heavy metals and diesel fuel contamination. Afr J Biotechnol 11(78): 14313–14319
38. Hough RL, Tye AM, Crout NMJ, McGrath SP, Zhang H, Young SD (2005) Evaluating a 'free ion activity model' applied to metal uptake by Lolium perenne L. grown in contaminated soils. Plant Soil 270:1–12
39. Ross SM (1994) Retention, transformation and mobility of toxic metals in soils. In: Ross SM (ed) Toxic metals in soil-plant systems. Wiley, New York, pp 63–152
40. Ross SM (1994) Toxic metals in soil–plant systems. Wiley, Chichester
41. Kayser A, Wenger K, Keller A, Attinger W, Felix HR, Gupta SK, Schulin R (2000) Enhancement of phytoextraction of Zn, Cd, and Cu from calcareous soil: the use of NTA and sulfur amendments. Environ Sci Technol 34:1778–1783
42. Angelova V, Ivanov K, Ivanova R (2003) Effect of chemical forms of lead, cadmium and zinc in polluted soils on their uptake by tobacco. J Environ Prot Ecol 4(2):316–327
43. Farrag K, Senesi N, Nigro F, Petrozza A, Palma A, Shaarawi S, Brunetti G (2012) Growth responses of crop and weed species to heavy metals in pot and field experiments. Environ Sci Pollut Res 19:3636–3644
44. Farrag K, Senesi N, Rovira PS, Brunetti G (2012) Effects of selected soil properties on phytoremediation applicability for heavy-metal-contaminated soils in the Apulia region, Southern Italy. Environ Monit Assess 184:6593–6606
45. Perfus-Barbeoch L, Leonhardt N, Vavasseur A, Forestier C (2002) Heavy metal toxicity: cadmium permeates through calcium channels and disturbs the plant water status. Plant J 32:539–548
46. Hall JL (2002) Cellular mechanisms for heavy metal detoxification and tolerance. J Exp Bot 53(366):1–11
47. Borišev M, Pajević S, Nikolić N, Orlović S, Župunski M, Pilipović A, Kebert M (2016) Magnesium and iron deficiencies alter Cd accumulation in *Salix viminalis* L. Int J Phytoremediation 18(2):164–170

48. Cieslinski G, Neilsen GH, Hogue EJ (1996) Effect of soil cadmium application and pH on growth and cadmium accumulation in roots, leaves and fruit of strawberry plants (*Fragaria* x *ananassa* Duch.). Plant Soil 180:267–276
49. Vidal M, Santos MJ, Abrao T, Rodriguez J, Rigol A (2009) Modeling competitive metal sorption in a mineral soil. Geoderma 149:189–198
50. Trakal L, Komárek M, Száková J, Tlustoš P, Tejnecký V, Drábek O (2012) Sorption behavior of Cd, Cu, Pb, and Zn and their interactions in phytoremediated soil. Int J Phytoremediation 14:806–819
51. Xin J, Huang B, Yang Z, Yuan J, Dai H, Qiu Q (2010) Responses of different water spinach cultivars and their hybrid to Cd, Pb and Cd-Pb exposures. J Hazard Mater 175:468–476
52. Hernández-Allica J, Garbisu C, Barrutia O (2007) Becerril JM EDTA-induced heavy metal accumulation and phytotoxicity in cardoon plants. Environ Exp Bot 60:26–32
53. Zhang GP, Fukami M, Sekimoto H (2000) Genotypic differences in effects of cadmium on growth and nutrient compositions in wheat. J Plant Nutr 23:1337–1350
54. Borišev M, Pajević S, Nikolić N, Pilipović A, Krstić B, Orlović S (2009) Phytoextraction of Cd, Ni, and Pb using four willow clones (*Salix* spp.). Pol J Environ Stud 18(4):553–561
55. Meers E, Lesage E, Lamsal S, Hopgood M, Vervaeke P, Tack FMG et al (2005) Enhanced phytoextraction: I. Effect of EDTA and citric acid on heavy metal mobility in a calcareous soil. Int J Phytoremediation 7(2):129–142
56. Meers E, Vandecasteele B, Ruttens A, Vangronsveld J, Tack FMG (2007) Potential of five willow species (*Salix* spp.) for phytoextraction of heavy metals. Environ Exp Bot 60:57–68
57. Derome J, Lindroos AJ (1998) Effect of heavy-metal contamination on macronutrient availability and acidification parameters in forest soil in the vicinity of the Harjavalta Cu-Ni smelter, SW Finland. Environ Pollut 99:225–232
58. Benavides MP, Gallego SM, Tomaro ML (2005) Cadmium toxicity in plants. Braz J Plant Phys 17:21–34
59. Emamverdian A, Ding Y, Mokhberdoran F, Xie Y (2015) Heavy metal stress and some mechanisms of plant defense response. Sci World J. Article ID 756120. http://dx.doi.org/10.1155/2015/756120
60. Yadav SK (2010) Heavy metals toxicity in plants: an overview on the role of glutathione and phytochelatins in heavy metal stress tolerance of plants. S Afr J Bot 76:167–179
61. Nikolić N, Kojić D, Pilipović A, Pajević S, Krstić B, Borišev M et al (2008) Responses of hybrid poplar to cadmium stress: photosynthetic characteristics, cadmium and proline accumulation, and antioxidant enzyme activity. Acta Biol Cracov Bot 50(2):95–103
62. Dai H-P, Shan C, Wei Y, Liang J-G, Yang T-X, Sa W-Q et al (2012) Subcellular localization of cadmium in hyperaccumulator *Populus* × *canescens*. Afr J Biotechnol 11:3779–3787
63. Clemens S (2006) Toxic metal accumulation, responses to exposure and mechanisms of tolerance in plants. Biochimie 88:1707–1719
64. Castiglione S, Franchin C, Fossati T, Lingua G, Torrigiani P, Biondi S (2007) High zinc concentrations reduce rooting capacity and alter metallothionein gene expression in white poplar (*Populus alba* L. cv. Villafranca). Chemosphere [Internet] 67(6):1117–1126. http://www.ncbi.nlm.nih.gov/pubmed/17223164
65. Macovei A, Ventura L, Donà M, Faè M (2010) Effect of heavy metal treatments on metallothionein expression profiles in white poplar (*Populus alba* L.) cell suspension cultures. Ann Univ Oradea Fascicula Biol Tom. XVII(2):274–279.
66. Salt DE, Rauser WE (1995) MgATP-dependent transport of phytochelatins across the tonoplast of oat roots. Plant Physiol 107:1293–1301
67. Bittsánszky A, Kömives T, Gullner G, Gyula G, Kiss J, Heszky L et al (2005) Ability of transgenic poplars with elevated glutathione content to tolerate zinc(2+) stress. Environ Int 31:251–254
68. Sanitá di Toppi L, Gabbrielli R (1999) Response to cadmium in higher plants. Environ Exp Bot 41:105–130
69. Ivanova L, Ronzhina D, Ivanov L, Stroukova L, Peuke A, Rennenberg H (2009) Chloroplast parameters differ in wild type and transgenic poplars overexpressing gsh1 in the cytosol. Plant Biol 11:625–630

70. Salamanca EJP, Medera-Parra CA, Avila-Williams CA, Rengifo-Gallego AL, Rios DA (2015) Phytoremediation using terrestrial plants. In: Ansari AA, Gill SS, Gill R, Lanza GR, Newman L (eds) Phytoremediation: management of environmental contaminants, vol 2. Springer, Cham, pp 305–319
71. Pilipović A, Orlović S, Nikolić N, Borišev M, Krstić B, Rončević S (2012) Growth and plant physiological parameters as markers for selection of poplar clones for crude oil phytoremediation. Šumarski list 136(5–6):273–281
72. Nikolić N, Borišev M, Pajević S, Arsenov D, Župunski M, Orlović S, Pilipović A (2015) Photosynthetic response and tolerance of three willow species to cadmium exposure in hydroponic culture. Arch Biol Sci 67(4):1411–1420. doi:10.2298/ABS150421120N
73. Küpper H, Parameswaran A, Leitenmajer B, Trtílek M, Šetlík I (2007) Cadmium-induced inhibition of photosynthesis and long-term acclimation to cadmium stress in the hyperaccumulator *Thlaspi caerulescens*. New Phytol 175(4):655–674
74. Katanić M, Pilipović A, Orlović S, Krstić B, Kovačević B, Pekeč S (2008) The influence of lead on the in vitro growth and concentration of photosynthetic pigments in shoots of the white poplar (*Populus alba*) clones. Lesnícky čas For J 54(Suppl 1):29–36
75. Klang-Westin E, Perttu K (2002) Effect of nutrient supply and soil cadmium concentration on cadmium removal by willow. Biomass Bioenergy 23:415–426
76. Donovan LA, Dudley SA, Rosenthal DM, Ludwig F (2007) Phenotypic selection on leaf water use efficiency and related ecophysiological traits for natural populations of desert sunflowers. Oecologia 152(1):13–25
77. Becerril JM, Gonzales-Marua C, Munoz-Rueda A, de Felipe MR (1989) Changes induced by cadmium and lead in gas exchange and water relations of clover and lucerne. Plant Physiol Biochem 27:913–918
78. Dickmann DI, Kuzovkina J (2014) Poplars and willows of the world, with emphasis on silviculturally important species. In: Isebrands JG, Richardson J (eds) Poplars and willows: trees for society and the environment. FAO and CABI, Croydon, pp 8–92
79. Kuzovkina YA, Vietto L (2014) An update on the cultivar registration of *Populus* and *Salix* (Salicaceae). Skvortsovia 1(2):133–148
80. Argus GW (1997) Infrageneric classification of New World *Salix* L. (Salicaceae). Syst Bot Monogr 52:1–121
81. Argus GW (2010) Salix. In: Flora of North America Editorial Committee (ed) Flora of North America, vol 7, Magnoliophyta: Salicaceae to Brassicaceae. Oxford University Press, New York, pp 23–51
82. Lauron-Moreau A, Pitre FE, Argus GW, Labrecque M, Brouillet L (2015) Phylogenetic relationships of American willows (Salix L., Salicaceae). Plos One 10(4):e0121965
83. Eckenwalder JE (2010) Populus. In: Flora of North America Editorial Committee (ed) Flora of North America North of Mexico. Oxford University Press, New York, pp 23–162
84. Rae AM, Street NR, Rodríguez-Acosta M (2007) *Populus* trees. In: Kole C (ed) Genome mapping and molecular breeding in plants, vol 7. Forest Trees. Springer, Berlin, pp 1–28
85. Gaudet M, Pietrini F, Beritognolo I, Iori V, Yacchini M, Massaci A et al (2011) Intraspecific variation of physiological and molecular response to cadmium stress in *Populus nigra* L. Tree Phys 31:1309–1318
86. Hamzeh M, Dayanandan S (2004) Phylogeny of *Populus* (Salicaceae) based on nucleotide sequences of chloroplast TRNT-TRNF region and nuclear rDNA. Am J Bot 91:1398–1408
87. Vyslouzilova M, Tlustos P, Szakova J, Vysloužilová M, Tlustoš P, Száková J (2003) Cadmium and zinc phytoextraction potential of seven clones of *Salix* spp. planted on heavy metal contaminated soils. Plant Soil Environ 49(12):542–547
88. Tlustoš P, Száková J, Vysloužilová M, Pavlíková D, Weger J, Javorská H (2007) Variation in the uptake of arsenic, cadmium, lead, and zinc by different species of willows *Salix* spp. grown in contaminated soils. Cent Eur J Biol 2(2):254–275
89. Cloutier-Hurteau B, Turmel MC, Mercier C, Courchesne F (2014) The sequestration of trace elements by willow (*Salix purpurea*)—which soil properties favor uptake and accumulation? Environ Sci Pollut Res 21:4759–4771

90. Sommerville AHC (1992) Willows in the environment. Proc Roy Soc Edinb B Biol Sci 98:215–244
91. Bedell JP, Capilla X, Girya C, Schwartzb C, Morelb JL, Perrodina Y (2009) Distribution, movement and availability of Cd and Zn in a dredged sediment cultivated with *Salix alba*. Environ Exp Bot 67:403–414
92. Mleczek M, Rutkowski P, Rissmann I, Kaczmarek Z, Golinski P, Szentner K et al (2010) Biomass productivity and phytoremediation potential of *Salix alba* and *Salix viminalis*. Biomass Bioenergy 34(9):1410–1418
93. Dickinson NM, Riddell-Black (2014) Phytoremediation and carbon sequestration of degraded lands. In: Isebrands JG, Richardson J (eds) Poplars and willows: trees for society and the environment. CABI, Boston
94. Lunáčková L, Masarovičová E, Král'Ová K, Streško V (2003) Response of fast growing woody plants from family Salicaceae to cadmium treatment. Bull Environ ContamToxicol 70(3):576–585
95. Riddell-Black D (1994) Heavy metal uptake by fast growing willow species. In: Aronsson O, Perttu K (eds) Willow vegetation filters for municipal wastewaters and sludges. A biological purification system. Swedish University Agricultural Sciences, Uppsala, pp 145–151
96. Landberg T, Greger M (1994) Can heavy metal tolerant clones of Salix be used as vegetation filters on heavy metal contaminated land? In: Aronsson P, Perttu K (eds) Willow vegetation filters for municipal wastewaters and sludges. A biological purification system. Swedish University Agricultural Sciences, Uppsala, pp 133–144
97. Landberg T, Greger M (1996) Differences in uptake and tolerance to heavy metals in *Salix* from unpolluted and polluted areas. Appl Geochem [Internet] 11(1–2):175–180. http://www.sciencedirect.com/science/article/pii/0883292795000828.
98. Lyyra S, Lima A, Merkle SA (2006) In vitro regeneration of *Salix nigra* from adventitious shoots. Tree Physiol 26(7):969–975
99. Lux A, Masarovicovà E, Liskova D, Sottnikova-Stefanovicova A, Lunackova L, Marcekova M (2002) Physiological and structural characteristics and in vitro cultivation of some willows and poplars. In: Proceedings of the Cost Action 837, Bordeaux, 25-27 April 2002
100. Capuana M (2011) Heavy metals and woody plants—biotechnologies for phytoremediation. iForest Biogeosci For [Internet] 4(1):7–15. http://www.sisef.it/iforest/?doi=10.3832/ifor0555-004
101. Confalonieri M, Balestrazzi A, Bisoffi S, Carbonera D (2003) In vitro culture and genetic engineering of *Populus* spp.: synergy for forest tree improvement. Plant Cell Tissue Organ Cult 72:109–138
102. Di Lonardo S, Capuana M, Arnetoli M, Gabbrielli R, Gonnelli C (2011) Exploring the metal phytoremediation potential of three *Populus alba* L. clones using an in vitro screening. Environ Sci Pollut Res 18:82–90
103. Watson C, Pulford ID, Riddell-Black D (1999) Heavy metal toxicity responses of two willow (*Salix*) varieties grown hydroponically: development of a tolerance screening test. Environ Geochem Health 21:359–364
104. YangW, Zhao F, Zhang X, Ding Z, Wang Y, Zhu Z et al Variations of cadmium tolerance and accumulation among 39 *Salix* clones: implications for phytoextraction. Environ Earth Sci [Internet] 2015;73(7):3263–3274. http://link.springer.com/10.1007/s12665-014-3636-4
105. Lux A, Šottníková A, Opatrná J, Greger M (2004) Differences in structure of adventitious roots in *Salix* clones with contrasting characteristics of cadmium accumulation and sensitivity. Physiol Plantarum 120:537–545
106. Vassilev A, Perez-Sanz A, Semanem B, Carleer R, Vangronsveld J (2005) Cadmium accumulation and tolerance of two *Salix* genotypes hydroponically grown in presence of cadmium. J Plant Nutr 28:2159–2177
107. Luković J, Merkulov L, Pajević S, Zorić L, Nikolić N, Borišev M et al (2012) Quantitative assessment of effects of cadmium on the histological structure of poplar and willow leaves. Water Air Soil Pollut [Internet] 23(6):2979–2993. http://link.springer.com/10.1007/s11270-012-1081-0

108. Cocozza C, Maiuro L, Tognetti R (2011) Mapping cadmium distribution in roots of *Salicaceae* through scanning electron microscopy with x-ray microanalysis. iForest 4:113–120
109. Vaculík M, Konlechner C, Langer I, Adlassnig W, Puschenreiter M, Lux A, Hauser MT (2012) Root anatomy and element distribution vary between two Salix caprea isolates with different Cd accumulation capacities. Environ Pollut 163:117–126
110. Sottnikova A, Lunackova L, Masarovicova E, Lux A, Stresko V (2003) Changes in the rooting and growth of willow and poplars induced by cadmium. Biol Plant 46:129–131
111. Cosio C, Vollenweider P, Keller C (2006) Localization and effects of cadmium in leaves of a cadmium-tolerant willow (*Salix viminalis* L.). Environ Exp Bot [Internet] 58(1–3):64–74. http://linkinghub.elsevier.com/retrieve/pii/S009884720500105X
112. Yang W, Wang Y, Zhao F, Ding Z, Zhang X, Zhu Z et al (2014) Variation in copper and zinc tolerance and accumulation in 12 willow clones: implications for phytoextraction. J Zhejiang Univ Sci B [Internet] 15(9):788–800. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4162880&tool=pmcentrez&rendertype=abstract
113. Dos SantosUtmazian MN, Wieshammer G, Vega R, Wenzel WW (2007) Hydroponic screening for metal resistance and accumulation of cadmium and zinc in twenty clones of willows and poplars. Environ Pollut [Internet] 148(1):155–165. http://linkinghub.elsevier.com/retrieve/pii/S0269749106005872
114. Zhivotovsky OP, Kuzovkina JA, Schulthess CP, Morris T, Pettinelli D, Ge M (2010) Hydroponic screening of willows (*Salix* L.) for lead tolerance and accumulation. Int J Phytoremediation 13:75–94
115. Klang-Westin E, Eriksson J (2003) Potential of *Salix* as phytoextractor for Cd on moderately contaminated soils. Plant Soil 249(1):127–137
116. Wieshammer G, Unterbrunner R, Garcia TB, Zivkovic MF, Puschenreiter M, Wenzel WW (2007) Phytoextraction of Cd and Zn from agricultural soils by *Salix* ssp. and intercropping of *Salix caprea* and *Arabidopsis halleri*. Plant Soil [Internet] 298(1–2):255–264. http://link.springer.com/10.1007/s11104-007-9363-9
117. Dos SantosUtmazian MND, Wenzel WW (2007) Cadmium and zinc accumulation in willow and poplar species grown on polluted soils. J Plant Nutr Soil Sci Fur Pflanzenernahrung Und Bodenkd [Internet] 170(2):265–272. <Go to ISI>://000246417400011
118. Pietrini F, Zacchini M, Iori V, Pietrosanti L, Ferretti M, Massacci A (2010) Spatial distribution of cadmium in leaves and its impact on photosynthesis: examples of different strategies in willow and poplar clones. Plant Biol (Stuttg) [Internet] 12(2):355–363. http://www.ncbi.nlm.nih.gov/pubmed/20398241
119. Vollenweider P, Cosio C, Günthardt-Goerg MS, Keller C (2006) Localization and effects of cadmium in leaves of a cadmium-tolerant willow (*Salix viminalis* L.). Environ Exp Bot [Internet] 58(1–3):25–40. http://linkinghub.elsevier.com/retrieve/pii/S0098847205001048
120. Kabata-Pendias A, Pendias H (2001) Trace elements in soils, 3rd edn. CRC Press, Boca Raton, p 413
121. Almeida A-AF, Valle RR, Mielke MS, Gomes FP (2007) Tolerance and prospection of phytoremediator woody species of Cd, Pb, Cu and Cr. Braz J Plant Physiol 19:83–98
122. Vandecasteele B, Meers E, Vervaeke P, De Vos B, Quataert P, Tack FMG (2005) Growth and trace metal accumulation of two *Salix* clones on sediment-derived soils with increasing contamination levels. Chemosphere 58(8):995–1002
123. Liu Y, Chen CG, Zhang J, Shi X, Wang R (2011) Uptake of cadmium from hydroponic solutions by willows (*Salix* spp.) seedlings. Afr J Biotechnol 10(72):16209–16218
124. Nissim WG, Hasbroucq S, Kadri H, Pitre FE, Labrecque M (2015) Potential of selected Canadian plant species for phytoextraction of trace elements from selenium-rich soil contaminated by industrial activity. Int J Phytoremediation [Internet] 17(8):745–52. http://www.tandfonline.com/doi/full/10.1080/15226514.2014.987370
125. Pulford ID, Riddell-Black D, Stewart C (2002) Heavy metal uptake by willow clones from sewage sludge-treated soil: the potential for phytoremediation. Int J Phytoremediation [Internet] 4(1):59. http://search.ebscohost.com/login.aspx?direct=true&db=a9h&AN=7262728&site=ehost-live

126. Borišev M, Krstić B, Orlović S, Pilipović A, Nikolić N, Pajević S, Župunski M (2013) Antagonism between Cd and Ni in willows phytoextraction test. In: 13th international scientific conference “Sakharov Readings 2013: Environmental Problems of XXIst Century”, May 16-17th, Minsk, The Book of Abstracts
127. Pajević S, Borišev M, Nikolić N, Luković J, Župunski M, Arsenov D, Orlović S (2014) Phytoextraction of elevated heavy metals in soil by using fast growing trees (*Salix* sp. and *Populus* sp.). In: The international bioscience conference IBSC 29-30 September 2014, Phuket, Proceedings, pp 13–18
128. Hrynkiewicz K, Dabrowska G, Baum C, Niedojadlo K, Leinweber P (2012) Interactive and single effects of ectomycorrhiza formation and Bacillus cereus on metallothionein MT1 expression and phytoextraction of Cd and Zn by willows. Water Air Soil Pollut 223(3): 957–968
129. Kuzovkina J (2014) Stress tolerance in North American willow species. In: Isebrands JG, Richardson J (eds) Poplars and willows: trees for society and the environment. CABI, Boston
130. Watson C, Pulford ID, Riddell-Black D (2003) Screening of willow species for resistance to heavy metals: comparison of performance in a hydroponics system and field trials. Int J Phytoremediation 5:351–365
131. Hermle S, Gunthardt-Goerg MS, Schulin R (2006) Effects of metal-contaminated soil on the performance of young trees growing in model ecosystems under field conditions. Environ Pollut 144:703–714
132. Evangelou M, Robinson B, Günthardt-Goerg M, Schulin R (2013) Metal uptake and allocation in trees grown on contaminated land: implications for biomass production. Int J Phytoremediation 15:77–90
133. Jensen JK, Holm PE, Nejrup J, Larsen MB, Borggaard OK (2009) The potential of willow for remediation of heavy metal polluted calcareous urban soils. Environ Pollut [Internet] 157(3):931–937. http://dx.doi.org/10.1016/j.envpol.2008.10.024
134. Li J-T, Baker AJM, Ye Z-H, Wang H-B, Shu W-S (2012) Phytoextraction of Cd-contaminated soils: current status and future challenges. Crit Rev Environ Sci Technol [Internet] 42(20):2113–2152. http://www.scopus.com/inward/record.url?eid=2-s2.0-84867723299&partnerID=tZOtx3y1
135. Kirkham MB (2006) Cadmium in plants on polluted soils: effects of soil factors, hyperaccumulation, and amendments. Geoderma 137:19–32
136. Vervaeke P, Luyssaert S, Mertens J, Meers E, Tack FM, Lust N (2003) Phytoremediation prospects of willow stands on contaminated sediment: a field trial. Environ Pollut [Internet] 126(2):275–282. http://linkinghub.elsevier.com/retrieve/pii/S0269749103001891
137. Algreen M, Trapp S, Rein A (2014) Phytoscreening and phytoextraction of heavy metals at Danish polluted sites using willow and poplar trees. Environ Sci Pollut Res [Internet] 21(15):8992–9001. http://link.springer.com/10.1007/s11356-013-2085-z
138. Katanić M, Kovačević B, Đorđević B, Kebert M, Pilipović A, Klašnja B et al (2015) Nickel phytoremediation potential of white poplar clones grown *in vitro*. Rom Biotechnol Lett 20:10085–10096
139. Kovačević B, Miladinović D, Orlović S, Katanić M, Kebert M, Kovinčić J (2013) Lead tolerance and accumulation in white poplar cultivated i*n vitro*. SEEFFOR 4:3–12
140. Bojarczuk K (2004) Effect of toxic metals on the development of poplar (*Populus tremula* L. × *P. alba* L.) cultured *in vitro*. Pol J Environ Stud 13:115–120
141. Nikolić N, Borišev M, Krstić B, Pajević S, Pilipović A, Orlović S. (2011) Tolerance of *Populus deltoides* to soil contamination. In: Proceedings of the “STREPOW” international workshop; 2011 Feb 23-24; Andrevlje, Novi Sad, Serbia, pp 231–236.
142. Migeon A, Richaud P, Guinet F, Blaudez D, Chalot M (2012) Hydroponic screening of poplar for trace element tolerance and accumulation. Int J Phytoremediation 14:350–361
143. Pilipović A, Orlović S, Nikolić N, Galić Z, Klašnja B, Borišev M, Krstić B (2011) The effect of heavy metal contamination on physiological parameters of five poplar (*Populus* sp.) clones. In: Proceedings of the “STREPOW” international workshop; 2011 Feb 23-24; Andrevlje, Novi Sad, Serbia; pp 237–243

144. Nikolić N, Krstić B, Orlović S, Pajević S, Borišev M, Pilipović A (2009) Bioindication and phytoremediation of cadmium and nickel contaminated environment using poplars. Экологический Вестник 3/4(9/10): 6–12
145. Sebastiani L, Scebba F, Tognetti R (2004) Heavy metal accumulation and growth responses in poplar clones Eridano (*Populus deltoides* × *maximowiczii*) and I-214 (*P.* × *euramericana*) exposed to industrial waste. Environ Exp Bot 52:79–88
146. Di Baccio D, Tognetti R, Sebastiani L, Vitagliano C (2003) Responses of *Populus deltoides* × *P. nigra* (*P.* × *euramericana*) clone I-214 to high zinc concentrations. New Phytol 159:443–452
147. Laureysens I, De Temmerman L, Hastir T, Van Gysel M, Ceulemans R (2005) Clonal variation in heavy metal accumulation and biomass production in a poplar coppice culture. II. Vertical distribution and phytoextraction potential. Environ Pollut 133:541–551
148. Simpson JA, Picchi G, Gordon AM, Thevathasan NV, Stanturf J, Nicholas I (2009) Short rotation crops for bioenergy systems. Task 30. Environmental benefits associated with short-rotation woody crops Technical Review No. 3 2009 IEA Bioenergy. http://www.ieabioenergytask43.org/Task_30_Web_Site/PDFs/Tech%20review%20No.%2003%20 for%20web.pdf. Accessed 9 Oct 2015
149. Dillen SY, Djomo SN, Al Afas N, Vanbeveren S, Ceulemans R (2013) Biomass yield and energy balance of a short rotation poplar coppice with multiple clones on degraded land during 16 years. Biomass Bioenergy 56:157–165
150. Rockwood DL, Naidu CV, Carter DR, Rahmani M, Spriggs TA, Lin C, Alker GR, Isebrands JG, Segrest SA (2004) Short-rotation woody crops and phytoremediation: opportunities for agroforestry? Agrofor Syst 61–62:51–63
151. Rédei K, Osváth-Bujtás Z, Veperdi I (2008) Black locust (Robinia pseudoacacia L.) improvement in Hungary: a review. Acta Silv Lign Hung 4:127–132
152. Mertens J, Van Nevel L, De Schrijver A, Piesschaert F, Oosterbean A, Tack FMG, Verheyen K (2007) Tree species effect on the redistribution of soil metals. Environ Pollut 149:173–181
153. Zhao X, Liu J, Xia X, Chu J, Wei Y, Shi S et al (2013) The evaluation of heavy metal accumulation and application of a comprehensive bio-concentration index for woody species on contaminated sites in Hunan, China. Environ Sci Pollut Res 21:5076–5085
154. Župunski M, Borišev M, Orlović S, Arsenov D, Nikolić N, Pilipović A et al (2015) Hydroponic screening of black locust families for heavy metals tolerance and accumulation. Int J Phytoremediation 18(6):583–591
155. Rockwood DL, Rudie AW, Ralph SA, Zhu JY, Winandy JE (2008) Energy product options for eucalyptus species grown as short rotation woody crops. Int J Mol Sci 9:1361–1378
156. Silva I, Novais R, Jham G, Barros N, Gebrim F, Nunes F et al (2004) Responses of eucalypt species to aluminum: the possible involvement of low molecular weight organic acids in the Al tolerance mechanism. Tree Physiol 24:1267–1277
157. Assareh MH, Shariat A, Ghamari-Zare A (2008) Seedling response of three *Eucalyptus* species to copper and zinc toxic concentrations. Caspian J Environ Sci 6:97–103
158. Fine P, Rathod PH, Beriozkin A, Mingelgrin U (2013) Uptake of cadmium by hydroponically grown, mature *Eucalyptus camaldulensis* saplings and the effect of organic ligands. Int J Phytoremediation 15:585–600
159. Pietrini F, Iori V, Bianconi D, Mughini G, Massacci A, Zacchini M (2015) Assessment of physiological and biochemical responses, metal tolerance and accumulation in two eucalypt hybrid clones for phytoremediation of cadmium-contaminated waters. J Environ Manage 162:221–231
160. Coupe SJ, Sallami K, Ganjian E (2013) Phytoremediation of heavy metal contaminated soil using different plant species. Afr J Biotechnol 12(43):6185–6192
161. Mughini G, Alianiello F, Benedetti A, Gras L, Gras M, Salvati L (2013) Clonal variation in growth, arsenic and heavy metal uptakes of hybrid Eucalyptus clones in a Mediterranean environment. Agrofor Syst 87:755–766
162. Wang Y, Bai S, Wu J, Chen J, Yang Y, Zhu X (2015) Plumbum/zinc accumulation in seedlings of six afforestation species cultivated in mine spoil substrate. J Trop For Sci 27(2):166–175

163. Asensio V, Vega F, Singh B, Covelo E (2013) Effects of tree vegetation and waste amendments on the fractionation of Cr, Cu, Ni, Pb and Zn in polluted mine soils. Sci Total Environ 443:446–453
164. Luo J, Qi S, Peng L, Xie X (2015) Phytoremediation potential of cadmium-contaminated soil by *Eucalyptus globulus* under different coppice systems. Bull Environ Contam Toxicol 94:321–325
165. Doumett S, Lamperi L, Checchini L, Azzarello E, Mugnai S, Mancuso S, Petruzzelli G, Bubba M (2008) Heavy metal distribution between contaminated soil and Paulownia tomentosa, in a pilot-scale assisted phytoremediation study: influence of different complexing agents. Chemosphere 72:1481–1490
166. Wang J, Zhang CB, Jin ZX (2009) The distribution and phytoavailability of heavy metal fractions in rhizosphere soils of *Paulownia fortunei* (seem) Hems near a Pb/Zn smelter in Guangdong, PR China. Geoderma 148(3–4):299–306
167. Doumett S, Fibbi D, Azzarello E, Mancuso S, Mugnai S, Petruzzelli G, Del Bubba M (2010) Influence of the application renewal of glutamate and tartrate on Cd, Cu, Pb and Zn distribution between contaminated soil and Paulownia tomentosa in a pilot-scale assisted phytoremediation Study. Int J Phytoremediation 13:1–17
168. Gomes M, Marques TCLLS, Carneiro MML, Soares Â (2012) Anatomical characteristics and nutrient uptake and distribution associated with the Cd-phytoremediation capacity of Eucalyptus camaldulenses Dehnh. J Soil Sci Plant Nutr 12:481–496
169. Arriagada C, Herrera M, Ocampo J (2005) Contribution of arbuscular mycorrhizal and saprobe fungi to the tolerance of Eucalyptus globulus to Pb. Water Air Soil Pollut 166:31–47
170. Leite FP (2001) Nutritional relationships and chemical characteristics alterations in soils of the Rio Doce Valley caused by eucalyptus cultivation. PhD thesis, Federal University of Viçosa, Viçosa, 72 p. In Portuguese with English abstract.
171. Caparros S, Diaz MJ, Ariza J, Lopez F, Jimenez L (2008) New perspectives for *Paulownia fortunei* L. valorisation of the autohydrolysis and pulping processes. Bioresour Technol 99:741–749
172. Azzarello E, Pandolfi C, Giordano C, Rossi M, Mugnai S, Mancuso S (2012) Ultramorphological and physiological modifications induced by high zinc levels in *Paulownia tomentosa*. Environ Exp Bot 81:11–17
173. Kopponen P, Utriainen M, Lukkari K, Suntioinen S (2001) Clonal differences in copper and zinc tolerance of birch in metal-supplemented soils. Environ Pollut 112:89–97
174. Bojarczuk K, Kieliszewska-Rokicka B (2010) Effect of ectomycorrhiza on Cu and Pb accumulation in leaves and roots of silver birch (*Betula pendula* Roth.) seedlings grown in metal-contaminated soil. Water Air Soil Pollut 207:227–240
175. Salt DE, Smith RD, Raskin I (1998) Phytoremediation. Annu Rev Plant Physiol Plant Mol Biol 49:643–668
176. Gisbert C, Clemente R, Navarro-Aviñó J, Carlos Baixauli C, Ginér A, Serrano R, Walker DJ, Pilar Bernal MP (2006) Tolerance and accumulation of heavy metals by *Brassicaceae* species grown in contaminated soils from Mediterranean regions of Spain. Environ Exp Bot 56:19–26
177. Bojarczuk K, Karolewski P, Oleksyn J, Kieliszewska-Rokicka B, Żytkowiak R, Tjoelker MG (2002) Effect of polluted soil and fertilisation on growth and physiology of silver birch (*Betula pendula* Roth.) seedlings. Pol J Environ Stud 11(5):483–492
178. Theriault G, Nkongolo K, Michael P (2014) Genetic and metal analyses of fragmented populations of Betula papyrifera (Marsh) in a mining reclaimed region: identification of population–diagnostic molecular marker. Ecol Evol 4(17):3435–3443
179. Wisłocka M, Krawczyk J, Klink A, Morrison L (2006) Bioaccumulation of heavy metals by selected plant species from uranium mining dumps in the Sudety Mts., Poland. Pol J Environ Stud 15(5):811–818
180. Rockwood DL, Cardellino R, Alker G, Lin C, Brown N, Spriggs T, Tsangaris S, Isebrands J, Hall R, Lange R, Nwokike B (2004) Fast-growing trees for heavy metal and chlorinated solvent phytoremediation. In: Magar VS, Kelley ME (eds) In situ and on-site bioremediation-2003. Proceedings of the seventh international in situ and on-site bioremediation symposium, Orlando, FL, June 2–5, 2003, Paper F-12, Battelle Press, Columbus

181. Sas-Nowosielska A, Kucharski R, Małkowski E, Pogrzeba M, Kuperberg JM, Kryński K (2004) Phytoextraction crop disposal—an unsolved problem. Environ Pollut 128:373–390
182. Šyc M, Pohořely M, Kameníková P, Habart J, Svoboda K, Punčochár M (2012) Willow trees from heavy metals phytoextraction as energy crops. Biomass Bioenergy 37:106–113
183. Keller C, Ludwig C, Davoli F, Wochele J (2005) Thermal treatment of metal-enriched biomass produced from heavy metal phytoextraction. Environ Sci Technol 39(9):3359–3367
184. Stals M, Carleer R, Reggers G, Schreurs S, Yperman J (2010) Flash pyrolysis of heavy metal contaminated hardwoods from phytoremediation: characterisation of biomass, pyrolysis oil and char/ash fraction. J Anal Appl Pyrolysis 89(1):22–29
185. Delpanque M, Collet S, Gratta FD, Schnuriger B, Gaucher B, Bert V (2013) Combustion of *Salix* used for phytoextraction: the fate of metals and viability of the process. Biomass Bioenerg 49:160–170
186. CL:AIRE (2004) Technical Bulletin TB7. Improving the reliability of contaminated land assessment using statistical methods: part 1. Basic principles and concepts. Rep. no., London
187. Punshon T, Dickinson NM (1999) Heavy metal resistance and accumulation characteristics in willows. Int J Phytoremediation 1:361–385
188. Glick BR (2003) Phytoremediation: synergistic use of plants and bacteria to clean up the environment. Biotechnol Adv 21:383–393
189. Kertész A, Madarász B (2014) Conservation agriculture in Europe. Int Soil Water Conserv Res 1(2):91–96
190. Chaves MM, Maroco JP, Pereira JS (2003) Understanding plant responses to drought—from genes to the whole plant. Funct Plant Biol 30:239–264

Chapter 3
Biological Approaches for Remediation of Metal-Contaminated Sites

A.P. Pinto, A. de Varennes, M.E. Lopes, and D. Martins Teixeira

3.1 Introduction

There is no standard definition of the term ‘heavy metal’, but it generally includes elements (both metals and metalloids) which are toxic and have an atomic density greater than 6 g cm^{-3}. This group includes both biologically essential elements such as cobalt (Co), copper (Cu), chromium (Cr), manganese (Mn) and zinc (Zn) and non-essential elements such as cadmium (Cd), lead (Pb) and mercury (Hg). Arsenic (As), boron (B) and selenium (Se) are usually included in this group although they are metalloids or non-metal [1]. The essential elements (for plants, microorganisms or animals) are required in low concentrations and hence are known as ‘trace elements’ or ‘micronutrients’. The non-essential metal (loid)s are phytotoxic and/or zootoxic and are widely known as ‘toxic elements’ [1–3]. Both groups are toxic to plants, animals and/or humans when present at high concentrations [4, 5].

A.P. Pinto (✉)
Chemistry Department of Science and Technology School, Évora University,
CLAV Rua Romão Ramalho no. 59, Évora 7000-671, Portugal

Institute of Mediterranean Agricultural and Environmental Sciences (ICAAM), Évora University, CLAV Rua Romão Ramalho no. 59, Évora 7000-671, Portugal
e-mail: app@uevora.pt

A. de Varennes
Linking Landscape, Environment, Agriculture and Food, Instituto Superior de Agronomia, University of Lisbon (TULisbon), Tapada da Ajuda, Lisbon 1349-017, Portugal
e-mail: adevarennes@isa.ulisboa.pt

M.E. Lopes • D.M. Teixeira
Chemistry Department of Science and Technology School, Évora University,
CLAV Rua Romão Ramalho no. 59, Évora 7000-671, Portugal

HERCULES Laboratory, Évora University, CLAV Rua Romão Ramalho no. 59,
Évora 7000-671, Portugal
e-mail: mel@uevora.pt; dmt@uevora.pt

A.A. Ansari et al. (eds.), *Phytoremediation*, DOI 10.1007/978-3-319-40148-5_3

Toxic elements can be released into the environment from natural and anthropogenic sources. The most significant natural sources are weathering of minerals, erosion and volcanic activity while anthropogenic sources include mining, smelting, electroplating, use in agriculture of pesticides, phosphatic fertilizers and biosolids, sludge dumping, industrial discharges, emissions from municipal waste incinerators, car exhausts, atmospheric deposition, etc. [1, 6–13]. Unlike organic substances, heavy metals (HMs) are essentially no biodegradable and therefore tend to accumulate in the environment and in the tissues of living organisms (bioaccumulation). Their concentration may increase as they pass from lower to higher trophic levels, a phenomenon known as biomagnification [13].

The total concentration of heavy metals in soils is commonly used to assess soil contamination [14, 15]. However, this does not provide an adequate indicator of their capacity to be absorbed by plants or soil organisms, nor does it predict the risk of their transfer along the food chain [15]. In fact, the bioavailability of heavy metals in soils depends on many factors such as pH, organic matter content, cation exchange capacity and the speciation of the metal. It has been shown that soils with larger concentrations of trace elements may be less toxic than those with smaller concentrations [16, 17]. Therefore, suitable indicators of soil pollution are still needed [1]. Remediation of soils contaminated with heavy metals remains a difficult challenge. The decision to remediate a soil depends on many factors, including the level of contamination, the risk posed by the soil, the present and future value of the soil and ultimately on political decisions which are influenced by the population and depend on the perception of the risk and willingness to pay the cost of remediation [1, 18].

There are two principal biological approaches for soil remediation: bioremediation systems that rely on microbial metabolism for site clean-up (next section) and phytoremediation, that utilizes natural processes harboured in (or stimulated by) plants. In most bioremediation processes, indigenous or introduced microbial communities include plant growth-promoting rhizobacteria (PGPR) [19], fungi [20], actinomycetes [21] and algae [22] to reduce, eliminate, contain and transform metals of different origins to more benign products [20]. The uptake of metals by plants or microorganisms is mainly influenced by their bioavailable fraction [1, 23, 24], which can be limited due to low solubility of metals in oxygenated matrices and sorption to soil particles. Metal availability and mobility in the rhizosphere is also influenced by rhizospheric microorganisms and root exudates. Acidification of the rhizosphere, exudation of organic acids and mechanisms assisting in the acquisition of phosphorus or micronutrients may enhance the bioavailability of certain heavy metals [1, 25].

Most of the metals are easily absorbed by plants and bioaccumulate in different organs where they can impair cell metabolism if present in sufficiently large amounts [3, 26]. Physiological mechanisms that may be affected include enzymatic activity, protein structure, water balance, respiration and ATP content, photosynthesis, plant division and morphogenesis [27–31]. A rather common consequence of heavy metal accumulation is the enhanced production of reactive oxygen species (ROS) due to interference of the metals with electron transport [32, 33]. Under abiotic and biotic stresses, the increased generation of ROS initiates signalling responses that include enzyme activation, programmed cell death and cellular damage [31, 34–36]. This increase in ROS exposes cells to oxidative stress leading to lipid peroxidation, deterioration of biological macromolecules such as DNA, dismantling of membranes and ion leakage [33, 37, 38].

Plants resort to a series of defence mechanisms to control uptake, accumulation and translocation of toxic elements and to detoxify them by excluding the free ionic forms from the cytoplasm. One commonly employed strategy relies on the entrapment of heavy metals in the apoplasm by binding them to exuded organic acids [33, 39] or to anionic groups present in cell walls, thus preventing uptake into root cells [33, 40, 41]. Most of the heavy metals that do enter the plant are kept in root cells, where they are detoxified by complexation with amino acids, organic acids or metal-binding peptides and/or by sequestration in vacuoles [33, 42]. This greatly restricts translocation to the aboveground organs, thus protecting the metabolically active photosynthetic cells from damage. A further defence mechanism generally adopted by exposed plants is the enhancement of cell antioxidant systems which counteract oxidative stress [33, 37, 43].

Plants can produce low molecular weight thiols that show high affinity for toxic metals [44, 45], the most important of which is glutathione (GSH) and cysteine. GSH metabolism is connected with cysteine and sulphur metabolism in plants, and GSH is a substrate for the synthesis of phytochelatins [45–49].

Phytochelatins (PCs) are a set of small, heavy metal-binding, cysteine-rich peptides with the general structure $(\gamma\text{-Glu-Cys})_n\text{Gly}$ ($n = 2$–11). They were first isolated from cell suspension cultures of a higher plant after exposure to Cd. Since then, PCs have been found in several eukaryotes, including higher plants, fungi and other organisms [45, 50, 51]. PCs are synthesized from GSH following exposure to heavy metals such as Cd, Hg, Cu, Zn, Pb, Ag, Bi, Au and Ni, with the involvement of α-glutamylcysteine transferase (EC 2.3.2.15), which is also called phytochelatin synthase [34, 45, 49, 52]. PCs form complexes with toxic metal ions in the cytosol and are subsequently transported into the vacuole by an ATP-dependent pathway [53]. Thus, toxic metals are swept away from the cytosol protecting plants from the deleterious effects they would cause otherwise [31, 54]. In fact, the vacuole is generally considered to be the main storage site for metals in plant cells, especially Cd and Zn, and compartmentalization of metals in the vacuole is an important part of the tolerance mechanism of some metal hyperaccumulator plants.

In conclusion, the detoxifying/sequestering mechanisms in plants consist mainly in heavy metal complexation with ligands and/or in their removal from the metabolically active cytoplasm by moving them into inactive compartments, mainly vacuoles and cell walls. Microorganisms have also to cope with situations where HMs are in excessive concentrations, and they developed various strategies to survive in heavy metal-polluted habitats, by developing different detoxifying mechanisms such as biosorption to cell walls, entrapment in extracellular capsules, controlled transport across the cell membrane, biotransformation through precipitation, complexation, and oxidation–reduction reactions, some of which can be exploited for bioremediation either ex situ or in situ [55–61].

Metal ions can be entrapped and subsequently biosorbed onto the binding sites present in the cellular structure of microorganisms. This method of uptake is independent of any biological metabolic activity and is known as ‘biosorption’ or ‘passive uptake'. The heavy metal can also cross the cell membrane through the cell metabolic cycle. This mode of metal uptake is referred to as 'active uptake'. Most of the studies dealing with microbial metal remediation via growing cells describe a biphasic uptake of metals, i.e. initial rapid phase of biosorption followed by slower, metabolism-dependent active uptake of metals [60]. The microbial cell walls, which mainly consist of polysaccharides, lipids and proteins, offer many functional groups that can bind metal ions, including carboxylate, hydroxyl, amino and phosphate groups [60, 62]. Among various microbe-mediated methods, the biosorption process seems to be more feasible for large-scale application compared to the 'active uptake' process because microbes will require addition of nutrients for their active uptake of heavy metals, which increases the biological oxygen demand or chemical oxygen demand in the waste. Further, it is very difficult to maintain a healthy population of microorganisms due to heavy metal toxicity and other environmental factors [60, 63, 64]. Fungi of the genera *Penicillium*, *Aspergillus* and *Rhizopus*, *Klebsiella oxytoca*, *Allescheriella* sp., *Stachybotrys* sp., *Phlebia* sp. *Pleurotus pulmonarius* and *Botryosphaeria rhodina* have metal-binding potential and are potential microbial agents for the removal of HMs [60, 65, 66].

Some examples are the remediation of Pb-contaminated soils by fungal species like *Aspergillus parasitica* and *Cephalosporium aphidicola* by biosorption process [67, 68], and Hg-resistant fungi (*Hymenoscyphus ericae*, *Neocosmospora vasinfecta* and *Verticillum terrestre*) which were able to biotransform a Hg (II) state to a nontoxic state [60, 69]. Sun et al. [70] evaluated the genetic diversity of endophytic bacteria from Cu-tolerant species of *Elshotzia apliendens* and *Commelina communis*, reporting increased dry weights of roots and aboveground tissues compared to uninoculated plants [70]. Further, they also reported significant amounts of (ranging from 63 to 125 %) Cu content in inoculated plants compared to uninoculated ones [60]. Microbes can also mobilize heavy metals from contaminated sites by leaching, chelation, methylation and redox transformation. Metals can never be destroyed, but these processes change their oxidation state or lead to the formation of organic complexes, so that the solubility is modified or they become less toxic [60, 71]. Two main mechanisms for development of resistance in bacteria are detoxification (transformation of the toxic metal state, making it less available) and active efflux pumping of the toxic metal from cells [60, 72]. The basic redox (oxidation and reduction) reactions take place in the soil; microorganisms act as an oxidizing agent for heavy metals and cause them to lose electrons, which are accepted by alternative electron acceptors (nitrate, sulphate and ferric oxides) [60].

3.2 Bioremediation: An Environmentally Friendly Strategy for the Remediation of a Wide Range of Heavy Metals

Multi-polluted soils are a growing global problem as heavy metals cannot be chemically or biologically degraded [73–75]. Different alternatives for their control rely on their removal or containment. There are essentially two approaches, described as

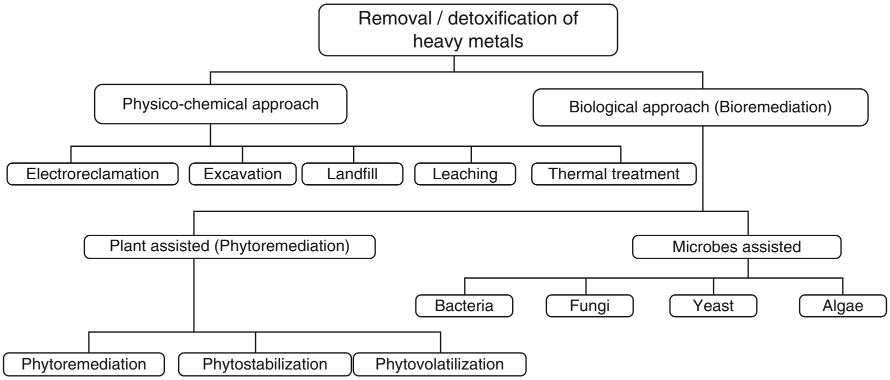


Fig. 3.1 Approaches used in the remediation of heavy metal-contaminated sites (Adapted from Zaidi A, Wani PA, Khan MS. Bioremediation: A Natural Method for the Management of Polluted Environment. Chapter 6, In: Zaidi A, Wani PA, Khan MS, editors. Toxicity of Heavy Metals to Legumes and Bioremediation. Wien, Austria: Springer-Verlag; 2012. p. 101–14 [20])

in situ and ex situ methods depending on whether the contaminated material is treated at the site or physically removed to be treated elsewhere. The rising popularity of in situ remediation over the past decade is due primarily to its relatively low cost and its capacity to destroy simultaneously a wide variety of organic pollutants, rather than transporting the target pollutants, minimizing risks to environmental and public health and potential liability associated with hazardous waste transportation and disposal [76]. The decontamination of soil and water from pollutants using microorganisms (*bioremediators*) is known as bioremediation. In the many forms of bioremediation (Fig. 3.1), microorganisms are utilized and managed through the control of environmental factors to reduce environmental pollution.

Microbial actions on heavy metal availability in soils include biosorption (metal sorption to cell surface by physicochemical mechanisms), bioleaching (mobilization through the excretion of organic acids or methylation reactions), biomineralization (immobilization through the formation of insoluble sulphides or polymeric complexes) and enzyme-catalyzed transformation by redox processes [75, 77, 78]. Biosorption seems to be the most common mechanism [75, 78, 79] as it does not have any energetic requirements. Intracellular processes include active transport, mediated by efflux pumps, sequestration, enzymatic transformation to other, less toxic chemical species, by redox reactions, methylation or alkylation/dealkylation, and/or reduction in the sensitivity of cellular targets to metal ions [80, 81].

Soil bioremediation constitutes a special challenge because of the heterogeneity of soils (or sediments), and also because well-adapted microorganisms are needed to bioremediate in specific environments [81, 82]. It is generally assumed that exposure to metals leads to the establishment of a tolerant or resistant microbial population [81, 83]. The common approach for in situ bioremediation is to engineer the environment to overcome limitations to natural degradation processes. For example, fertilizers and oxygen can be injected into hydrocarbon-contaminated aquifers to overcome the insufficient supply of nutrients and electron acceptors [76]. Therefore, one of the first steps in bioremediation is to conduct a complete site assessment to identify the factors that limit natural degradation processes. This information is then used to design approaches to overcome such limitations and enhance specific biocatalytic activities in situ [76].

There is considerable variability from site to site on how bioremediation works although all engineered bioremediation systems share a common feature—they rely on the stimulation and maintenance of microbial metabolism. However, some strategies may be applicable to one site but not to another, depending on the contamination scenario and the local hydrogeochemical conditions. Therefore, successful implementation of bioremediation may require integration of a broad range of sciences (including biochemistry, microbiology, mechanics and geosciences) and engineering principles (including mathematical modelling, systems analysis and reactor design) [76]. It is possible for bioremediation to occur under natural conditions but it can be stimulated, *e.g.* by the application of fertilizers (*biostimulation*) and even through the addition of microbial strains to the medium, enhancing the effectiveness of the microbial population to decompose contaminants [84]. Thus, there are two main bioremediation approaches: (a) *biostimulation*, which involves the addition of nutrients, electron acceptors (or electron donors) and sometimes auxiliary substrates to stimulate the growth and activity of specific indigenous microbial populations; and (b) *bioaugmentation*, which refers to the addition of exogenous, specialized microorganisms with enhanced capabilities to degrade the target pollutant [76].

An enormous range of polluting materials may be addressed, including heavy metals, hydrocarbons (*e.g.* from oil-spills), polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs), heterocyclics, pharmaceutical substances and pesticides. Microorganisms can also be induced to oxidize or reduce metals (*e.g.* Cr^{3+}, Cd^{2+}, Pb^{2+}, Cu^{2+}, Zn^{2+}) and various radionuclides (*e.g.* Cs^{+}, Sr^{2+}) to alter the valence of such inorganic pollutants for enhanced immobilization [76]. Microorganisms act on heavy metals through two main mechanisms: *bioadsorption*, which is a metabolism-independent binding, and *bioaccumulation*, which is an energy-dependent flux into the cell.

The tolerance capability of heavy metal-resistant microorganisms depends on their action as biosorbent and, therefore, on the presence of proper functional groups, with a high affinity for binding metals, at the cell wall. In this sense, both bacteria and fungi have surface compounds useful for sorption. In fact, when dead cells are used as bioremediation agents it is the only feasible process for them. In contrast, living cells can also act via other different strategies, which positively affect the efficiency of the process, as well as the self-replenishment capacity of the population [58, 75, 78].

According to Fomina et al. [85], fungi are usually more tolerant to metallic elements and have a higher surface to volume ratio than bacteria. Several authors have reported the specific properties of fungi for bioremediation processes, regarding both bioaccumulation and biosorption applications [75, 86, 87]. Among the actions that fungi can exert on metals, some of them ascribable exclusively or mostly to fungal species, are the complexation with cell wall chitin and other polysaccharides, as well as glycoproteins [75, 88], the synthesis of metallothioneins and other functional groups containing thiol [75, 89], the production of organic acids [90] or the compartmentalization in vacuoles [75, 91], which results in three main mechanisms: biosorption on the surface, intracellular uptake and chemical transformation [75, 92].

3.2.1 Ex Situ Versus In Situ Bioremediation

Since heavy metals cannot be chemically or biologically degraded [73–75], the different alternatives for their control proposed over the last decades rely on their removal or containment, both via ex situ and in situ strategies. Ex situ methods are usually quite expensive and cause structural and functional disturbances to soils [75, 93], while in situ techniques have been described as time-consuming and with inconsistent results [75, 94]. Among in situ strategies, physical, chemical and biological methodologies can be applied. Although both physical and chemical processes are extensively applied, they have proved to be not fully satisfactory as they are not completely effective in reducing toxicity, especially when the concentration of pollutants in materials is below 100 mg L^{-1} [75, 95]. They may also have a high polluting potential on account of the use of some toxic reagents [75, 96, 97]. In contrast, biological reclamation of heavy metal-polluted environments is increasingly gaining attention as it is considered as a clean and non-environmentally disruptive method and an efficient strategy for the remediation of a wide range of metals [75, 98, 99].

Ex situ treatment is usually selected when rapid removal of the contamination is required [76] or for the treatment of highly contaminated materials or when hydrophobic pollutants (such as PAHs and PCBs) are not effectively removed by in situ treatments due to low bioavailability. Ex situ bioremediation includes the use of aboveground bioreactors to treat contaminated soil (in slurry bioreactors) or groundwater (in conventional suspended or attached growth bioreactors) that has been extracted from the contaminated site [76]. The general approach is to excavate the contaminated soil and to treat it in slurry or solid phase [76]. For slurry treatment, water (about 60–80 % by weight) and nutrients are added to the contaminated soil in a batch reactor to obtain a slurry. Amendments may include seed bacteria or fungi, acids or bases to adjust the pH, and surfactants to enhance the dissolution of hydrophobic pollutants. Mixing, aeration and temperature are typically controlled in slurry bioreactors to optimize microbial activity and enhance solubilization of organic pollutants. However, this represents additional costs associated with energy and waste material and water handling. After treatment is completed, the slurry is dewatered and disposed of [76].

Solid phase ex situ bioremediation is typically less expensive than when slurry bioreactors are used but it is a slower process due to decreased ability to control the process. Solid phase treatment is often accomplished through landfarming by spreading and conditioning of the contaminated soil in special facilities that are lined with impermeable membranes to prevent the migration of contaminated leachate to the underlying soil and groundwater. Moisture and nutrients (and sometimes microorganisms) can be provided by sprinklers or sprayers, whereas oxygen is provided by mixing and tilling, or through air pumps that can be installed at the bottom of the contaminated soil pile. To facilitate aeration, straw or other bulking agents may be added [76]. In the case of hydrocarbon-contaminated soils, the lighter constituents are typically removed by evaporation, and the remaining aliphatic and aromatic compounds are degraded by a wide variety of microorganisms (e.g. *Pseudomonas*, *Corynebacterium* and *Mycobacterium*) [76].

In situ bioremediation occurs below the ground surface, where the contaminated zone becomes the bioreactor [76]. This process often relies on manipulation of soil conditions to overcome specific limitations to natural degradation processes and increase the rate and extent of pollutant degradation. This can be accomplished through biostimulation and/or bioaugmentation [76]. In situ bioremediation can be implemented as an aerobic or an anaerobic treatment. Aerobic treatments require the addition of oxygen, which can be injected into the contaminated zone (along with nutrients) using air spargers (especially for shallow contamination) or hydrogen peroxide dissolved in the nutrient solution. In anaerobic processes, organic substrates or hydrogen gas can be injected as electron donors (e.g. acetate, lactate, methanol or hydrogen gas) in concentrations that exceed the biochemical oxygen demand to stimulate anaerobiosis. Nutrients are also typically injected to satisfy the physiological requirements of anaerobes. For both aerobic and anaerobic biostimulation, the location, depth and number of injection wells depend on the hydrogeologic characteristics of the sites and the contamination profile [76].

3.2.2 Methods, Principles and Application of Bioremediation

There is a wide variety of bioremediation techniques that have been developed in the last decade (Table 3.1).

3.2.2.1 Natural Attenuation

Natural attenuation, also known as passive remediation, intrinsic (bio)remediation, or bioattenuation, are terms used to describe a collection of in situ physical, chemical and biological processes that, under favourable conditions, act without human intervention to reduce the mass, toxicity, mobility, volume or concentration of contaminants in soils or groundwater [100–103]. These processes include biodegradation, dispersion, dilution, sorption, volatilization and (bio) chemical stabilization. Biodegradation is frequently considered to be the primary mechanism for attenuation of contaminants [104]. Target contaminants includes metals, fuels, non-halogenated VOCs, SVOCs and pesticides [103]. Natural attenuation may be a proactive approach when verification and monitoring of natural remediation

Table 3.1 Bioremediation methodologies—principles and applications

Methods	Principles	Applications
Natural attenuation	Existence of indigenous microorganisms capable of degrading the contaminants	Diesel-oil-contaminated soil [130]
		PAHs-contaminated soil [316]
		Natural attenuation of heavy metal contamination in soils [317]
		Bioremediation process of PAHs-contaminated soil at field and laboratory scales [318]
		Natural attenuation of contaminated soils: state of the art in Europe [104]
		Natural attenuation of carbofuran-contaminated soil [129]
		Natural attenuation of heavy metals (Cd, Cr and Pb) in a water table aquifer [319]
		Arsenic-contaminated soils [320]
		Microbial remediation of an aged PAHs-contaminated soil [321]
		Soil mesocosm studies on atrazine bioremediation [322]
		Natural attenuation of arsenic by immobilization in nanostructured hematite [323]
		Natural attenuation of 1,2,4-trichlorobenzene in a shallow aquifer [324]
		Natural attenuation in alkylbenzene-polluted soil [325]
Bioaugmentation	Addition of exogenous microorganisms with the ability of degrading the contaminants that are recalcitrant to the indigenous microbiota	Pentachlorophenol-containing aquifers [326]
		Polycyclic aromatic hydrocarbons (PAHs) [327]
		Pseudomonas sp. strain was used for bioaugmentation of atrazine-contaminated soils in open soil microcosms [328]
		Bioaugmentation strategy of a heavy metal-contaminated soil, spiked with diesel oil [329]
		Bioaugmentation of diesel oil and heavy metal co-contaminated soil [330]
		Bioaugmentation of a carbofuran-contaminated soil [129]
		Bioremediation of water co-contaminated with 1,2-dichloroethane and heavy metals [132]
		Bioremediation of Cr(VI) and lindane in soil by actinobacteria [331]
		Bioremediation of soils contaminated with PAHs at fungal-inoculated microcosms [332]
		Bioremediation of soils heavily contaminated with PAHs with *Phanerochaete velutina* [332]
		Soil multi-polluted with Pb and PAHs [333]

(continued)

Table 3.1 (continued)

Methods	Principles	Applications
		The dynamics of indigenous bacterial community and the role of exogenous *P. phragmitetus* BB were utilized to intensify the remediation of Cr(VI)-contaminated soil [334]
		Bioremediation of TNT-contaminated soil with fungi [335]
		Bioaugmentation of a burned woodland soil contaminated by toxic hydrocarbons [135]
		In situ treatment of a diesel-polluted soil [336]
		Restoration of metal-contaminated estuary with autochthonous rhizobacteria [337]
Biostimulation	Addition of nutrients that stimulate the growth and development of indigenous microorganisms, increasing their metabolic activity, thus increasing the degradation rate	Effluent contaminated by surfactants and diesel oil [338]
		Diesel-oil-contaminated soil [130]
		Uranium-contaminated aquifer [131]
		Acidic and alkaline soils heavily contaminated with radionuclides (mainly U and Ra) and non-ferrous metals (mainly Cu, Zn, Cd and Pb) were treated in situ using the activity of the indigenous soil microflora under suitable changes of some essential environmental factors such as pH and water, oxygen and nutrients [132]
		Biostimulation strategy of an aged diesel-oil-contaminated soil [339]
		Bioremediation of water co-contaminated with 1,2-dichloroethane and heavy metals [133]
		Bioremediation of a crude oil-contaminated soil under microcosm study [134]
		Immobilization of Cd in soil by bioaugmentation of UV-mutated *Bacillus subtilis* 38 assisted by biostimulation [112–114]
		Biostimulation of a burned woodland soil contaminated by toxic hydrocarbons [135]
Bioleaching	Specific microorganisms like *Thiobacillus ferrooxidans* and *T. thiooxidans* promote metal solubilization	Heavy metal-contaminated soil [137, 138]
		Dissolution of metals [340]
		Fractionation behaviour of heavy metals in soil during bioleaching with *Acidithiobacillus thiooxidans* [138]
		Bioleaching of heavy metals from a contaminated soil in an industrial area using metabolites, mainly weak organic acids, produced by *Aspergillus niger* [341]
		Bioleaching of heavy metal-contaminated sediment by pure and mixed cultures of *Acidithiobacillus* spp. [342]

Methods	Principles	Applications
		A combination of bioleaching and bioprecipitation for deep removal of contaminating metals from dredged sediment [343]
		Bioleaching of heavy metals from a contaminated soil using indigenous *Penicillium chrysogenum* strain F1 [344]
		Bioleaching of heavy metals in a mixture of contaminated soil and slag by using indigenous *Penicillium chrysogenum* [345]
		Bioleaching of heavy metals from contaminated sediments (effect of sediment size) [346]
		Bioleaching of a low-grade nickel-copper sulphide [347]
		In situ treatment of an acidic leached cinnamonic forest soil heavily contaminated with radionuclides (mainly U and Ra) and non-ferrous metals (mainly Cu, Zn and Cd), due to the joint action of the soil microorganisms and leach solutions used to irrigate the soils (mainly acidophilic chemolithotrophic bacteria and diluted sulphuric acid) [348]
		Bioleaching of highly concentrated arsenic mine tailings by *Acidithiobacillus ferrooxidans* [349]
		The effects of typical strains of *Acidithiobacillus* attached cells in chalcopyrite bioleaching [350]
		Bioleaching of heavy metals from contaminated alkaline sediment by auto- and heterotrophic bacteria in a stirred tank reactor [351]
		Bioleaching of heavy metals from spent household batteries using *Acidithiobacillus ferrooxidans* [352]
		Bioleaching of heavy metals using *Acidithiobacillus thiooxidans* in a slurry bubble column bioreactor [353]
		Bioleaching of arsenic and heavy metals from mine tailings by pure and mixed cultures of *Acidithiobacillus* spp. [354]
		Bioleaching of heavy metal-contaminated soils using *Burkholderia* sp. Z-90 [355]
Phytoremediation	Use of plants to extract, sequestrate or decontaminate terrestrial or aquatic environments	Heavy metal accumulation and phytostabilization potential of tree fine roots in a contaminated soil [227]
		In situ phytostabilization of heavy metal polluted soils using *Lupinus luteus* inoculated with metal-resistant plant growth-promoting rhizobacteria [356]
		Phytoremediation of zinc-contaminated soils using silver birch (*Betula pendula* Roth) [357]

(continued)

Table 3.1 (continued)

Methods	Principles	Applications
		Comparative bioremediation of heavy metals and petroleum hydrocarbons co-contaminated soil by natural attenuation, phytoremediation, bioaugmentation and bioaugmentation-assisted phytoremediation [358]
		A field-scale study of cadmium phytoremediation in a contaminated agricultural soil [359]
		The hyperaccumulator *Sedum plumbizincicola* harbours metal-resistant endophytic bacteria that improve its phytoextraction capacity in multi-metal-contaminated soil [360]
		Phytoextraction of heavy metal from tailing waste using Napier grass [361]
		Earthworm populations of highly metal-contaminated soils restored by fly ash-aided phytostabilization [362]
Biofilters	Application of bacteria in filters for the decontamination of polluted water and wastes	Water contaminated by trichloroethylene [363]
		Effluent contaminated by silver nanoparticles [364]
		Waste contaminated by Cu^{2+} and Cd^{2+}[365]
		Removal of *Clostridium perfringens*, *Escherichia coli* and F-RNA coliphages by stormwater biofilters [366]
		Removal of heavy metals (Cu, Zn) in a stormwater retention biofilter [367]
Biopiling	The material to be treated is piled over an aerated system and nutrients are added to it	Hydrocarbon-contaminated soil [368]
		Bioremediation of diesel-contaminated soils by biopile technique [369]
		Bioremediation of soils contaminated with PAHs, petroleum, pesticides, chlorophenols and heavy metals by composting [295]
		Bioremediation of petroleum hydrocarbon contaminated soils by biopiling techniques [311]
Biosorption	Adsorption of metals and other ions by the use of biological materials	Biosorption of cadmium by *Trichoderma reesei* [370]
		Biosorption of Pb (II) and Cd (II) ions from aqueous solution [371]
		The potential of a biofilter containing a mixture of dried micro-algal/bacterial biomass for removing heavy metals (Cu^{2+}, Cd^{2+}) from dilute electroplating waste [365]
		Biosorption of multiple heavy metals (cadmium, chromium, mercury and lead) by *Bacillus subtilis* [111–114]
		Biosorption of heavy metals on *Citrus maxima* peel, passion fruit shell and sugarcane bagasse in a fixed-bed column [372]

		Biosorption of Cr (VI) by *Typha angustifolia* in wastewater solutions [373]
		Biosorption of heavy metals by algal biomass review [278]
		Chitosan-based biosorbents for heavy metal removal from aqueous solutions [374]
		Biosorption of several metals by *Phallusia nigra* in an aquatic environment [375]
		Adsorption behaviour of unmodified yeast cells of *Saccharomyces cerevisiae* to remove Pb(II) and Cu(II) ions from aqueous solutions [264]
		Use of algae (micro and macro) in raw or modified forms as biosorbents for water or wastewater decontamination [376]
		Removal of Cu (II) by *Cystoseira crinitophylla* biomass in batch and continuous flow biosorption [267]
		Biosorption of Cd^{2+} and Zn^{2+} by bacteria isolated from soils collected from a zinc mine [261]
Bioventing	Combination of venting of soil to remove the volatile compounds with bioremediation that uses oxygen to degrade the organic contaminants	Bioventing remediation and ecotoxicity evaluation of phenanthrene-contaminated soil [377]
		Bioventing tests in contaminated residual granitic soils [378]
		Volatilization and bioremediation of toluene-contaminated soil [379]
		Sustainable wind-driven bioventing at a petroleum hydrocarbon-impacted site [380]
Composting	Nutrients are added to soil that is mixed to increase aeration and activation of indigenous microorganisms	Involvement of microbial populations during the composting of olive mill wastewater sludge [381]
		Biodegradation of phenolic compounds in creosote treated wood waste by a composting microbial culture augmented with the fungus *Thermoascus aurantiacus* [382]
		Compost as a source of microbial isolates for the bioremediation of heavy metals: in vitro selection [75]
		Heavy metals removal using an integrated composting vermicomposting process [383]
		Bioremediation of soils contaminated with polycyclic aromatic hydrocarbons, petroleum, pesticides, chlorophenols and heavy metals by composting [295]
		Metal loss during the composting and vermi composting of Municipal Solid Waste employing *Eudrilus eugeniae* [384]

(continued)

Table 3.1 (continued)

Methods	Principles	Applications
Landfarming	Soil is organized in piles and is periodically turned over by agricultural practices to stimulate the degradation by indigenous microorganisms	Oil-contaminated soils [385–388]
		Remediation of hydrocarbon-contaminated soils [389]
		Allium cepa bioassay to evaluate landfarming before and after the addition of rice hulls to accelerate organic pollutants biodegradation [309]
		Bioremediation of weathered petroleum hydrocarbon-contaminated soil [390]
		Horizontal and vertical contaminant gradients in an old landfarming field for oil refinery waste [391]
Rhizoremediation	The plant releases exudates that will increase the rhizospheric microorganisms that will help plant growth and the degradation of contaminants	Soil contaminated by trichloroethylene [392]
		Polychlorinated biphenyl rhizoremediation by *Pseudomonas fluorescens* F113 [393]
		Soil microbial response during the phytoremediation of a PAHs-contaminated soil [394, 395]
Biosparging	Involving the injection of air below the water table to increase groundwater oxygen concentrations and enhance the rate of biological degradation of contaminants by naturally occurring bacteria	Cometabolic air sparging field demonstration with propane to remediate trichloroethene and *cis*-dichloroethene [396]
		Crude oil-contaminated soil washing in air sparging-assisted stirred tank reactor using biosurfactants [397]
		In situ biosparging to remediate a petroleum-hydrocarbon spill site [315]
		Enhancement of the microbial community biomass and diversity during air sparging bioremediation of a soil highly contaminated with kerosene and BTEX [398]
		A dynamic two-phase flow model for air sparging [399]
Bioreactors	Slurry reactors (aqueous reactors)	Bioremediation of crude oil-contaminated soil using slurry-phase biological treatment [400]
		Influence of bioaugmentation in ex situ bioremediation of pyrene-contaminated soil in bio-slurry phase reactor operated in periodic discontinuous batch mode [401]
		Influence of bioaugmentation in biodegradation of PAHs-contaminated soil in bio-slurry phase reactor [402]
		Ex situ remediation of polluted soils by absorptive polymers, and a comparison of slurry and two-phase partitioning bioreactors for ultimate contaminant degradation [403]

processes are carried out [102, 103]. When indigenous microorganisms are not able to effectively degrade the contaminants of concern, specific degraders can be added to the impacted areas. This technique is called bioaugmentation.

3.2.2.2 Bioaugmentation

In bioaugmentation, a group of natural microbial strains or a genetically engineered variant, with enhanced capabilities to degrade the target pollutant, is introduced to treat contaminated soils or waters. It involves microbes like bacteria, protozoa, nematodes, rotifers and fungi capable of degrading organic compounds [103]. The microbial cultures are typically grown separately under well-defined conditions to perform a specific remediation task in a given environment (in situ or in a bioreactor). The manipulation of soil microbial communities has been utilized in agriculture since the 1800s when nitrogen-fixing *Rhizobium* spp. inocula were added to legumes [105]. Bioaugmentation is often used to enhance the degradation of recalcitrant contaminants that cannot be mineralized by the indigenous consortium even under optimum conditions. The introduced microorganism increases the indigenous population's degradation capacity, hence the term bioaugmentation.

Two factors limit the use of added microbial cultures in a land treatment unit: (a) nonindigenous microorganisms rarely compete well enough with an indigenous population to be able to develop and sustain useful population levels; and (b) most soils with long-term exposure to biodegradable wastes already have indigenous microorganisms that are effective degraders if the land is well managed [106]. The benefits of bioaugmentation have been demonstrated in field trials for a wide variety of recalcitrant contaminants, including MTBE [107], carbon tetrachloride [108] and TCE [109–111]. Whereas a competent indigenous consortium can develop over time at some contaminated sites (resulting in the eventual degradation of some persistent organic pollutants), bioaugmentation results in shorter acclimation periods and faster degradation, often with less objectionable by-products. Furthermore, if a rapid response is important, relying on small numbers of indigenous microorganisms may not be appropriate. In such cases, bioaugmentation can enhance the reliability and rate of the clean-up process.

In wastewater treatment, bioaugmentation of activated sludge systems with specialized bacterial strains can improve several aspects of the treatment processes, such as improved flocculation and degradation of recalcitrant compounds. This is relatively easy to accomplish because the added microorganisms can be readily mixed in the reactor and reaction conditions can be manipulated to enhance their survival and performance [76]. Bioaugmentation of aquifers is a more challenging task. Critical issues include survival of added strains, their distribution throughout the contaminated zone (which is hindered by the porous medium), and low concentration of nutrients and target contaminants that serve as substrates to the added microorganisms. Fungi, which are larger than bacteria, are restricted to reactor or surface soil applications, whereas bacteria are more adaptable to subsurface applications. In addition to the challenge of delivering the inoculant to the desired subsurface location, the survival of exogenous microorganisms may be hindered by abiotic and biological stress. These include fluctuating or extreme temperatures, pH, water activity, low nutrient levels, toxic pollutant concentrations, and competition with indigenous microorganisms [105]. Also, some of the bacteria used for bioaugmentation (e.g. *Burkholderia cepacia*) could, under certain conditions, exhibit pathogenic properties [76].

Bioaugmentation is environmentally friendly and hence it could be an ideal option to lower the hazardous effects of heavy metals without destroying soil properties [112–114]. A problem encountered in bioaugmentation is the toxicity of heavy metals, which presents a stress on the bioaugmented microflora. Therefore, a crucial step is acquiring bacterial species with high tolerance to heavy metals. The main bacteria breeding methods include domestication, mutagenesis and genetic engineering. However, the domestication of strains is time-consuming and inefficient, and genetic engineering is complex and still in its early stages of development. Thus, mutation breeding is a promising option to enhance the activity of strains. Several recombination biotechnologies that increase the frequency of spontaneous mutation, such as X-ray irradiation, ultraviolet (UV) irradiation, ethyl methane sulfonate (EMS) treatment and HNO_2 induction, have been developed [115, 116].

The UV-irradiation mutation is the simplest and most effective physical mutation method, which has been widely used in microbial industry [117–121]. However, the technology has seldom been used in the mutation of microorganisms to improve their resistance and affinity for heavy metals [122, 123]. Another limitation of bioaugmentation is that the contaminated sites are usually nutrient deficient and cannot support the rapid growth of the introduced population [124]. A strategy to stimulate the metabolism and proliferation of microorganisms is the addition of nutrients, i.e. biostimulation [125]. Several matrixes, including pure nutrients, such as glucose, and wastes from agriculture [126] and industry [127], have been tested. The combined technology of bioaugmentation assisted by biostimulation integrates the efficacy of both technologies and suggests a promising approach for the bioremediation of heavy metal-contaminated soils.

3.2.2.3 Biostimulation

Biostimulation is an in situ treatment which involves the addition of nutrients, electron acceptors (or electron donors) and sometimes auxiliary substrates to stimulate the growth and activity of specific indigenous microbial populations [76]. Various organic amendments such as biogas slurry, farmyard manure [128], spent mushroom compost [128], rice straw and corncob [106] were used to stimulate the activity of indigenous microorganisms to enhance degradation of target pollutants. The amended materials should contain suitable nutrients to stimulate the activity of indigenous microorganisms and may even contain a large number of degradative microbes [129]. These technologies are designed primarily to treat soil and groundwater contamination by fuels, non-halogenated VOCs, SVOCs, metals and pesticides [130–135].

The technology requires the presence of indigenous organisms capable of degrading the contaminants of interest [103].

Biostimulation is commonly selected for the clean-up of hydrocarbon-contaminated sites, often through the addition of oxygen and macronutrients such as nitrogen and phosphorus. Bioaugmentation is often unnecessary in such cases because indigenous bacteria that degrade hydrocarbons under aerobic conditions are ubiquitous in nature. Indeed, hydrocarbons have a natural pyrolitic origin and have been in contact with microorganisms throughout evolutionary periods of time, exerting selective pressure for the development of a wide variety of degradation pathways [76].

3.2.2.4 Bioleaching

Bioleaching of metals is another example of the use of microorganisms in the decontamination of aqueous solutions and soils. It consists on the recovery of metals by some microorganisms capable of changing their solubility [136]. For example, White et al. [137] used sulphuric acid produced by sulphur-oxidizing bacteria followed by precipitation of the leachate metals (Cd, Co, Cr, Mn, Ni and Zn) by sulphate-reducing bacteria, demonstrating that the metals could be leached from the soil. Kumar and Nagendran [138] reported that this methodology is useful for an efficient removal of heavy metals from soils. A very well-known case is the use of *Thiobacillus* that is able to perform microbiological leaching of metals such as copper, silver, uranium and zinc due to oxidation, by releasing protons which can replace the metals adsorbed to soil particles, or by oxidation inducing the solubilization of metals [136].

3.3 Phytoremediation

Phytoremediation uses plants and associated soil microorganisms to remove or reduce contaminants in different environmental matrices (air, soil and water). Plants can be used to treat most classes of contaminants—toxic metals, radionuclides and recalcitrant organic pollutants, like chlorinated pesticides, organophosphate insecticides, petroleum hydrocarbons (BTEX), polynuclear aromatic hydrocarbons (PAHs), sulfonated aromatics, phenolics, nitroaromatics and explosives, polychlorinated biphenyls (PCBs) and chlorinated solvents (TCE, PCE) [10, 139–142]. This method is often complementary to traditional bioremediation techniques, based exclusively on the use of microorganisms [71, 143–145]. Plants have been successfully used not only for soil remediation but also to treat municipal and industrial wastewaters [19, 146–149] and even some information is available on their application to treat air [31, 150].

It represents a sustainable technology for the remediation of slightly to moderately contaminated sites and contributes to the improvement of the physical (erosion control), chemical (nutrient levels and organic matter content) and biological (biodiversity and enhanced microbial biomass and activity) quality of the soil [151, 152]. Low costs of phytoremediation are an advantage compared to conventional technologies together with lower environmental impacts. Thus, phytoremediation has a high potential because it is a cost-effective, efficient, environment- and eco-friendly and solar-driven remediation strategy [9, 13, 152–160]. It is seen as having great promise compared to conventional, civil-engineering methods and several recent comprehensive reviews summarizing the most important aspects of phytoremediation are available [31, 33, 161–166]. The term 'phytoremediation' encompasses several technological subsets, i.e. phytoextraction, phytofiltration, phytostabilization, phytovolatilization, phytodegradation, rhizodegradation and phytodesalination [10, 13, 24, 167] involving various physiological and biochemical mechanisms [84, 140, 166].

Plant–microbe interactions have gained considerable attention due to the potential of microbes to accumulate metals from polluted environments [166, 168, 169], positively influencing plants by improving growth and root development, which in turn increases plant tolerance to various environmental stresses [166, 170–172]. Bacteria that promote plant growth (PGP) can improve limiting factors such as metal solubility, level of contamination and soil chemistry [166, 173]. These may be free-living, live in symbiotic associations or live as endophytic bacteria [174]. Some important genera of PGP bacteria include *Serratia, Bacillus*, *Pseudomonas*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Klebsiella*, *Beijerinckia*, *Flavo-bacterium* and *Gluconacetobacter* [166, 175, 176]. The PGP bacteria improve plant growth by atmospheric nitrogen fixation (diazotrophs), phytohormone production, specific enzymatic activities and plant protection from diseases by the production of antibiotics or other pathogen-depressing substances such as siderophores and chelating agents [177]. In addition, some PGP bacteria have the ability to decrease the level of ethylene in plants, which increases plant growth [166].

Consequently, vigorous and healthier plants are better able to phytoremediate heavy metals in contaminated soils [178]. In addition to plant growth, PGP bacteria also have the ability to affect metal mobility and availability to the plant through the release of chelating agents, acidification, phosphate solubilization and redox changes [166, 179–182]. Moreover, these bacteria developed metal tolerance mechanisms including exclusion, biosorption, active removal, precipitation or bioaccumulation both in external and intracellular spaces [183]. GPR (Plant Growth-Promoting Rhizobacteria) such as *Agrobacterium*, *Alcaligenes* (*Ralstonia*), *Arthrobacter*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Serratia*, *Pseudomonas* and *Rhizobium* [184–188] are particularly interesting since they increase both the rate of metals accumulated by plants and the plant biomass. *Pseudomonas* are well-known producers of siderophores involved in the modification of metal speciation in soils. Siderophores synthesized by *P. fluorescens* improved Fe uptake by tomatoes [189], barley [190], vine, maize [191, 192] and peanut [193]. Sun et al. [70] evaluated the genetic diversity of endophytic bacteria from Cu-tolerant species of *Elshotzia apliendens* and *Commelina communis*, reporting increased dry weights of roots and aboveground tissues compared to uninoculated plants [70]. They also reported greater amounts of Cu in inoculated plants compared to uninoculated ones [60].

This is not always a favourable effect, as increased uptake of toxic metals such as Cd, Cr, Cu, Ni, Pb and Zn was observed when soils were inoculated with pseudomonas [188, 194–199]. Some hydroxamate siderophores, such as desferrioxamine B, can complex Pb [200], potentially helping Pb uptake by plants, while complexation of Cd, Cu and Zn is much higher with nitrilotriacetate [188, 201]. However, the response of microbial communities to heavy metals depends on the concentration and availability of heavy metals and is a complex process which is controlled by multiple factors, such as type of metal, the nature of the matrix and microbial species [60].

Phytoremediation may be divided into several remediation technologies, the most important of which as described in more detail in the next sections.

3.3.1 Phytoextraction

Phytoextraction, also known as phytoaccumulation, phytoabsorption or phytosequestration, is the uptake of contaminants from soil, water or sediments by plant roots and their translocation and accumulation in aboveground biomass, i.e. shoots or any other harvestable plant parts [13, 24, 61, 202, 203]. For this purpose, plants capable of accumulating heavy metals are grown on contaminated sites and the metal-rich aboveground biomass is harvested, resulting in the removal of a fraction of the contaminant. Metal translocation to shoots is a crucial physiological process as it is much simpler to harvest shoots than roots [13, 173, 204]. Phytoextraction is the main and most useful phytoremediation technique for the removal of heavy metals and metalloids from polluted soils [13, 205, 206]. It is also the most promising for commercial applications [13].

The success of phytoextraction as a potential environmental clean-up technology depends on many factors like soil properties, bioavailability of heavy metals (which depends on speciation of the heavy metals), as well as the plant ability to absorb and accumulate metals in its aboveground parts [13, 24]. Two different approaches have been tested for phytoextraction of heavy metals: (1) The use of hyperaccumulators, which produce comparatively less aboveground biomass but accumulate target heavy metals to a greater extent; (2) The application of other plants, such as *Brassica juncea* (L.) Czern. (Indian mustard), which accumulate target heavy metals to a lesser extent but produce more aboveground biomass so that overall accumulation is comparable to that of hyperaccumulators [13, 207, 208].

3.3.1.1 Hyperaccumulators

Some plants are able to accumulate large amounts of metals in their aboveground tissues—they are called hyperaccumulators. Content of specific metals in these plants exceeds levels that are actually required for normal growth and development. Hyperaccumulators belong to distantly related families, but share the ability to grow on metalliferous soils and accumulate metals in levels far in excess of those found in the majority of species, without suffering phytotoxic effects [33]. Three basic hallmarks distinguish hyperaccumulators from related non-hyperaccumulating plants: a strongly enhanced rate of heavy metal uptake, a faster root-to-shoot translocation and a greater ability to detoxify and sequester heavy metals in leaves [33].

About 450 angiosperm species have been identified as hyperaccumulators, of which about 25 % belong to the Brassicaceae family and, in particular, to the genera *Thlaspi* and *Alyssum* [33, 209]. These also include the highest number of Ni hyperaccumulating taxa [33, 210]. Zinc hyperaccumulators are less numerous and include *Arabidopsis halleri* L., and species of *Thlaspi* among the Brassicaceae [211] and *Sedum alfredii* Hance (Crassulaceae) [212]. *A. halleri*, *S. alfredii*, *Corydalis pterygopetala* Handel-Mazzetti, together with *Thlaspi caerulescens* J.&C. Presl. and *T. praecox* Wulfen are recognized species that hyperaccumulate Cd as well as Zn [33, 213, 214]. *Solanum nigrum* L. (Solanaceae) was reported as being the fifth Cd hyperaccumulator [215].

Species hyperaccumulating Se are distributed in genera of different families, including the Fabaceae, Asteraceae, Rubiaceae, Brassicaceae, Scrophulariaceae and Chenopodiaceae [216]. Some plants other than angiosperms are *Isatis cappadocica* Desv. and *Hesperis persica* Boiss. [217, 218], a number of brake ferns belonging to the genus *Pteris* have also been found to hyperaccumulate As [219–221]. Many studies focus on the genetics and biochemistry of metal uptake, transport and storage in hyperaccumulator plants, so that transgenic plants with improved phytoremediation capability can be obtained. Significant progress in understanding the mechanisms governing metal hyperaccumulation has been made in the last decade through comparative physiological, genomic and proteomic studies of hyperaccumulators and related non-hyperaccumulator plants. A great number of studies were carried out with *T. caerulescens* and *A. halleri*, which became model plants for these studies [33, 222, 223]. However, tolerance and accumulation of heavy metals, and thus the phytoextraction potential, is controlled by many genes, so that genetic manipulations to improve these traits is not an easy task, requiring extensive changes in the expression of a number of genes that have to be previously identified. This means that functions and regulations of genes involved in metal uptake, translocation and detoxification/sequestration mechanisms need to be fully understood [33].

3.3.1.2 Fast Growing Species

Many plant species are being investigated to determine their usefulness for phytoextraction, especially fast growing crops capable of accumulating a large biomass [24]. Nonetheless, the use of crops for phytoextraction of heavy metals also presents some disadvantages, in particular the risk of contamination of the food chain. In no case should field crops grown for phytoremediation purposes be used as animal feed or for direct human consumption [13, 23]. The harvested biomass is usually incinerated or composted and rarely recycled for reuse [24, 224]. The ideal plant to be used in phytoextraction should possess multiple traits like the ability to grow in many climatic and edaphic conditions, a fast growth and large biomass, and capacity to accumulate a range of heavy metals in its harvestable parts [24, 225]. Fast growing trees, like *Populus* spp. and *Salix* spp., are ideal candidates for phytoextraction due to their extensive root systems, high rates of water uptake and transpiration, rapid growth and large biomass. They can be harvested with subsequent resprouting without disturbing the site [24, 47, 226–228]. However, the use of trees requires excavation and disposal of roots at the end of the process [24, 229].

3.3.1.3 Phycoremediation

The term phycoremediation is applied when microalgae, macroalgae and cyanobacteria are used for the removal or biotransformation of pollutants, including nutrients and xenobiotics from wastewater and CO_2 from waste air. In both type of technologies, it is important to assess the fate of pollutants (i.e. metals) within the various compartments of the treatment system: roots, stems, fronds or leaves, microbial biomass and water column [230].

3.3.2 Phytostabilization

Unlike phytoextraction, phytostabilization or phytoimmobilization stabilizes contaminants through the establishment of a plant cover on contaminated sites. This reduces the mobility of contaminants within the vadose zone, thereby reducing off-site contamination by wind erosion or leaching [231]. In phytostabilization, plants (often with soil amendments) are used to reduce the solubility or toxicity of contaminants, primarily through modification of the physicochemical conditions to reduce labile metal pools, through accumulation by roots or immobilization within the rhizosphere [3, 232, 233]. Plants immobilize metals through sorption by roots, precipitation, complexation or changes in metal valence [12, 13, 234, 235]. Metals of different valences vary in toxicity; by excreting redox enzymes, plants skillfully convert hazardous metals to less toxic states. For example, reduction of Cr(VI) to Cr(III) has been widely reported, the latter being both less mobile and less toxic [13, 99].

Phytostabilization has proved useful for the treatment of Pb, As, Cd, Cr, Cu and Zn-contaminated soils [236] and has also been successful in addressing metals and other inorganic contaminants in sediments [237]. A typical scenario in which phytostabilization may be considered is represented by metalliferous sites (e.g. abandoned mining sites, smelter sites) where the presence of wastes and mine tailings results in severe pollution and poses aesthetic impacts on the local environment [238, 239]. Thus, phytostabilization is usually applied on highly polluted areas, where the removal of metals by phytoextraction would be too slow [239, 240]. Phytostabilization is also advantageous when decontamination strategies are impractical because of the extent of the contaminated area or the lack of adequate funding [241]. Trees are expected to be suitable for extensive and long-term phytoremediation or phytostabilization due to their large root systems and high transpiration rates [47, 242]. In addition to the direct stabilization of the soil by root systems, the vegetation cover decreases the risk of soil loss by wind and water erosion. Leaf fall adds significant amounts of organic matter to the surface layers, promoting nutrient cycling and soil aggregation and increasing the water-holding capacity. The large amount of water removed from the soil by the transpiration stream decreases the downward flow through the soil, helping to reduce the amounts of heavy metals that are transferred to surface and groundwater [47, 243].

The risk of environmental hazard will be reduced by choosing tree species that do not accumulate heavy metals because access to the contaminating metals through the aboveground tissues, in particular leaves, will be reduced [47, 243]. Selecting appropriate tree species is thus crucial for achieving a successful phytostabilization. Moreover, trees may enhance metal leaching due to soil acidification and production of dissolved organic matter. Hence, it is important to select tree species that do not acidify the soil and keep the metals mostly in their root systems, with little translocation [243].

This technique can be adapted to a variety of sites and situations, with different conditions (e.g. soil pH, salinity, soil texture, metal levels and contaminant types) through the careful selection not only of the appropriate plant species but also by application of amendments [168, 232]. There are thus two major components in the phytostabilization process: the plant itself and the amendments added to the system [167].

Some plants have a natural ability to immobilize pollutants by providing a region around the roots where these are precipitated and stabilized, i.e. they alter the speciation of soil metals. Therefore, plants reduce the mobility and bioavailability of pollutants in the environment either by immobilization or by prevention of migration, thus rendering them less harmful [3, 49, 244, 245]. For instance, species tolerant to low pH such as *Cynodon dactylon* (L.) Pers., *Juncus usitatus* L.A.S. Johnson and *Lomandra longifolia* Labill. were identified as having the potential to be used in phytostabilization programmes [246]. Metal-tolerant species like *Carduus pycnocephalus* L., *Dasypyrum villosum* L. P. Candargy, *Ferula communis* L., *Silybum marianum* (L.) Gaertn., *Sinapis arvensis* L. and *Stipa austroitalica* Martinivsky also promote metal stabilization and soil conservation as a result of their excluder behaviour [247]. Moreover, pioneer plants can be ideal species for the phytostabilization of mine tailings [246], e.g. *Atriplex halimus* L. *subsp. schweinfurthii* has potential for phytostabilization of Cd-contaminated saline soils [248].

Jatropha curcas L. is another candidate to cover and reclaim metalloid and metal-contaminated soils [249], and *Alnusrugosa* (Du Roi) Spreng. was used in tailings and to re-establish forest ecosystems [250]. Zou et al. [251] reported that *Athyrium wardii* (Hook.) H. Christ has the potential to stabilize Pb-contaminated soils. For phytostabilization of metals, a combination of trees and grasses may work best. Fast-transpiring trees maintain an upward flow to prevent downward leaching, while grasses with their dense root systems prevent wind erosion and lateral runoff. In addition, grasses do not accumulate metals in their shoots to such an extent as do dicots, thus minimizing exposure of wildlife to toxic elements [163, 233].

3.3.3 Phytovolatilization

While elements like Cd, Cr, Co, Cu, Ni, Pb and Zn can be treated by phytostabilization or phytoextraction, As, Hg and Se can be removed by phytovolatilization because they can form volatile chemical species through reduction and methylation reactions [140, 252]. Phytovolatilization involves the uptake of contaminants from soil or water and their transformation into volatile compounds and transfer into the atmosphere [3]. This technique is less useful for the removal of heavy metals as these must (a) be taken up by plants through roots, (b) pass through the xylem to the leaves (c) be converted into volatile compounds, and (d) volatilize to the atmosphere. Despite these limitations, this technique has been reported to be useful for the removal of Hg from polluted soils. Mercury is regarded as one of the ‘priority hazardous substances’ by the Agency for Toxic Substances and Disease Registry (ATSDR) because of its toxicity, mobility and long residence time in the atmosphere [253]. Once Hg is released into the atmosphere it can be retained for between 6 and 24 months and be transported over tens of thousands of kilometers before eventual re-deposition on the Earth’s surface [254]. Both natural and anthropogenic activities emit Hg to the atmosphere [255].

The volatilization of Se by plants and soil microbes is also an important component of phytoremediation. Plants take up Se mostly as selenate, selenite and/or organic Se. The uptake of selenate and organic Se is driven metabolically, whereas the uptake of selenite may have a passive component [256]. Selenate is highly bioavailable, and it is the soluble form of Se commonly found in soils and subsurface drainage waters. Volatile Se compounds (mainly dimethyl selenide and dimethyl diselenide) form through biomethylation, a biological process in which microorganisms and/or plants convert inorganic Se into methylated volatile Se compounds [257]. Plant species differ substantially in their ability to take up and volatilize Se. Plant species from the Brassicaceae family were particularly effective Se volatilizers [256]. It seems that the ability to volatilize Se is associated with the ability to accumulate Se in plant tissues—the rate of Se volatilization by different plant species was strongly correlated with the plant tissue Se concentration [256, 258].

Various technologies are available to remediate soils contaminated with As, including phytovolatilization. Plants absorb As through phosphate transporters and translocate it to the aboveground parts (shoots and leaves). The amount of arsenic translocated from roots to shoots indicates the phytoremediation efficiency of that plant, but most plants store more than 90 % of the As in their roots. Few plants have the ability to translocate high amounts of As [259]. *Pteris vittata* L. has the highest ability to accumulate and translocate As [219], and it is considered an efficient As-hyperaccumulator also capable of volatilizing As. An alternative that is being considered for phytovolatilization is the use of genetic engineering to integrate genes from other organisms to enhance the phytovolatilization capabilities of plants [253].

In conclusion, phytovolatilization uses plants that remove volatile contaminants (e.g. Hg, As and Se) from terrestrial or aqueous systems and facilitate their conversion to volatile forms for release to the atmosphere, redistributing the pollutant into a much larger land area after deposition where its concentration does not comprise risk. However, phytovolatilization should be avoided in sites near population centres and in places with unique meteorological conditions that promote the rapid deposition of volatile compounds. Hence, the consequences of releasing the metals to the atmosphere need to be considered carefully before adopting this method as a remediation tool.

3.4 Other Treatments

3.4.1 Biosorption

Biosorption is the passive uptake of heavy metals from aqueous solutions by biological materials (biosorbents) [260, 261]. Both living and dead microorganisms [262–264] can be involved as metal uptake may be a combination of a metabolism independent physical process, followed by bioaccumulation [264, 265]. Various types of biomass have been used for the study of biosorption, including algae [266, 267], bacteria [55, 267] and fungi [268–270]. The advantages of the use of marine algae as a biosorbent have been extensively reported, as their macroscopic structures offer a convenient basis for the production of biosorbent particles suitable for sorption process applications [267, 269].

Mechanisms of metal adsorption usually include physical adsorption, ion exchange, chelation, complexation and micro-precipitation [270–272]. Since biosorption involves a variety of metabolism-independent processes, taking place essentially in the cell wall, the mechanisms responsible for the metal binding differ according to the biomass type. The cell wall is the first effective compartment for adsorbing heavy metals because it contains many anionic functional groups capable of binding metals, such as peptidoglycan, teichoic acids, phospholipids and lipopolysaccharides [261, 272–274]. Several review articles discussed the performance of these biosorbents in metal biosorption [272, 274–279].

Use of biosorption is an attractive method for heavy metal removal from metal-laden effluents due to low cost and high efficiency of the process [264]. For example, when metal concentration in the feed does not exceed 100 mg^{-1} biosorption has been reported to be superior than existing wastewater treatment techniques [264, 280]. Schiewer and Wong [281] found biosorption techniques to be the only ones capable of providing drinking water at a very low cost. Despite increased understanding of biosorption and abundance of research in this field, an industrially relevant method for biosorption technology has not been fully realized yet [95, 263, 264, 282–284]. Most of the studies in the field deal with batch equilibrium studies relating adsorbate, adsorbent, and operating conditions. Removal of heavy metals in continuous mode was earlier reported as a preferred choice in some metal adsorption studies. For instance, Kapoor and Viraraghavan [285] used immobilized cells of *Aspergillus niger* in a continuous operation for the removal of metals from solutions containing Cd, Cu, Pb and Ni. Marques et al. [286] used a fixed-bed reactor for

Cd removal using immobilized cells of an industrial strain of *Saccharomyces cerevisiae*.

There are certain potential limitations of continuous fixed bed adsorption: low density of fungal biomass reported to be problematic in fixed-bed operation due to clogging and subsequent release of biomass in the treated wastewater. Immobilization of biomass also causes mass transfer limitations by hindering the access of the metals to the biosorbent sites compared to suspended biosorbents [264, 280, 287]. Moreover, since the regeneration capacity of immobilized cells is limited, biomass needs to be frequently replaced, which is a costly process. However, continuous operation is the only viable way of treating large volumes of wastewater in a reasonable time, and this is where most of the bench scale batch biosorption studies are limited in their scope.

3.4.2 Bioventing

Bioventing is one of the most common in situ treatment techniques. This technology is designed primarily to treat soil contamination by fuels, non-halogenated volatile organic compounds (VOCs) and semi-volatile organic compounds (SVOCs), pesticides and herbicides. The technology requires the presence of indigenous organisms capable of degrading the contaminants of interest, as well as nutrients necessary for their growth. Air and nutrients are supplied through wells to contaminated soils. It employs low air flow rates to provide only the amount of oxygen necessary for the biodegradation while minimizing volatilization and release of contaminants to the atmosphere. Alternatively, vacuum pumps are used to pull air through the contaminated soil. Ideally, the soil moisture should be kept at 40–85 % of the water-holding capacity. Care must be taken so that water infiltration does not saturate the porous medium, which would hinder soil permeability to gas flow [76]. Bioventing is a medium- to long-term remediation technology. Clean-up ranges from 6 months to 5 years [76]. It works for simple hydrocarbons and can be used where the contamination is deep under the surface [106].

3.4.3 Composting

Composting is an aerobic process that utilizes microbes to clean up or stabilize pollutants in agricultural and municipal solid wastes through the degradation of biodegradable compounds. It has been considered as one of the most cost-effective technologies for soil remediation [288–295]. Contaminated soils or sediments are mixed with uncontaminated amendments (bulking agents), such as manure, straw, wood chips or grasses, to create biologically active decomposing environments. Although compost piles are exposed to the atmosphere, the interior is often anaerobic due to the oxygen demand of the contaminants and amendments. Thus, air should be injected or drawn through the composting pile to supply O_2 and remove evaporated water. Compost piles are also subjected to intermittent mixing using specially manufactured equipment that is capable of turning the pile over onto itself. Temperatures can increase to 60–70 °C due to the exothermic nature of biodegradation, and mixing, aeration and water addition help dissipate excess heat that could be inhibitory to biodegradation [103, 106].

Organic matter from the composting process improves soil quality and fertility [296–300] as microbes had the ability to degrade various organic pollutants and impose excellent passivation effect on heavy metals [293, 295, 301, 302]. Remediation of contaminated soils by composting or compost addition mainly relies on two mechanisms [303]: (a) adsorption by organic matter and (b) degradation by microorganisms, in particular bacteria and fungi [295]. The effect of compost addition on remediation of metal-contaminated soils depends on the type of compost, pollution level and soil type [304]. Farrell and Jones [305] reported that all types of composts decreased the levels of heavy metals in soil solution, due to increased soil pH, and reduced solubility of heavy metals [73, 74]. Composting is appropriate to treat soils contaminated with munitions wastes that contain 2,4,6-trinitrotoluene (TNT) and other contaminants that are susceptible to co-metabolic reduction [306]. Composting of soils contaminated with polycyclic aromatic hydrocarbon (PAH) compounds is also common. In these systems, degradation is accomplished through non-specific oxidation by lignolytic fungi (e.g. *Phaenerochaeta chrysosporium, Phaenerochaetasordida* and *Trametes hirsuta*) and non-specific oxygenase enzymes that also participate in the decomposition of complex organic matter [76].

3.4.4 Landfarming

Landfarming is a simple technique in which contaminated soil is excavated and spread over a prepared bed and periodically tilled to mix and aerate the material until pollutants are degraded. The goal is to stimulate indigenous biodegradative microorganisms and facilitate their aerobic degradation of contaminants. In general, the practice is limited to the treatment of the superficial 10–35 cm soil layer. Since landfarming has the potential to reduce monitoring and maintenance costs, as well as clean-up liabilities, it has received much attention as a disposal alternative [103, 106, 307, 308]. Sometimes, in cases of very shallow contamination, the top layer may simply be tilled without requiring any excavation. Liners or other methods may be used to control leachate. This technology is designed primarily to treat soil contamination by fuels, non-halogenated VOCs, SVOCs, pesticides and herbicides. The process may be applied to halogenated organic compounds but is less effective. Although the technology is very simple and inexpensive, it does require large amount of space, and reduction in contaminant concentrations may sometimes be due to volatilization rather than biodegradation [103, 309, 310].

3.4.5 Biopiles

Biopiles are a hybrid of landfarming and composting. Essentially, engineered cells are constructed as aerated composted piles. Typically used for treatment of surface contamination with petroleum hydrocarbons, they are a refined version of landfarming that tend to control physical losses of the contaminants by leaching and volatilization. Biopiles provide a favourable environment for indigenous aerobic and anaerobic microorganisms [106, 311].

3.4.6 Biosparging

This technology is designed primarily to treat groundwater contaminated with fuels, non-halogenated VOCs including BTEX, SVOCs, pesticides and herbicides and involves the injection of air under pressure below the water table to increase groundwater oxygen concentrations at the same time that it strips volatile organic compounds (VOCs) into the unsaturated zone, which can subsequently be removed by a vapour-capture system. Compressed air is injected directly into the contaminated zone through a series of wells, enhancing the rate of biological degradation of contaminants by naturally occurring indigenous organisms capable of degrading the contaminants of interest [103, 312–314]. Biosparging increases mixing in the saturated zone and thereby increases the contact between soil and groundwater. The ease and low cost of installing small-diameter air injection points allows considerable flexibility in the design and construction of the systems [106, 315].

The effectiveness of biosparging depends primarily on two factors. First, the partitioning characteristics of the contaminants that can be present in the vapour, dissolved and sorbed phases. In particular, vapour/dissolved phase partitioning is a significant factor in determining the rate at which dissolved constituents can be transferred to the vapour phase. Second, the permeability of the soil determines the rate at which air can be injected into the saturated zone. This, in turn, determines the mass transfer rate of the contaminants from the dissolved to the vapour phase [76]. The process works best for contaminants that are both volatile and biodegradable, such as BTEX. If the contaminants are soluble and/or nonvolatile, they must be biodegradable [76].

3.4.7 Bioreactors

These technologies involve the treatment of excavated soil in the controlled environment of a bioreactor. Slurry reactors (bioreactors) or aqueous reactors are used for ex situ treatment of contaminated soil or water pumped up from a contaminated plume. Excavated soil is processed to separate stones and rubble and then mixed with water to a predetermined concentration depending upon the concentration of contaminants, rate of biodegradation and physical nature of the soils. Usually, slurries contain 10–40 % of solids. Electron acceptors and nutrients are added to the reactor and parameters such as pH and temperature are controlled to optimize biological processes. The mixture may also be inoculated with specific organisms if a suitable population is not present. Targeted contaminants include pesticides, explosives, petroleum hydrocarbons and other organic compounds [103, 106]. In general, the rate and extent of biodegradation are greater in a bioreactor system than in situ because the contained environment is more manageable and hence more controllable and predictable [106]. Therefore, bioreactors are favoured over in situ biological techniques for heterogeneous soils, low permeability soils, areas where underlying groundwater would be difficult to capture, or when faster treatments are required [103].

3.4.8 Controlled Solid Phase Treatment

This process includes preparation of treatment beds, biotreatment cells, and soil piles or composting. Moisture, heat, nutrients, oxygen and pH can be controlled to enhance biodegradation. These technologies differ from landfarming as the treatment processes are often enclosed to control gas emissions. Typically, excavated material is mixed with soil amendments and placed on a treatment area that includes leachate collection systems and some aeration [103]. Like landfarming, these technologies require a lot of space and excavation of contaminated material. One advantage, however, of contained ex situ methods is that toxic by-products or metabolites formed during the biodegradation process (e.g. vinyl chloride from TCE) are contained [103].

3.5 Conclusions and Future Perspectives

The contamination of soils and waters with toxic substances, in particular metals, has become one of the most important environmental problems throughout the world. Metal toxicity affects crop yields, microbial biomass and soil fertility. Soils polluted with heavy metals pose a serious health hazard to humans as well as plants and animals, and often require soil remediation. Physical and chemical methods for clean-up and restoration of heavy metal-contaminated soils present serious limitations like high cost, irreversible changes in soil properties, destruction of native soil microflora and creation of secondary pollution problems. Advances in biotechnological tools and techniques and their application in biological systems to enhance bioremediation processes helped overcome limitations associated with traditional bioremediation processes.

Bioremediation is one of the green approaches to clean the planet. It is promising, efficient, eco-friendly and cost-effective. Compared to chemical-physical methods, bioremediation shows many advantages. Studies on this field are increasing and the mechanisms used are becoming clearer. Using transgenic technology is a tendency; however, genetic pollution must be taken into consideration. Microbes, in many cases, are more efficient in accumulating and absorbing heavy metals because of their huge specific surface area. Furthermore, genetic engineering of microbes is easier and more mature than in plant cells. Therefore, using transgenic technology to create an optimum plant + soil + microbes combination would be a promising way in future remediation approaches.

Research aimed at understanding the interactive roles between plants roots and soil microorganisms will help scientists to utilize their integrative capacity for soil decontamination. Genetic evaluation of hyperaccumulators growing in metal-contaminated soils and associated microorganisms may reveal key control genes that trigger the cascade of responses that provide tolerance and accumulation. Such genes could be the ultimate candidates for overexpression, producing the complete hyperaccumulator profile in high-biomass crops. The discovery of metal-related genes with the aid of genome sequencing will open up new avenues for the creation of transgenic plants having desired properties that would help in establishing phytoremediation technologies for environmental clean-up. In view of the current trends of integration of scientific knowledge worldwide, it is hoped that many challenging questions about commercial application of bioremediation will be also answered in the future.

References

1. Pinto AP, Varennes A, Fonseca R, Martins Teixeira D (2015) Phytoremediation of soils contaminated with heavy metals: techniques and strategies. In: Ansari AA, Gill SS, Gill R, Lanza GR, Newman L (eds) Management of environmental contaminants, vol 1. Springer, Wien, pp 133–155
2. Verkleij JAC, Prast JE (1990) Cadmium tolerance and co-tolerance in Silene vulgaris. New Phytol 111:637–645
3. Ashraf M, Ozturk M, Ahmad MSA (2010) Toxins and their phytoremediation. In: Ashraf M, Ozturk M, Ahmad MSA (eds) Plant adaptation and phytoremediation. Springer, New York, pp 1–32
4. Adriano DC (2001) Trace elements in terrestrial environments: biogeochemistry, bioavailability and risks of metals. Springer, Berlin
5. Park JH, Lamb D, Paneerselvam P, Choppala G, Bolan N, Chung JW (2011) Role of organic amendments on enhanced bioremediation of heavy metal(loid) contaminated soils. J Hazard Mater 185:549–574
6. Pendias AK (2001) Trace elements in soils and plants. CRC, New York
7. Modaihsh A, Al-Swailem M, Mahjoub M (2004) Heavy metal contents of commercial inorganic fertilizer used in the Kingdom of Saudi Arabia. Agric Mar Sci 9:21–25. http://hdl.handle.net/123456789/2334
8. Yang XE, Feng Y, He Z, Stoffella PJ (2005) Molecular mechanisms of heavy metal hyperaccumulation and phytoremediation. J Trace Elem Med Biol 18:339–353

9. Chehregani A, Malayeri BE (2007) Removal of heavy metals by native accumulator plants. Int J Agric Biol 9:462–465
10. Fulekar M, Singh A, Bhaduri AM (2009) Genetic engineering strategies for enhancing phytoremediation of heavy metals. Afr J Biotechnol 8:529–535
11. Sabiha-Javied, Mehmood T, Tufai M, Irfan N (2009) Heavy metal pollution from phosphate rock used for the production of fertilizer in Pakistan. Microchem J 91:94–9
12. Wuana RA, Okieimen FE (2011) Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation. ISRN Ecol :1–20
13. Ali H, Khan E, Sajad MA (2013) Phytoremediation of heavy metals—concepts and applications. Chemosphere 91:869–881
14. Sheppard SC, Gaudet C, Sheppard MI, Cureton PM, Wong MP (1992) The development of assessment and remediation guidelines for contaminated soils, a review of the science. Can J Soil Sci 72:359–394
15. Dai J, Becquer T, Rouiller JH, Reversat G, Bernhard-Reversat F, Lavelle P (2004) Influence of heavy metals on C and N mineralisation and microbial biomass in Zn-, Pb-, Cu-, and Cd-contaminated soils. Appl Soil Ecol 25:99–109
16. Alvarenga P, Gonçalves AP, Fernandes RM, de Varennes A, Vallini G, Duarte E, Cunha-Queda AC (2009) Organic residues as immobilizing agents in aided phytostabilization: (I) effects on soil chemical characteristics. Chemosphere 74:1292–1300
17. Alvarenga P, Palma P, Varennes A, Cunha-Queda AC (2012) A contribution towards the risk assessment of soils from the Sao Domingos Mine (Portugal): chemical, microbial and ecotoxicological indicators. Environ Pollut 161:50–56
18. Lorestani B, Cheraghi M, Yousefi N (2012) The potential of phytoremediation using hyperaccumulator plants: a case study at a lead-zinc mine site. Int J Phytoremediation 14:786–795
19. Khan MS, Zaidi A, Wani PA, Oves M (2009) Role of plant growth promoting rhizobacteria in the remediation of metal contaminated soils. Environ Chem Lett 7:1–19
20. Zaidi A, Wani PA, Khan MS (2012) Bioremediation: a natural method for the management of polluted environment (Chapter 6). In: Zaidi A, Wani PA, Khan MS (eds) Toxicity of heavy metals to legumes and bioremediation. Springer, Wien, pp 101–114
21. El-Syed OH, Refaat HM, Swellam MA, Amer MM, Attwa AI, El-Awady ME (2011) Bioremediation of zinc by *Streptomyces aureofacienes*. J Appl Sci 11:873–877
22. Huq SMI, Abdullah MB, Joardar JC (2007) Bioremediation of arsenic toxicity by algae in rice culture. Land Contam Reclamat 15:327–333
23. Vamerali T, Bandiera M, Mosca G (2010) Field crops for phytoremediation of metal-contaminated land. A review. Environ Chem Lett 8:1–17
24. Bhargava A, Carmona FF, Bhargava M, Srivastava S (2012) Approaches for enhanced phytoextraction of heavy metals. J Environ Manage 105:103–120
25. Chaney RL, Angle JS, Broadhurst CL, Peters CA, Tappero RV, Sparks DL (2007) Improved understanding of hyperaccumulation yields commercial phytoextraction and phytomining technologies. J Environ Qual 36:1429–1443
26. Wang WS, Shan XQ, Wen B, Zhang SZ (2003) Relationship between the extractable metals from soils and metals taken up by maize roots and shoots. Chemosphere 53:523–530
27. Shao HB, Chu LY, Lu ZH, Kang CM (2008) Primary antioxidant free radical scavenging and redox signaling pathways in higher plant cells. Int J Biol Sci 4:8–14
28. Sharma SS, Dietz KJ (2009) The relationship between metal toxicity and cellular redox imbalance. Trends Plant Sci 14:43–50
29. Ahmad P, Umar S, Sharma S (2010) Mechanism of free radical scavenging and role of phytohormones in plants under abiotic stresses. In: Ashraf M, Ozturk M, Ahmad MSA (eds) Plant adaptation and phytoremediation. Springer, New York
30. Guerra F, Gainza F, Pérez R, Zamudio F (2011) Phytoremediation of heavy metals using poplars (Populus Spp): a glimpse of the plant responses to copper, cadmium and zinc stress. In: Golubev IA (ed) Handbook of phytoremediation. Nova Science, New York, pp 387–414
31. Kadukova J, Kavuličova J (2011) Phytoremediation of heavy metal contaminated soils—plant stress assessment. In: Golubev IA (ed) Handbook of phytoremediation. Nova Science, New York, pp 185–222

32. Pagliano C, Raviolo M, DallaVecchia F, Gabbrielli R, Gonnelli C, Rascio N et al (2006) Evidence for PSII-donor-side damage and photoinhibition induced by cadmium treatment on rice (Oryza sativa L.). J Photochem Photobiol B: Biol 84:70–78
33. Rascio N, Navari-Izzo F (2011) Heavy metal hyperaccumulating plants: how and why do they do it? And what makes them so interesting? Plant Sci 180:169–181
34. Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci 7:405–410
35. Neil S, Desikan R, Hancock J (2002) Hydrogen peroxide signalling. Curr Opin Plant Biol 5:388–395
36. Pitzschke A, Hirt H (2006) Mitogen-activated protein kinases and reactive oxygen species signaling in plants. Plant Physiol 141:351–356
37. Navari-Izzo F, Quartacci MF, Pinzino C, Dalla Vecchia F, Sgherri C (1998) Thylakoid-bound and stromal antioxidative enzymes in wheat treated with excess of copper. Physiol Plant 104:630–638
38. Navari-Izzo F, Pinzino C, Quartacci MF, Sgherri C (1999) Superoxide and hydroxyl radical generation, and superoxide dismutase in PSII membrane fragments from wheat. Free Radic Res 31:S3–S9
39. Watanabe T, Osaki M (2002) Mechanism of adaptation to high aluminium condition in native plant species growing in acid soils: a review. Commun Soil Sci Plant Anal 33:1247–1260
40. Dalla Vecchia F, La Rocca N, Moro I, De Faveri S, Andreoli C, Rascio N (2005) Morphogenetic, ultrastructural and physiological damages suffered by submerged leaves of *Elodea canadensis* exposed to cadmium. Plant Sci 168:329–338
41. Rascio N, Dalla Vecchia F, La Rocca N, Barbato R, Pagliano C, Raviolo M et al (2008) Metal accumulation and damage in rice (c.v. Vialone nano) seedlings exposed to cadmium. Environ Exp Bot 62:267–278
42. Hall JL (2002) Cellular mechanisms for heavy metal detoxification and tolerance. J Exp Bot 53:1–11
43. Sgherri C, Cosi E, Navari-Izzo F (2003) Phenols and antioxidative status of Raphanus sativus grown in copper excess. Physiol Plant 118:21–28
44. Bricker TJ, Pichtel J, Brown HJ, Simmons MJ (2001) Phytoextraction of Pb and Cd from a superfund soil: effects of amendments and croppings. J Environ Sci Health A 36:1597–1610
45. Yadav SK (2010) Heavy metals toxicity in plants: an overview on the role of glutathione and phytochelatins in heavy metal stress tolerance of plants. S Afr J Bot 76:167–179
46. Arisi ACM, Mocquot B, Lagriffoul A, Mench M, Foyer CH, Jouanin L (2000) Responses to cadmium in leaves of transformed poplars overexpressing γ-glutamylcysteine synthetase. Physiol Plant 109:143–149
47. Pulford ID, Watson C (2003) Phytoremediation of heavy metal-contaminated land by trees—a review. Environ Int 29:529–540
48. Freeman JL, Persans MW, Nieman K, Albrecht C, Peer W, Pickering IJ et al (2004) Increased glutathione biosynthesis plays a role in nickel tolerance in Thlaspi nickel hyperaccumulators. Plant Cell 16:2176–2191
49. Prasad MNV, Pratas J, Freitas H (2006) Trace elements in plants and soils of abandoned mines in portugal: significance for phytomanagement and biogeochemical prospecting. In: Prasad MNV, Sajwan KS, Naidu R (eds) Trace elements in the environment: biogeochemistry, biotechnology and bioremediation. CRC, Boca Raton, pp 507–522
50. Gekeler W, Grill E, Winnacker EL, Zenk MH (1998) Algae sequester heavy metals via synthesis of phytochelatin complexes. Arch Microbiol 150:197–202
51. Grill E, Thumann J, Winnacker EL, Zenk MH (1988) Induction of heavy metal binding phytochelatins by inoculation of cell cultures in standard media. Plant Cell Rep 7:375–378
52. Hirata K, Tsuji N, Miyamoto K (2005) Biosynthetic regulation of phytochelatins, heavy metal-binding peptides. J Biosci Bioeng 100:593–599
53. Salt DE, Rauser WE (1995) MgATP-dependent transport of phytochelatins across the tonoplast of oat roots. Plant Physiol 107:1293–1301

54. Cobbett C, Goldsbrough P (2002) Phytochelatins and metallothioneins: roles in heavy metal detoxification and homeostasis. Annu Rev Plant Biol 53:159–182
55. Veglio F, Beolchini F, Gasbarro A (1997) Biosorption of toxic metals: an equilibrium study using free cells of Arthrobacter sp. Process Biochem 32:99–105
56. Gadd GM (2000) Bioremedial potential of microbial mechanisms of metal mobilization and immobilization. Curr Opin Biotechnol 11:271–279
57. Lim PE, Mak KY, Mohamed N, Noor AM (2003) Removal and speciation of heavy metals along the treatment path of wastewater in subsurface-flow constructed wetlands. Water Sci Technol 48:307–313
58. Malik A (2004) Metal bioremediation through growing cells. Environ Int 30:261–278
59. Lin CC, Lin HL (2005) Remediation of soil contaminated with the heavy metal (Cd^{2+}). J Hazard Mater 122:7–15
60. Dixit R, Wasiullah, Malaviya D, Pandiyan K, Singh UB, Sahu A et al (2015) Bioremediation of heavy metals from soil and aquatic environment: an overview of principles and criteria of fundamental processes. Sustainability 7:2189–2212
61. Singh NK, Singh RP (2016) Potential of plants and microbes for the removal of metals: eco-friendly approach for remediation of soil and water (Chapter 19). In: Ahmad P (ed) Plant metal interaction emerging remediation techniques. Elsevier, Amsterdam, pp 469–482
62. Scott JA, Karanjkar AM (1992) Repeated cadmium biosorption by regenerated Enterobacter aerogenes biofilm attached to activated carbon. Biotechnol Lett 14:737–740
63. Ajmal M, Rafaqat AK, Bilquees AS (1996) Studies on removal and recovery of Cr (VI) from electroplating wastes. Water Res 30:1478–1482
64. Dilek FB, Gokcay CF, Yetis U (1998) Combined effects of Ni(II) and Cr(VI) on activated sludge. Water Res 32:303–312
65. Volesky B, Holan ZR (1995) Biosorption of heavy metals. Biotechnol Prog 11:235–250
66. Huang C, Huang CP (1996) Application of *Aspergillus oryzae* and *Rhizopus oryzae* for Cu (II) removal. Water Res 30:1985–1990
67. Tunali S, Akar T, Oezcan AS, Kiran I, Oezcan A (2006) Equilibrium and kinetics of biosorption of lead(II) from aqueous solutions by *Cephalosporium aphidicola*. Sep Purif Technol 47:105–112
68. Akar T, Tunali S, Cabuk A (2007) Study on the characterization of lead (II) biosorption by fungus *Aspergillus parasiticus*. Appl Biochem Biotechnol 136:389–406
69. Kelly DJA, Budd K, Lefebvre DD (2006) The biotransformation of mercury in pH-stat cultures of microfungi. Can J Bot 84:254–260
70. Sun LN, Zhang YF, He LY, Chen ZJ, Wang QY, Qian M et al (2010) Genetic diversity and characterization of heavy metal-resistant-endophytic bacteria from two copper-tolerant plant species on copper mine wasteland. Bioresour Technol 101:501–509
71. Garbisu C, Alkorta I (2001) Phytoextraction: a cost-effective plant-based technology for the removal of metals from the environment. Bioresour Technol 77:229–236
72. Silver S (1996) Bacterial heavy metal resistance: new surprises. Annu Rev Microbiol 50:753–789
73. Pérez de Mora A, Burgos P, Madejón E, Cabrera F, Jaeckel P, Schloter M (2006) Microbial community structure and function in soil contaminated by heavy metals: effects on plant growth and different amendments. Soil Biol Biochem 38:327–341
74. Pérez-de-Mora E, Burgos MP, Cabrera F (2006) Trace element availability and plant growth in a mine-spill contaminated soil under assisted natural remediation I. Soils. Sci Total Environ 363:28–37
75. Vargas-García MC, López MJ, Suárez-Estrella F, Moreno J (2012) Compost as a source of microbial isolates for the bioremediation of heavy metals: in vitro selection. Sci Total Environ 431:62–67
76. Alvarez PJJ, Illman WA (2006) Bioremediation technologies. In: Schnoor JL, Zehnder, (eds) Bioremediation and natural attenuation. Process fundamentals and mathematical models. An environmental science and technology. A Wiley-Interscience series of texts and monographs. Wiley, Hoboken, pp 351–455

77. Lloyd JR (2002) Bioremediation of metals: the application of microorganisms that make and break minerals. Microbiol Today 29:67–69
78. Bezza FA, Chirwa EMN (2015) Production and applications of lipopeptide biosurfactant for bioremediation and oil recovery by *Bacillus subtilis* CN2. Biochem Eng J 101:168–178
79. Haferburg G, Kothe E (2007) Microbes and metals: interactions in the environment. J Basic Microbiol 47:453–467
80. Gadd GM (2001) Accumulation and transformation of metals by microorganisms. In: Rehm HJ (ed) Biotechnology, a multi-volume comprehensive treatise, references, vol 10. Wiley-VCH Verlag, Weinheim, pp 225–264
81. Colin VL, Villegas LB, Abate CM (2012) Indigenous microorganisms as potential bioremediators for environments contaminated with heavy metals. Int Biodeterior Biodegrad 69:28–37
82. Tabak HH, Lens P, Van Hullebusch ED, Dejonghe W (2005) Developments in bioremediation of soils and sediments polluted with metals and radionuclides-1. Microbial processes and mechanisms affecting bioremediation of metal contamination and influencing metal toxicity and transport. Rev Environ Sci Biotechnol 4:115–156
83. Viti C, Giovannetti L (2001) The impact of chromium contamination on soil heterotrophic and photosynthetic microorganisms. Ann Microbiol 51:201–213
84. Rhodes CJ (2013) Applications of bioremediation and phytoremediation. Sci Prog 96:417–427
85. Fomina M, Charnock J, Bowen AD, Gadd GM (2007) X-ray absorption spectroscopy (XAS) of toxic metal mineral transformations by fungi. Environ Microbiol 9:308–321
86. Kumar R, Bhatia D, Singh R, Bishnoi NR (2012) Metal tolerance and sequestration of Ni(II), for treatment and disposal of heavy metals polluted sediments. J Environ Sci Zn(II) and Cr(VI) ions from simulated and electroplating wastewater in batch pro- 2009;21:877–83. cess: kinetics and equilibrium study. Int Biodeterior Biodegrad 66:82–90
87. Taştan BE, Ertuğrul S, Dönmez G (2010) Effective bioremoval of reactive dye and heavy metals by *Aspergillus versicolor*. Bioresour Technol 101:870–876
88. Skorik YA, Pestov AV, Yatluk YG (2010) Evaluation of various chitin-glucan derivatives from *Aspergillus niger* as transition metal adsorbents. Bioresour Technol 101:1769–1775
89. Guimarães-Soares L, Felicia H, Bebianno MJ, Cassio F (2006) Metal-binding proteins and peptides in the aquatic fungi *Fontanospora fusiramosa* and *Flagellopora curta* exposed to severe metal stress. Sci Total Environ 372:148–156
90. Arwidsson Z, Johansson E, Von Kronhelm T, Allard B, Van Hees P (2010) Remediation of metal contaminated soil by organic metabolites from fungi I—production of organic acids. Water Air Soil Pollut 205:215–226
91. Sun F, Shao Z (2007) Biosorption and bioaccumulation of lead by *Penicillium sp. Psf-2* isolated from the deep sea sediment of the Pacific Ocean. Extremophiles 11:853–858
92. Leitão AL (2009) Potential of Penicillium species in the bioremediation field. Int J Environ Res Public Health 6:1393–1417
93. Helmisaari HS, Salemaa M, Derome J, Kiikkilä O, Uhligh C, Nieminem T (2007) Remediation of heavy metal-contaminated forest soil using recycled organic matter and native woody plants. J Environ Qual 36:1145–1153
94. Farrell M, Perkins WT, Hobbs PJ, Griffith GW, Jones D (2010) Migration of heavy metals in soil as influenced by compost amendments. Environ Pollut 158:55–64
95. Wang J, Chen C (2009) Biosorbents for heavy metals removal and their future. Biotechnol Adv 27:196–226
96. Neagoe A, Merten D, Iordache V, Büchel G (2009) The effect of bioremediation methods involving different degrees of soil disturbance on the export of metals by leaching and by plant uptake. Chem Erde-Geochem 69(S2):57–73
97. Shi W, Shao H, Li H, Shao M, Su S (2009) Progress in the remediation of hazardous heavy metal-polluted soils by natural zeolite. J Hazard Mater 170:1–6
98. Wei SH, Zhou QX, Wang X, Cao W, Ren LP, Song YF (2004) Potential of weed species applied to remediation of soils contaminated with heavy metals. J Environ Sci 16:868–873

99. Wu G, Kang H, Zhang X, Shao H, Chu L, Ruan C (2010) A critical review on the bioremoval of hazardous heavy metals from contaminated soils: issues, progress, eco-environmental concerns and opportunities. J Hazard Mater 174:1–8
100. USEPA (1999) Use of monitored natural attenuation at superfund, RCRA corrective action, and underground storage tank sites. Oswer Directive. 9200.4-17P. US Environmental Protection Agency, Office of Research and Development, Cincinnati
101. Boparai HK, Shea PJ, Comfort SD, Machacek TA (2008) Sequencing zerovalent iron treatment with carbon amendments to remediate agrichemical-contaminated soil. Water Air Soil Pollut 193:189–196
102. Khan FI, Husain T, Hejazi R (2004) An overview and analysis of site remediation technologies. J Environ Manag 71:95–122
103. Juwarkar AA, Misra RR, Sharma JK (2014) Recent trends in bioremediation. In: Parmar N, Singh A (eds) Geomicrobiology and biogeochemistry. Springer, Wien, pp 81–100
104. Declercq I, Cappuyns V, Duclos Y (2012) Monitored natural attenuation (MNA) of contaminated soils: state of the art in Europe—a critical evaluation. Sci Total Environ 426:393–405
105. Gentry TJ, Rensing C, Pepper IL (2004) New approaches for bioaugmentation as a remediation technology. Crit Rev Environ Sci Technol 34(5):447–494
106. Vidali M (2001) Bioremediation. An overview. Pure Appl Chem 73:1163–1172
107. Salanitro JP, Johnson P, Spinnler GE, Maner PM, Wisniewski HL, Bruce C (2000) Field scale demonstration of enhanced MTBE bioremediation through aquifer bioaugmentation and oxygenation. Environ Sci Technol 34(19):4152–4162
108. Dybas MJ, Barcelona M, Bezborodnikov S, Davies S, Forney L, Heuer H et al (1998) Pilot-scale evaluation of bioaugmentation for in-situ remediation of a carbon tetrachloride-contaminated aquifer. Environ Sci Technol 32:3598–3611
109. Duba AG, Jackson KJ, Jovanovich MC, Knapp RB, Taylor RT (1996) TCE remediation using in-situ, resting-state bioaugmentation. Environ Sci Technol 30:1982–1989
110. Ellis DE, Lutz EJ, Odom JM, Buchanan J, Ronald J, Bartlett CL et al (2000) Bioaugmentation for accelerated in situ anaerobic bioremediation. Environ Sci Technol 34:2254–2260
111. Major DW, McMaster ML, Cox EE, Lee BJ, Gentry EE, Hendrickson E et al (2002) Successful field demonstration of bioaugmentation to degrade PCE and TCE to ethene. In: Sixth international in situ and on-site remediation symposium; 2001 Jun 4–7; San Diego, California. Battelle Press, Columbus
112. Wang T, Sun H, Jiang C, Mao H, Zhang Y (2014) Immobilization of Cd in soil and changes of soil microbial community by bioaugmentation of UV-mutated *Bacillus subtilis 38* assisted by biostimulation. Eur J Soil Biol 65:62–69
113. Wang T, Sun H, Mao H, Zhang Y, Wang C, Zhang Z et al (2014) The immobilization of heavy metals in soil by bioaugmentation of a UV-mutant *Bacillus subtilis 38* assisted by NovoGro biostimulation and changes of soil microbial community. J Hazard Mater 278:483–490
114. Wang Y, Peng B, Yang Z, Tang C, Chen Y, Liao Q et al (2014) Treatment of Cr(VI) contaminated water with *Pannonibacter phragmitetus* BB. Environ Earth Sci 71(10):4333–4339
115. El-Bestawy E, El-Kheir EA, Abd El-Fatah HI, Hassouna SM (1998) Enhancement of bacterial efficiency for metal removal using mutation techniques. World J Microbiol Biotechnol 14:853–856
116. Valls M, De Lorenzo V (2002) Exploiting the genetic and biochemical capacities of bacteria for the remediation of heavy metal pollution. FEMS Microbiol Rev 26:327–338
117. Krishnaswamy R, Wilson DB (2000) Construction and characterization of an *Escherichia coli* strain genetically engineered for Ni(II) bioaccumulation. Appl Environ Microbiol 66:5383–5386
118. Zhao XW, Zhou MH, Li QB, Lu YH, He N, Sun DH, Deng X (2005) Simultaneous mercury bioaccumulation and cell propagation by genetically engineered *Escherichia coli*. Process Biochem 40:1611–1616
119. Yang YN, Ren N, Xue JM, Yang J, Rong BL (2007) Mutation effect of MeV protons on bioflocculant bacteria *Bacillus cereus*. Nucl Instrum Methods Phys Res, Sect B 262:220–224

120. Brierley JA (2008) A perspective on developments in biohydrometallurgy. Hydrometallurgy 94:2–7
121. Qiu G, Liu X, Zhou H (2008) Microbial community structure and function in sulfide ore bioleaching systems. Trans Nonferrous Metals Soc China 18:1295–1301
122. Jiang C, Sun H, Sun T, Zhang Q, Zhang Y (2009) Immobilization of cadmium in soils by UV-mutated *Bacillus subtilis* 38 bioaugmentation and NovoGro amendment. J Hazard Mater 167:1170–1177
123. Yin H, He B, Lu X, Peng H, Ye J, Yang F (2008) Improvement of chromium biosorption by UV–HNO cooperative mutagenesis in *Candida utilis*. Water Res 42:3981–3989
124. Reddy KR, Chinthamreddy S, Saichek RE, Cutright TJ (2003) Nutrient amendment for the bioremediation of a chromium-contaminated soil by electrokinetics. Energy Sources 25:931–943
125. Roane TM, Josephson KL, Pepper IL (2001) Dual-bioaugmentation strategy to ination on soil microbes and enhanced the proliferation of both enhance remediation of cocontaminated soil. Appl Environ Microbiol 67:3208–3215
126. Pal S (2010) Use of bio-resources for remediation of soil pollution. Nat Resour 01:110–125
127. Juwarkar AA, Jambhulkar HP (2008) Restoration of fly ash dump through biological interventions. Environ Monit Assess 139:355–365
128. Kadian N, Gupta A, Satya S, Mehta RK, Malik A (2008) Biodegradation of herbicide (atrazine) in contaminated soil using various bioprocessed materials. Bioresour Technol 99:4642–4647
129. Pimmata P, Reungsang A, Plangklang P (2013) Comparative bioremediation of carbofuran contaminated soil by natural attenuation, bioaugmentation and biostimulation. Int Biodeterior Biodegrad 85:196–204
130. Margesin R, Schinner F (2001) Bioremediation (natural attenuation and biostimulation) of diesel-oil-contaminated soil in an Alpine glacier skiing area. Appl Environ Microbiol 67:3127–3133
131. Anderson RT, Vrionis HA, Ortiz-Bernad I, Resch CT, Long PE, Dayvault R et al (2003) Stimulating the in situ activity of Geobacter species to remove uranium from the groundwater of a uranium-contaminated aquifer. Appl Environ Microbiol 69:5884–5891
132. Groudev S, Spasova I, Nicolova M, Georgiev P (2010) In situ bioremediation of contaminated soils in uranium deposits. Hydrometallurgy 104:518–523
133. Arjoon A, Olaniran AO, Pillay B (2013) Enhanced 1,2-dichloroethane degradation in heavy metal co-contaminated wastewater undergoing biostimulation and bioaugmentation. Chemosphere 93:1826–1834
134. Roy AS, Baruah R, Borah M, Singh AK, Boruah HPD, Saikia N et al (2014) Bioremediation potential of native hydrocarbon degrading bacterial strains in crude oil contaminated soil under microcosm study. Int Biodeterior Biodegrad 94:79–89
135. Andreolli M, Lampis S, Brignoli P, Vallini G (2015) Bioaugmentation and biostimulation as strategies for the bioremediation of a burned woodland soil contaminated by toxic hydrocarbons: a comparative study. J Environ Manage 153:121–131
136. Kavamura VN, Esposito E (2010) Biotechnological strategies applied to the decontamination of soils polluted with heavy metals. Biotechnol Adv 28:61–69
137. White C, Shaman AK, Gadd GM (1998) An integrated microbial process for the bioremediation of soil contaminated with toxic metals. Nat Biotechnol 16:572–575
138. Kumar RN, Nagendran R (2009) Fractionation behavior of heavy metals in soil during bioleaching with *Acidithiobacillus thiooxidans*. J Hazard Mater 169:1119–1126
139. McGrath SP, Zhao FJ, Lombi E (2002) Phytoremediation of metals, metalloids, and radionuclides. Adv Agron 75:1–56
140. Wu L, Luo Y, Song J (2007) Manipulating soil metal availability using EDTA and low-molecular-weight organic acids. In: Willey N (ed) Methods in biotechnology, vol 23, Phytoremediation: methods and reviews. Humana Press Inc., Totowa, pp 290–303
141. Jadia CD, Fulekar MH (2009) Phytoremediation of heavy metals: recent techniques. Afr J Biotechnol 8:921–928

142. Wu L, Li Z, Han C, Liu L, Teng Y, Sun X et al (2012) Phytoremediation of soil contaminated with cadmium, copper and polychlorinated biphenils. Int J Phytoremediation 14:570–584
143. Ouyang Y (2002) Phytoremediation: modelling plant uptake and contaminant transport in the soil-plant-atmosphere continuum. J Hydrology 266:66–82
144. Schwitzguébel J-P (2002) Hype or hope: the potential of phytoremediation as an emerging green technology. Fed Facil Environ J 13:109–125
145. Abhilash PC, Pandey VC, Srivastava P, Rakesh PS, Chandran S, Singh N et al (2009) Phytofiltration of cadmium from water by *Limnocharis flava* (L.) Buchenau grown in free-floating culture system. J Hazard Mater 170:791–797
146. Newman JM, Clausen JC, Neafsey JA (2000) Seasonal performance of a wetland constructed to process dairy milkhouse wastewater in Connecticut. Ecol Eng 14:181–198
147. Dunne EJ, Culleto N, O'Donovan G, Harrington R, Olsen AE (2005) An integrated constructed wetland to treat contaminants and nutrients from dairy farmyard dirty water. Ecol Eng 24:221–234
148. Chavan PV, Dennett KE, Marchand EA, Gustin MS (2007) Evaluation of small-scale constructed wetland for water quality and Hg transformation. J Hazard Mater 149:543–547
149. Zurita F, De Anda J, Belmont MA (2009) Treatment of domestic wastewater and production of commercial flowers in vertical and horizontal subsurface-flow constructed wetlands. Ecol Eng 35:861–869
150. Liu Y-J, Mu Y-J, Zhu Y-G, Ding H, Arens NC (2007) Which ornamental plant species effectively remove benzene from indoor air? Atmos Environ 41:650–654
151. Maegher R (2000) Phytoremediation of toxic elemental and organic pollutants. Curr Opin Plant Biol 3:153–162
152. Willscher S, Mirgorodsky D, Jablonski L, Ollivier D, Merten D, Büchel G et al (2013) Field scale phytoremediation experiments on a heavy metal and uranium contaminated site, and further utilization of the plant residues. Hydrometallurgy 131–132:46–53
153. Clemens S (2001) Developing tools for phytoremediation: towards a molecular understanding of plant metal tolerance and accumulation. Int J Occup Med Environ Health 14:235–239
154. Kawahigashi H (2009) Transgenic plants for phytoremediation of herbicides. Curr Opin Biotechnol 20:225–230
155. Willscher S, Wittig J, Bergmann H, Büchel G, Merten D, Werner P (2009) Phytoremediation as an alternative way for the treatment of large, low heavy metal contaminated sites: application at a former uranium mining area. Adv Mater Res 71–73:705–708
156. Saier MH, Trevors JT (2010) Phytoremediation. Water Air Soil Pollut 205:61–63
157. Kalve S, Sarangi BK, Pandey RA, Chakrabarti T (2011) Arsenic and chromium hyperaccumulation by an ecotype of *Pteris vittata*—prospective for phytoextraction from contaminated water and soil. Curr Sci 100:888–894
158. Sarma H (2011) Metal hyperaccumulation in plants: a review focusing on phytoremediation technology. J Environ Sci Technol 4:118–138
159. Singh A, Prasad SM (2011) Reduction of heavy metal load in food chain: technology assessment. Rev Environ Sci Biotechnol 10:199–214
160. Vithanage M, Dabrowska BB, Mukherjee B, Sandhi A, Bhattacharya P (2012) Arsenic uptake by plants and possible phytoremediation applications: a brief overview. Environ Chem Lett 10:217–224
161. Navari-Izzo F, Quartacci MF (2001) Phytoremediation of metals: tolerance mechanisms against oxidative stress. Minerva Biotechnol 13:23–83
162. McGrath SP, Zhao FJ (2003) Phytoextraction of metals and metalloids from contaminated soils. Curr Opin Biotechnol 14:277–282
163. Pilon-Smits E (2005) Phytoremediation. Annu Rev Plant Biol 56:15–39
164. Kidd P, Barceló J, Bernal MP, Navari-Izzo F, Poschenrieder C, Shileve S et al (2009) Trace element behaviour at the root–soil interface: implications in phytoremediation. Environ Exp Bot 67:243–259

165. Schwitzguébel J-P, Kumpiene J, Comino E, Vanek T (2009) From green to clean: a promising approach towards environmental remediation and human health for the 21st century. Agrochimica 4(LIII-N):209–237
166. Ullah A, Heng S, Farooq M, Munis H, Fahad S, Yang X (2015) Phytoremediation of heavy metals assisted by plant growth promoting (PGP) bacteria: a review. Environ Exp Bot 117:28–40
167. Marques APGC, Rangel AOSS, Castro PML (2009) Remediation of heavy metal contaminated soils: phytoremediation as a potentially promising clean-up technology. Crit Rev Environ Sci Technol 39:622–654
168. Jing Y, He Z, Yang X (2007) Role of soil rhizobacteria in phytoremediation of heavy metal contaminated soils. J Zhejiang Univ Sci 8:192–207
169. Glick BR (2010) Using soil bacteria to facilitate phytoremediation. Biotechnol Adv 28:367–374
170. Gamalero E, Trotta A, Massa N, Copetta A, Martinotti MG, Berta G (2004) Impact of two fluorescent pseudomonads and an arbuscular mycorrhizal fungus on tomato plant growth root architecture, and P acquisition. Mycorrhiza 14:185–192
171. Glick BR (2004) Bacterial ACC, deaminase and the alleviation of plant stress. Adv Appl Microbiol 56:291–312
172. Hadi F, Bano A (2010) Effect of diazotrophs (Rhizobium and Azobactor) on growth of maize (*Zea mays* L.) and accumulation of lead (Pb) in different plant parts. Pak J Bot 42:4363–4370
173. Tangahu BV, Abdullah SRS, Basri H, Idris M, Anuar N, Mukhlisin M (2011) Review on heavy metals (As, Pb, and Hg) uptake by plants through phytoremediation. Int J Chem Eng 2011:1–31
174. Glick BR (2012) Plant growth-promoting bacteria: mechanisms and applications. Scientifica 2012:15 pages
175. Dardanelli MS, Manyani H, González-Barroso S, Rodríguez-Carvajal MA, Gil-Serrano AM, Espuny MR et al (2010) Effect of the presence of the plant growth promoting rhizobacterium (PGPR) *Chryseobacterium balustinum Aur9* and salt stress in the pattern of flavonoids exuded by soybean roots. Plant Soil 328:483–493
176. Nadeem SM, Zahir ZA, Naveed M, Asghar HN, Arshad M (2010) Rhizobacteria capable of producing ACC-deaminase may mitigate salt stress in wheat. Soil Sci Soc Am J 74:533–542
177. Kamnev AA, Lelie VD (2000) Chemical and biological parameters as tools to evaluate and improve heavy metal phytoremediation. Biosci Rep 20:239–258
178. Ma Y, Rajkumar M, Luo Y, Freitas H (2011) Inoculation of endophytic bacteria on host and non-host plants-effects on plant growth and Ni uptake. J Hazard Mater 195:230–237
179. Yan-de J, Zhen-li H, Xiao-e Y (2007) Role of soil rhizobacteria in phytoremediation of heavy metal contaminated soils. J Zhejiang Univ Sci B 8:192–207
180. Gadd GM (2004) Microbial influence on metal mobility and application for bioremediation. Geoderma 122:109–119
181. Gadd GM (2005) Microorganisms in toxic metal polluted soils. In: Buscot F, Varma A (eds) Microorganisms in soils: roles in genesis and functions. Springer, Berlin, pp 325–356
182. Gadd GM (2010) Metals: minerals and microbes: geomicrobiology and bioremediation. Microbiology 156:609–643
183. Rajkumar M, Ae N, Freitas H (2009) Endophytic bacteria and their potential to enhance heavy metal phytoextraction. Chemosphere 77:153–160
184. O'Sullivan DJ, O'Gara F (1992) Traits of fluorescent *Pseudomonas* spp. Involved in suppression of plant root pathogens. Microbiol Rev 56:662–676
185. Höflich G, Wiehe W, Kuhn G (1994) Plant growth stimulation by inoculation with symbiotic and association rhizosphere microorganisms. Experientia 50:897–905
186. Carlot M, Giacomini A, Casella S (2002) Aspects of plante-microbe interactions in heavy metal polluted soil. Acta Biotechnol 22:13–20
187. Glick BR (2003) Phytoremediation: synergistic use of plants and bacteria to clean up the environment. Biotechnol Adv 21:383–393

188. Lebeau T, Braud A, Jézéquel K (2008) Performance of bioaugmentation-assisted phytoextraction applied to metal contaminated soils: a review. Environ Pollut 153:497–522
189. Duss F, Mozafar A, Oertli JJ, Jäggi W (1986) Effect of bacteria on the iron uptake of axenically-cultured roots of Fe-efficient and Fe-inefficient tomatoes (Lycopersicon esculentum Mill.). J Plant Nutr 9:587–598
190. Duijff BJ, Kogel WJ, Bakker PAHM, Schippers B (1994) Influence of pseudobactin-358 on the iron nutrition of barley. Soil Biol Biochem 26:1681–1688
191. Sharma A, Johri BN (2003) Combat of iron-deprivation through a plant growth promoting fluorescent *Pseudomonas* strain GRP3A in mung bean (Vigna radiata L. Wilzeck). Microbiol Res 158(1):77–81
192. Sharma A, Johri BN (2003) Growth promoting influence of siderophore-producing *Pseudomonas* strains GRP3A and PRS9 in maize (Zea mays L.) under iron limiting conditions. Microbiol Res 158(3):243–248
193. Jurkevitch E, Chen Y, Hadar Y (1988) Involvement of bacterial siderophores in the remedy of lime-induced chlorosis in peanut. Soil Sci Soc Am J 52:1032–1037
194. Abou-Shanab R, Angle J, Chaney R (2006) Bacterial inoculants affecting nickel uptake by Alyssum murale from low, moderate and high Ni soils. Soil Biol Biochem 38:2882–2889.
195. Whiting SN, Souza MP, Terry N (2001) Rhizosphere bacteria mobilize Zn for hyperaccumulation by *Thlaspi caerulescens*. Environ Sci Technol 35:3144–3150
196. Braud A, Jezequel K, Vieille E, Tritter A, Lebeau T (2006) Changes in extractability of Cr and Pb in a polycontaminated soil after bioaugmentation with microbial producers of biosurfactants, organic acids and siderophores. Water Air Soil Pollut Focus 6:261–279
197. Duponnois R, Kisa M, Assigbetse K, Prin Y, Thioulouse J, Issartel M et al (2006) Fluorescent pseudomonads occurring in Macrotermes subhyalinus mound structures decrease Cd toxicity and improve its accumulation in sorghum plants. Sci Total Environ 370:391–400
198. Wu CH, Wood TK, Mulchandani A, Chen W (2006) Engineering plante microbe symbiosis for rhizoremediation of heavy metals. Appl Environ Microbiol 72:1129–1134
199. Wu SC, Cheung KC, Luo YM, Wong MH (2006) Effects of inoculation of plant growth-promoting rhizobacteria on metal uptake by Brassica juncea. Environ Pollut 140:124–135
200. Dubbin WE, Ander EL (2003) Influence of microbial hydroxamate siderophores on Pb(II) desorption from a-FeOOH. Appl Geochem 18:1751–1756
201. Neubauer U, Nowack B, Furrer G, Schulin R (2000) Heavy metal sorption on clay minerals affected by the siderophore desferrioxamine B. Environ Sci Technol 34:2749–2755
202. Sekara A, Poniedzialeek M, Ciura J, Jedrszczyk E (2005) Cadmium and lead accumulation and distribution in the organs of nine crops: implications for phytoremediation. Pol J Environ Stud 14:509–516
203. Rafati M, Khorasani N, Moattar F, Shirvany A, Moraghebi F, Hosseinzadeh S (2011) Phytoremediation potential of *Populus alba* and *Morus alba* for cadmium, chromuim and nickel absorption from polluted soil. Int J Environ Res 5:961–970
204. Zacchini M, Pietrini F, Mugnozza GS, Iori V, Pietrosanti L, Massacci A (2009) Metal tolerance, accumulation and translocation in poplar and willow clones treated with cadmium in hydroponics. Water Air Soil Pollut 197:23–34
205. Cherian S, Oliveira MM (2005) Transgenic plants in phytoremediation: recent advances and new possibilities. Environ Sci Technol 39:9377–9390
206. Milic D, Lukovic J, Ninkov J, Zeremski-Skoric T, Zoric L, Vasin J et al (2012) Heavy metal content in halophytic plants from inland and maritime saline areas. Cent Eur J Biol 7:307–317
207. Robinson BH, Leblanc M, Petit D, Brooks RR, Kirkman JH, Gregg PEH (1998) The potential of *Thlaspi caerulescens* for phytoremediation of contaminated soils. Plant Soil 203:47–56
208. Tlustoš P, Szakova J, Hrubŷ J, Hartman I, Najmanova J, Nedělnik J et al (2006) Removal of As, Cd, Pb, and Zn from contaminated soil by high biomass producing plants. Plant Soil Environ 52:413–423

209. Raskin I, Kumar PBAN, Dushenkov S, Salt DE (1994) Bioconcentration of heavy metals by plants. Curr Opin Biotechnol 5:285–290
210. Brooks RR (ed) (1998) Plants that hyperaccumulate heavy metals. CAB International, Wallingford
211. Baker AJM, Brooks RR (1989) Terrestrial higher plants which hyperaccumulate metallic elements—a review of their distribution, ecology and phytochemistry. Biorecovery 1:81–126
212. Yang XE, Long XX, Ye HB, He ZL, Calvert DV, Stoffella PJ (2004) Cadmium tolerance and hyperaccumulation in a new Zn hyperaccumulating plant species (*Sedum alfredii* Hance). Plant Soil 259:181–189
213. Yanqun Z, Yuan L, Jianjun C, Haiyan C, Li Q, Schvartz C (2005) Hyperaccumulation of Pb, Zn and Cd in herbaceous grown on lead–zinc mining area in Yunnan, China. Environ Int 31:755–762
214. Sun Q, Ye ZH, Wang XR, Wong MH (2007) Cadmium hyperaccumulation leads to an increase of glutathione rather than phytochelatins in the cadmium hyperaccumulator *Sedum alfredii*. J Plant Physiol 164:1489–1498
215. Sun R, Zhou Q, Jin C (2006) Cadmium accumulation in relation to organic acids in leaves of *Solanum nigrum* L. as a newly found cadmium hyperaccumulator. Plant Soil 285:125–134
216. Reeves RD, Baker AJM (2000) Metal-accumulating plants. In: Raskin I, Ensley BD (eds) Phytoremediation of toxic metals: using plants to clean up the environment. Wiley, New York, pp 193–229
217. Karimi N, Ghaderian SM, Raab A, Feldmann J, Meharg AA (2009) An arsenic accumulating, hypertolerant brassica, *Isatis cappadocica*. New Phytol 184:41–47
218. Karimi N, Ghaderian SM, Maroofi H, Schat H (2010) Analysis of arsenic in soil and vegetation of a contaminated area in Zarshuran, Iran. Int J Phytoremediation 12:159–173
219. Ma LQ, Komar KM, Tu C, Zhang WH, Cai Y, Kennelley ED (2001) A fern that hyperaccumulates arsenic: a hardy, versatile, fast-growing plant helps to remove arsenic from contaminated soils. Nature 409:579
220. Zhang W, Cai Y, Downum KR, Ma LQ (2004) Thiol synthesis and arsenic hyperaccumulation in *Pteris vittata* (Chinese brake fern). Environ Pollut 131:337–345
221. Wang HB, Wong MH, Lan CY, Baker AJ, Qin YR, Shu WS et al (2007) Uptake and accumulation of arsenic by 11 Pteris taxa from southern China. Environ Pollut 145:225–233
222. Milner MJ, Kochian LV (2008) Investigating heavy-metal hyperaccumulation using *Thlaspi caerulescens* as a model system. Ann Bot 102:3–13
223. Krämer U (2010) Metal hyperaccumulation in plants. Annu Rev Plant Biol 61:517–534
224. Prasad MNV, Freitas H (2003) Metal hyperaccumulation in plants e biodiversity prospecting for phytoremediation technology. Electron J Biotechnol 6:275–321
225. Seth CS (2012) A review on mechanisms of plant tolerance and role of transgenic plants in environmental clean-up. Bot Rev 78:32–62
226. Meers E, Vandecasteele B, Ruttens A, Vangronsveld J, Tack FMG (2007) Potential of five willow species (*Salix* spp.) for phytoextraction of heavy metals. Environ Exp Bot 60:57–68
227. Brunner I, Luster J, Günthardt-Goerg MS, Frey B (2008) Heavy metal accumulation and phytostabilisation potential of tree fine roots in a contaminated soil. Environ Pollut 152:559–568
228. Domínguez MT, Marañón T, Murillo JM, Schulin R, Robinson BH (2008) Trace element accumulation in woody plants of the Guadiamar Valley, SW Spain: a large-scale phytomanagement case study. Environ Pollut 152:50–59
229. Mench M, Lepp N, Bert V, Schwitzguébel JP, Gawronski SW, Schröder P et al (2010) Successes and limitations of phytotechnologies at field scale: outcome, assessment and outlook from COST Action 859. J Soil Sediments 10:1039–1070
230. Olguín EJ, Sánchez-Galván G (2010) Aquatic phytoremediation: novel insights in tropical and subtropical regions. Pure Appl Chem 82:27–38
231. Bolan NS, Park JH, Robinson B, Naidu R, Huh KY (2011) Chapter four—phytostabilization: a green approach to contaminant containment. Adv Agron 112:145–204

232. Berti WR, Cunningham SD (2000) Phytos tabilizat ion of metals: phytoremediation of toxic metals using plants to clean up the environment. In: Raskin I, Ensley BD (eds) Phytoremediation of toxic metals: using plants to clean up the environment. Wiley, New York, pp 71–88
233. Hooda V (2007) Phytoremediation of toxic metals from soil and waste water. J Environ Biol 28(2):367–376
234. Barcelo J, Poschenrieder C (2003) Phytoremediation: principles and perspectives. Contrib Sci 2:333–344
235. Ghosh M, Singh SP (2005) A review on phytoremediation of heavy metals and utilization of it's by products. Appl Ecol Environ Res 3:1–18
236. Etim EE (2012) Phytoremediation and its mechanisms: a review. I\nt J Environ Bioenergy 2(3):120–136
237. USEPA (2000) Introduction to phytoremediation. EPA 600/R-99/107, U.S. Environmental Protection Agency, Office of Research and Development, Cincinnati
238. Wong MH (2003) Ecological restoration of mine degraded soils, with emphasis on metal contaminated. Chemosphere 50:775–780
239. Marchiol L, Fellet G, Poščić F, Zerbi G (2011) A decade of research on phytoremediation in north-east Italy: lessons learned and future directions. In: Golubev A (ed) Handbook of phytoremediation. Nova Science, Hauppauge, pp 137–184
240. Dickinson NM, Baker AJM, Doronila A, Laidlaw S, Reeves RD (2009) Phytoremediation of inorganics: realism and synergies. Int J Phytoremediation 11:97–114
241. Santibáñez C, Verdugo C, Ginocchio R (2008) Phytostabilization of copper mine tailings with biosolids: implications for metal uptake and productivity of Lolium perenne. Sci Tot Environ 395:1–10
242. Van Nevel L, Mertens J, Staelens J, De Schrijver A, Tack FMG, De Neve S et al (2011) Elevated Cd and Zn uptake by aspen limits the phytostabilization potential compared to five other tree species. Ecol Eng 37:1072–1080
243. Mertens J, Vervaeke P, De Schrijver A, Luyssaert S (2004) Metal uptake by young trees from dredged brackish sediment: limitations and possibilities for phytoextraction and phytostabilisation. Sci Total Environ 326:209–215
244. Raskin I, Robert D, Smith RD, Salt DE (1997) Phytoremediation of metals: using plants to remove pollutants from the environment. Curr Opin Biotechnol 8:221–226
245. Yao Z, Li J, Xie H, Yu C (2012) Review on remediation technologies of soil contaminated by heavy metals. The 7th international conference on waste management and technology. Procedia Environ Sci 16:722–729
246. Liu Y-J, Liu Q-J, Ding H (2011) Reviews on soil pollution, risks, sources and phytoremediation involving metal contaminants. In: Golubev I (ed) Handbook of phytoremediation. Nova Science, New York
247. Brunetti G, Soler-Rovira P, Farrag K, Senesi N (2009) Tolerance and accumulation of heavy metals by wild plant species grown in contaminated soils in Apulia region, Southern Italy. Plant Soil 318:285–298
248. Nedjimi B, Daoud Y (2009) Cadmium accumulation in *Atriplex halimus* subsp. *schweinfurthii* and its influence on growth, proline, root hydraulic conductivity and nutrient uptake. Flora 204:316–324
249. Yadav SK, Juwarkar AA, Kumar GP et al (2009) Bioaccumulation and phyto-translocation of arsenic, chromium and zinc by *Jatropha curcas* L.: impact of dairy sludge and biofertilizer. Bioresour Technol 100:4616–4622
250. Mehta P (2005) Evaluating the potential of alder-Frankia symbionts for the remediation and revegetation of oil sands tailings. Masters Abstracts International, vol 45, p 99
251. Zou T, Li T, Zhang X, Yu H, Luo H (2011) Lead accumulation and tolerance characteristics of Athyrium wardii (Hook.) as a potential phytostabilizer. J Hazard Mater 186:683–689
252. Nabais C, Gonçalves SC, Freitas H (2007) Phytoremediation in Portugal: present and future. In: Willey N (ed) Phytoremediation. Methods and reviews. Methods in biotechnology. Humana Press Inc., Totowa, pp 405–422

253. Wang J, Feng X, Anderson CWN, Xing Y, Shang L (2012) Remediation of mercury contaminated sites—a review. J Hazard Mater 221–222:1–18
254. Dastoor AP, Larocque Y (2004) Global circulation of atmospheric mercury: a modelling study. Atmos Environ 38:147–161
255. Selin NE (2009) Global biogeochemical cycling of mercury: a review. Annu Rev Environ Resour 34:43–63
256. Zayed A, Pilon-Smits E, Souza M, Lin ZQ, Terry N (2000) Remediation of selenium-polluted soils and waters by phytovolatilization. In: Terry N, Bañuelos G (eds) Phytoremediation of contaminated soil and water. CRC, Boca Raton, pp 61–83
257. Bañuelos GS, Lin ZQ (2007) Acceleration of selenium volatilization in seleniferous agricultural drainage sediments amended with methionine and casein. Environ Pollut 150:306–312
258. Terry N, Bañuelos G (eds) (2000) Phytoremediation of contaminated soil and water. CRC, New York
259. Rahman IMM, Hossain MM, Begum ZA, Rahman MA, Hasegawa H (2011) Eco-environmental consequences associated with chelant-assisted phytoremediation of metal-contaminated soil. In: Golubev IA (ed) Handbook of phytoremediation. Nova Science, New York, pp 709–722
260. Volesky B (1999) Biosorption for the next century. Process Metall 9:161–170
261. Limcharoensuk T, Sooksawat S, Sumarnrote A, Awutpet T, Kruatrachue M, Pokethitiyook P, Auesukaree C (2015) Bioaccumulation and biosorption of Cd^{2+} and Zn^{2+} by bacteria isolated from a zinc mine in Thailand. Ecotoxicol Environ Saf 122:322–330
262. Tobin JM, White C, Gadd GM (1994) Metal accumulation by fungi: applications in environmental biotechnology. J Ind Microbiol 1:126–130
263. Fomina M, Gadd GM (2014) Biosorption: current perspectives on concept, definition and application. Bioresour Technol 160:3–14
264. Amirnia S, Ray MB, Margaritis A (2015) Heavy metals removal from aqueous solutions using *Saccharomyces cerevisiae* in a novel continuous bioreactor–biosorption system. Chem Eng J 264:863–872
265. Wehrheim B, Wettern M (1994) Biosorption of cadmium, copper and lead by isolated mother cell walls and whole cells of *Chlorella fusca*. Appl Microbiol Biotechnol 41:725–728
266. Davis TA, Volesky B, Vieira RHSF (2000) Sargassum seaweed as biosorbent for heavy metals. Water Res 34:4270–4278
267. Christoforidis AK, Orfanidis S, Papageorgiou SK, Lazaridou AN, Favvas EP, Mitropoulos AC (2015) Study of Cu(II) removal by *Cystoseira crinitophylla* biomass in batch and continuous flow biosorption. Chem Eng J 277:334–340
268. Kapoor A, Viraraghavan T, Cullimore DR (1999) Removal of heavy metals using the fungus *Aspergillus niger*. Bioresour Technol 70:95–104
269. Vieira RHSF, Volesky B (2000) Biosorption: a solution to pollution? Int Microbiol 3:17–24
270. Veglio F, Beolchini F (1997) Removal of metals by biosorption: a review. Hydrometallurgy 44:301–316
271. Abdolali A, Guo WS, Ngo HH, Chen SS, Nguyen NC, Tung KL (2014) Typical lignocellulosic wastes and by-products for biosorption process in water and wastewater treatment: a critical review. Bioresour Technol 160:57–66
272. Vijayaraghavan K, Balasubramanian R (2015) Is biosorption suitable for decontamination of metal-bearing wastewaters? A critical review on the state-of-the-art of biosorption processes and future directions. J Environ Manage 160:283–296
273. Beveridge T, Murray R (1976) Uptake and retention of metals by cell walls of *Bacillus subtilis*. J Bacteriol 127:1502–1518
274. Vijayaraghavan K, Yun Y-S (2008) Bacterial biosorbents and biosorption. Biotechnol Adv 26:266–291
275. Kapoor A, Viraraghavan T (1995) Fungal biosorption-an alternative treatment option for heavy metal bearing wastewaters: a review. Bioresour Technol 53:195–206
276. Davis TA, Volesky B, Mucci A (2003) A review of the biochemistry of heavy metal biosorption by brown algae. Water Res 37:4311–4330

277. Crini G (2005) Recent developments in polysaccharide-based materials used as adsorbents in wastewater treatment. Prog Polym Sci 30:38–70
278. He J, Chen JP (2014) A comprehensive review on biosorption of heavy metals by algal biomass: materials, performances, chemistry, and modelling simulation tools. Bioresour Technol 160:67–78
279. Kumar SK, Dahmas HU, Won E-J, Lee J-S, Shin K-H (2015) Microalgae—a promising tool for heavy metal remediation. Ecotoxicol Environ Saf 113:329–352
280. Cassidy MB, Lee H, Trevors JT (1996) Environmental applications of immobilized microbial cells: a review. J Ind Microbiol 16:79–101
281. Schiewer S, Wong MH (2000) Ionic strength effects in biosorption of metals by marine algae. Chemosphere 41:271–282
282. Wang J, Chen C (2006) Biosorption of heavy metals by *Saccharomyces cerevisiae*: a review. Biotechnol Adv 24:427–451
283. Volesky B, Naja G (2007) Biosorption technology: starting up an enterprise. Int J Technol Transf Commer 6:196–211
284. Lesmana SO, Febriana N, Soetaredjo FE, Sunarso J, Ismadji S (2009) Studies on potential applications of biomass for the separation of heavy metals from water and wastewater. Biochem Eng J 44:19–41
285. Kapoor A, Viraraghavan T (1998) Removal of heavy metals from aqueous solutions using immobilized fungal biomass in continuous mode. Water Res 32(6):1968–1977
286. Marques P, Pinheiro HM, Rosa MF (2007) Cd(II) removal from aqueous solution by immobilised waste brewery yeast in fixed-bed and airlift reactors. Desalination 214:343–351
287. Tsezos M (1990) Engineering aspects of metal binding by biomass. In: Ehrlich HL, Brierley CL (eds) Microbial MINERAL RECOVERY. McGraw-Hill, New York, pp 325–340
288. Fernandez JM, Plaza C, Hernandez D, Polo A (2007) Carbon mineralization in an and soil amended with thermally-dried and composted sewage sludges. Geoderma 137:497–503
289. Alburquerque JA, Gonzalvez J, Tortosa G, Baddi GA, Cegarra J (2009) Evaluation of "alperujo" composting based on organic matter degradation, humification and compost quality. Biodegradation 20:257–270
290. Gandolfi I, Sicolo M, Franzetti A, Fontanarosa E, Santagostino A, Bestetti G (2010) Influence of compost amendment on microbial community and ecotoxicity of hydrocarbon-contaminated soils. Bioresour Technol 101:568–575
291. Huang DL, Zeng G, Feng CL, Hu S, Jian XY, Tang L et al (2008) Degradation of lead contaminated lignocellulosic waste by *Phanerochaete chrysosporium* and the reduction of lead toxicity. Environ Sci Technol 42:4946–4951
292. Huang DL, Zeng G, Feng CL, Hu S, Lai C, Zhao MH et al (2010) Changes of microbial population structure related to lignin degradation during lignocellulosic waste composting. Bioresour Technol 101:4062–4067
293. Yu Z, Zeng GM, Chen YN, Zhang JC, Yu Y, Li H et al (2011) Effects of inoculation with *Phanerochaete chrysosporium* on remediation of pentachlorophenol-contaminated soil waste by composting. Process Biochem 46:1285–1291
294. Zeng GM, Yu Z, Chen YN, Zhang JC, Li H, Yu M et al (2011) Response of compost maturity and microbial community composition to pentachlorophenol (PCP)-contaminated soil during composting. Bioresour Technol 102:5905–5911
295. Chen M, Xu P, Zeng G, Yang C, Huang D, Zhang J (2015) Bioremediation of soils contaminated with polycyclic aromatic hydrocarbons, petroleum, pesticides, chlorophenols and heavy metals by composting: applications, microbes and future research needs. Biotechnol Adv 33:745–755
296. Pedra F, Polo A, Ribeiro A, Domingues H (2007) Effects of municipal solid waste compost and sewage sludge on mineralization of soil organic matter. Soil Biol Biochem 39:1375–1382
297. Kästner M, Mahro B (1996) Microbial degradation of polycyclic aromatic hydrocarbons in soils affected by the organic matrix of compost. Appl Microbiol Biotechnol 44:668–675
298. Lu LH, Zeng GM, Fan CZ, Ren XJ, Wang C, Zhao QR et al (2013) Characterization of a laccase-like multicopper oxidase from newly isolated *Streptomyces* sp C1 in agricultural waste compost and enzymatic decolorization of azo dyes. Biochem Eng J 72:70–76

299. Zeng G, Zhang J, Chen Y, Yu Z, Yu M, Li H et al (2011) Relative contributions of archaea and bacteria to microbial ammonia oxidation differ under different conditions during agricultural waste composting. Bioresour Technol 102:9026–9032
300. Zhang J, Zeng G, Chen Y, Yu M, Huang H, Fan C et al (2013) Impact of *Phanerochaete chrysosporium* inoculation on indigenous bacterial communities during agricultural waste composting. Appl Microbiol Biotechnol 97:3159–3169
301. Samanta SK, Singh OV, Jain RK (2002) Polycyclic aromatic hydrocarbons: environmental pollution and bioremediation. Trends Biotechnol 20:243–248
302. Watanabe K (2001) Microorganisms relevant to bioremediation. Curr Opin Biotechnol 12:237–241
303. Puglisi E, Cappa F, Fragoulis G, Trevisan M, Del Re AA (2007) Bioavailability and degradation of phenanthrene in compost amended soils. Chemosphere 67:548–556
304. van Herwijnen R, Hutchings TR, Al-Tabbaa A, Moffat AJ, Johns ML, Ouki SK (2007) Remediation of metal contaminated soil with mineral-amended composts. Environ Pollut 150:347–354
305. Farrell M, Jones DL (2010) Use of composts in the remediation of heavy metal contaminated soil. J Hazard Mater 175:575–582
306. Zhang C, Hughes JB, Nishino SF, Spain JC (2000) Slurry-phase biological treatment of 2,4-dinitrotoluene and 2,6-dinitrotoluene: role of bioaugmentation and effects of high dinitrotoluene concentration. Environ Sci Technol 34:2810–2816
307. Harmsen J, Rulkens WH, Sims RC, Rijtema PE, Zweers AJ (2007) Theory and application of landfarming to remediate polycyclic aromatic hydrocarbons and mineral oil contaminated sediments; beneficial reuse. J Environ Qual 36:1112–1122
308. Maciel BM, Santos ACF, Dias JCT, Vidal RO, Dias RJC, Gross E et al (2009) Simple DNA extraction protocol for a 16S rDNA study of bacterial diversity in tropical landfarm soil used for bioremediation of oil waste. Genet Mol Res 8:375–388
309. Souza TS, Hencklein FA, Angelis DF, Gonçalves RA, Fontanetti CS (2009) The Allium cepa bioassay to evaluate landfarming soil, before and after the addition of rice hulls to accelerate organic pollutants biodegradation. Ecotoxicol Environ Saf 72:1363–1368
310. Sanscartier D, Reimer K, Zeeb B, George K (2010) Management of hydrocarbon contaminated soil through bioremediation and landfill disposal at a remote location in Northern Canada. Can J Civil Eng 37:147–155
311. Smith E, Thavamani P, Ramadass K, Naidu R, Srivastava P, Megharaj M (2015) Remediation trials for hydrocarbon-contaminated soils in arid environments: evaluation of bioslurry and biopiling techniques. Int Biodeterior Biodegrad 101:56–65
312. Adams JA, Reddy KR (2003) Extent of benzene biodegradation in saturated soil column during air sparging. Ground Water Monit Rem 23:85–94
313. Wu YW, Huang GH, Chakma A, Zeng GM (2005) Separation of petroleum hydrocarbons from soil and groundwater through enhanced bioremediation. Energy Sources 27:221–232
314. Brar SK, Verma M, Surampalli RY, Misra K, Tyagi RD, Meunier N et al (2006) Bioremediation of hazardous wastes—a review. Pract Period Hazard Toxic Radioact Waste Manage 10:59–72
315. Kao CM, Chen CY, Chen SC, Chien HY, Chen YL (2008) Application of in situ biosparging to remediate a petroleum-hydrocarbon spill site: field and microbial evaluation. Chemosphere 70:1492–1499
316. Conte P, Agretto A, Spaccini R, Piccolo A (2005) Soil remediation: humic acids as natural surfactants in the washings of highly contaminated soils. Environ Pollut 135:515–522
317. Vázquez S, Hevia A, Moreno E, Esteban E, Peñalosa JM, Carpena RO (2011) Natural attenuation of residual heavy metal contamination in soils affected by the Aznalcóllar mine spill, SW Spain. J Environ Manage 92:2069–2075
318. Lors C, Damidot D, Ponge J-F, Périé F (2012) Comparison of a bioremediation process of PAHs in a PAH-contaminated soil at field and laboratory scales. Environ Pollut 165:11–17
319. Kedziorek MAM, Etchebers O, Reynal-Preud'homme C, Bourg ACM (2013) Natural attenuation of heavy metals (Cd, Cr, and Pb) in a water table aquifer underlying an industrial site. Procedia Earth Planet Sci 7:89–92

320. Das S, Jean J-S, Kar S, Chou M-L, Chen C-Y (2014) Screening of plant growth-promoting traits in arsenic-resistant bacteria isolated from agricultural soil and their potential implication for arsenic bioremediation. J Hazard Mater 272:112–120
321. Xu Y, Sun G-D, Jin J-H, Liu Y, Luo M, Zhong Z-P, Liu Z-P (2014) Successful bioremediation of an aged and heavily contaminated soil using a microbial/plant combination strategy. J Hazard Mater 264:430–438
322. Sagarkar S, Nousiainen A, Shaligram S, Björklöf K, Lindström K, Jørgensen KS, Kapley A (2014) Soil mesocosm studies on atrazine bioremediation. J Environ Manage 139:208–216
323. Freitas ETF, Montoro LA, Gasparon M, Ciminelli VST (2015) Natural attenuation of arsenic in the environment by immobilization in nanostructured hematite. Chemosphere 138:340–347
324. Dong WH, Zhang P, Lin XY, Zhang Y, Tabouré A (2015) Natural attenuation of 1,2,4-trichlorobenzene in shallow aquifer at the Luhuagang's landfill site, Kaifeng, China. Sci Total Environ 505:216–222
325. Martínez-Pascual E, Grotenhuis T, Solanas AM, Viñas M (2015) Coupling chemical oxidation and biostimulation: effects on the natural attenuation capacity and resilience of the native microbial community in alkylbenzene-polluted soil. J Hazard Mater 300:135–143
326. Stormo KE, Crawford RL (1992) Preparation of encapsulated microbial cells for environmental applications. Appl Environ Microbiol 58:727–730
327. Jacques RJS, Okeke BC, Bento FM, Teixeira AS, Peralba MCR, Camargo FAO (2008) Microbial consortium bioaugmentation of a polycyclic aromatic hydrocarbons contaminated soil. Bioresour Technol 99:2637–2643
328. Lima D, Viana P, André S, Chelinho S, Costa C, Ribeiro R et al (2009) Evaluating a bioremediation tool for atrazine contaminated soils in open soil microcosms: the effectiveness of bioaugmentation and biostimulation approaches. Chemosphere 74:187–192
329. Alisi C, Musella R, Tasso F, Ubaldi C, Manzo S, Cremisini C, Sprocati AR (2009) Bioremediation of diesel oil in a co-contaminated soil by bioaugmentation with a microbial formula tailored with native strains selected for heavy metals resistance. Sci Total Environ 407:3024–3032
330. Sprocati AR, Alisi C, Tasso F, Marconi P, Sciullo A, Pinto V et al (2012) Effectiveness of a microbial formula, as a bioaugmentation agent, tailored for bioremediation of diesel oil and heavy metal co-contaminated soil. Process Biochem 47:1649–1655
331. Polti MA, Aparicio JD, Benimeli CS, Amoroso MJ (2014) Simultaneous bioremediation of Cr(VI) and lindane in soil by Actinobacteria. Int Biodeterior Biodegrad 88:48–55
332. Winquist E, Björklöf K, Schultz E, Räsänen M, Salonen K, Anasonye F et al (2014) Bioremediation of PAH-contaminated soil with fungi—from laboratory to field scale. Int Biodeterior Biodegrad 86:238–247
333. García-Delgado C, Yunta F, Eymar E (2015) Bioremediation of multi-polluted soil by spent mushroom (*Agaricus bisporus*) substrate: polycyclic aromatic hydrocarbons degradation and Pb availability. J Hazard Mater 300:281–288
334. Wang Y, Peng B, Yang Z, Chai L, Liao Q, Zhang Z, Li C (2015) Bacterial community dynamics during bioremediation of Cr(VI)-contaminated soil. Appl Soil Ecol 85:50–55
335. Anasonye F, Winquist E, Räsänen M, Kontro J, Björklöf K, Vasilyeva G et al (2015) Bioremediation of TNT contaminated soil with fungi under laboratory and pilot scale conditions. Int Biodeterior Biodegrad 105:7–12
336. Ramírez EM, Camacho JV, Rodrigo MA, Cañizares P (2015) Combination of bioremediation and electrokinetics for the in-situ treatment of diesel polluted soil: a comparison of strategies. Sci Total Environ 533:307–316
337. Mesa J, Rodríguez-Llorentea ID, Pajueloa E, Piedras JMB, Caviedes MA, Redondo-Gómez S, Mateos-Naranjo E (2015) Moving closer towards restoration of contaminated estuaries: bioaugmentation with autochthonous rhizobacteria improves metal rhizoaccumulation in native Spartina marítima. J Hazard Mater 300:263–271
338. Margesin R, Schinner F (1998) Low-temperature bioremediation of a waste water contaminated with anionic surfactants and fuel oil. Appl Microbiol Biotechnol 49:482–486

339. Dias RL, Ruberto L, Hernández E, Vázquez SC, Lo Balbo A, Del Panno MT, Mac Cormack WT (2012) Bioremediation of an aged diesel oil-contaminated Antarctic soil: evaluation of the "on site" biostimulation strategy using different nutrient sources. Int Biodeterior Biodegrad 75:96–103
340. Mishra D, Kim D-J, Ralph DE, Ahn J-G, Rhee Y-A (2008) Bioleaching of metals from spent lithium ion secondary batteries using *Acidithiobacillus ferrooxidans*. Waste Manag 28:333–338
341. Ren W-X, Li P-J, Geng Y, Li X-J (2009) Biological leaching of heavy metals from a contaminated soil by *Aspergillus niger*. J Hazard Mater 167:164–169
342. Akinci G, Guven DE (2011) Bioleaching of heavy metals contaminated sediment by pure and mixed cultures of *Acidithiobacillus* spp. Desalination 268:221–226
343. Fang D, Zhang R, Zhou L, Li J (2011) A combination of bioleaching and bioprecipitation for deep removal of contaminating metals from dredged sediment. J Hazard Mater 192:226–233
344. Deng X, Chai L, Yang Z, Tang C, Tong H, Yuan P (2012) Bioleaching of heavy metals from a contaminated soil using indigenous *Penicillium chrysogenum* strain F1. J Hazard Mater 233–234:25–32
345. Deng X, Chai L, Yang Z, Tang C, Wang Y, Shia Y (2013) Bioleaching mechanism of heavy metals in the mixture of contaminated soil and slag by using indigenous *Penicillium chrysogenum* strain F1. J Hazard Mater 248–249:107–114
346. Guven DE, Akinci G (2013) Effect of sediment size on bioleaching of heavy metals from contaminated sediments of Izmir Inner Bay. J Environ Sci 25:1784–1794
347. Li S, Zhong H, Hu Y, Zhao J, He Z, Gu G (2014) Bioleaching of a low-grade nickel–copper sulfide by mixture of four thermophiles. Bioresour Technol 153:300–306
348. Groudev S, Georgiev P, Spasova I, Nicolova M (2014) Decreasing the contamination and toxicity of a heavily contaminated soil by in situ bioremediation. J Geochem Explor 144:374–379
349. Park J, Han Y, Lee E, Choi U, Yoo K, Song Y, Kim H (2014) Bioleaching of highly concentrated arsenic mine tailings by *Acidithiobacillus ferrooxidans*. Sep Purif Technol 133:291–296
350. Yang H, Feng S, Xin Y, Wang W (2014) Community dynamics of attached and free cells and the effects of attached cells on chalcopyrite bioleaching by *Acidithiobacillus* sp. Bioresour Technol 154:185–191
351. Zhu J-Y, Zhang J-X, Li Q, Han T, Hu Y-H, Liu X-D et al (2014) Bioleaching of heavy metals from contaminated alkaline sediment by auto- and heterotrophic bacteria in stirred tank reactor. Trans Nonferrous Metals Soc China 24:2969–2975
352. Bajestani MI, Mousavi SM, Shojaosadati SA (2014) Bioleaching of heavy metals from spent household batteries using *Acidithiobacillus ferrooxidans*: statistical evaluation and optimization. Sep Purif Technol 132:309–316
353. Shahrabi-Farahani M, Yaghmaei S, Mousavi SM, Amiri F (2014) Bioleaching of heavy metals from a petroleum spent catalyst using *Acidithiobacillus thiooxidans* in a slurry bubble column bioreactor. Sep Purif Technol 32:41–49
354. Nguyen VK, Lee MH, Park HJ, Lee J-U (2015) Bioleaching of arsenic and heavy metals from mine tailings by pure and mixed cultures of *Acidithiobacillus* spp. J Ind Eng Chem 21:451–458
355. Yang Z, Zhanga Z, Chaia L, Wang Y, Liu Y, Xiao R (2016) Bioleaching remediation of heavy metal-contaminated soils using *Burkholderia* sp. Z-90. J Hazard Mater 301:145–152
356. Dary M, Chamber-Pérez MA, Palomares AJ, Pajuelo E (2010) *In situ* phytostabilisation of heavy metal polluted soils using *Lupinus luteus* inoculated with metal resistant plant-growth promoting rhizobacteria. J Hazard Mater 177:323–330
357. Dmuchowskia W, Gozdowski D, Brągoszewsk P, Baczewska AH, Suwara I (2014) Phytoremediation of zinc contaminated soils using silver birch (*Betula pendula* Roth). Ecol Eng 71:32–35

358. Agnello AC, Bagard M, van Hullebuscha ED, Esposito G, Huguenota D (2015) Comparative bioremediation of heavy metals and petroleum hydrocarbons co-contaminated soil by natural attenuation, phytoremediation, bioaugmentation and bioaugmentation-assisted phytoremediation. Sci Total Environ. doi:10.1016/j.scitotenv.2015.10.061
359. Khaokaew S, Landrot G (2015) A field-scale study of cadmium phytoremediation in a contaminated agricultural soil. Chemosphere 138:883–887
360. Ma Y, Oliveira RS, Nai F, Rajkumar M, Luo Y, Rocha I et al (2015) The hyperaccumulator *Sedum plumbizincicola* harbors metal-resistant endophytic bacteria that improve its phytoextraction capacity in multi-metal contaminated soil. J Environ Manage 156:62–69
361. Ma C, Ming H, Lin C, Naidu R, Bolan B (2016) Phytoextraction of heavy metal from tailing waste using Napier grass. CATENA 136:74–83. Bech J, editor. Section 1: Special issue on Reclamation of mining site soils, Part I. and Lei, TW, Yu XX, Zhuang J, editors. Section 2: Special Issue on Understanding hydrological and erosion processes under changing environment
362. Grumiaux F, Demuynck S, Pernin C, Leprêtre A (2015) Earthworm populations of highly metal-contaminated soils restored by fly ash-aided phytostabilisation. Ecotoxicol Environ Saf 113:183–190
363. Taylor RT, Duba AG, Durham WB, Hanna ML, Jackson KJ, Jovanovich MC et al (1992) In situ bioremediation of trichloroethylene-contaminated water by a resting-cell methanotrophic microbial filter. In: Conference of the international in situ bioremediation; Ontario, pp 20–24
364. Duran N, Marcato PD, De Souza GIH, Alves OL, Esposito E (2007) Antibacterial effect of silver nanoparticles produced by fungal process on textile fabrics and their effluent treatment. J Biomed Nanotechnol 3:203–208
365. Loutseti S, Danielidis DB, Economou-Amilli A, Katsaros C, Santas R, Santas P (2009) The application of a micro-algal/bacterial biofilter for the detoxification of copper and cadmium metal wastes. Bioresour Technol 100:2099–2105
366. Li YL, Deletic A, Alcazar L, Bratieres K, Fletcher TD, McCarthy DT (2012) Removal of *Clostridium perfringens*, *Escherichia coli* and F-RNA coliphages by stormwater biofilters. Ecol Eng 49:137–145
367. Vezzaro L, Eriksson E, Ledin A, Mikkelsen PS (2012) Quantification of uncertainty in modelled partitioning and removal of heavy metals (Cu, Zn) in a stormwater retention pond and a biofilter. Water Res 46:6891–6903
368. Mohn W, Radziminski C, Fortin M-C, Reimer K (2001) On site bioremediation of hydrocarbon- contaminated arctic tundra soils in inoculated biopiles. Appl Microbiol 57:242–247
369. Chemlal R, Tassist A, Drouiche M, Lounici H, Drouiche N, Mameri N (2012) Microbiological aspects study of bioremediation of diesel-contaminated soils by biopile technique. Int Biodeterior Biodegrad 75:201–206
370. Kim SK, Park CB, Koo Y-M, Yun HS (2003) Biosorption of cadmium and copper ions by *Tri-choderma reesei* RUT C30. J Ind Eng Chem 9:403–406
371. Sari A, Tuzen M (2009) Kinetic and equilibrium studies of biosorption of Pb(II) and Cd(II) from aqueous solution by macrofungus (Amanita rubescens) biomass. J Hazard Mater 164:1004–1011
372. Chao H-P, Chang C-C, Nieva A (2014) Biosorption of heavy metals on Citrus maxima peel, passion fruit shell, and sugarcane bagasse in a fixed-bed column. J Ind Eng Chem 20:3408–3414
373. Chen YLC, Hong XQ, He H, Luo HW, Qian TT, Li RZ et al (2014) Biosorption of Cr (VI) by Typha angustifolia: mechanism and responses to heavy metal stress. Bioresour Technol 160:89–92
374. Wang J, Chen C (2014) Chitosan-based biosorbents: modification and application for biosorption of heavy metals and radionuclides. Bioresour Technol 160:129–141
375. Jaffar AHA, Tamilselvi M, Soban Akram A, Kaleem Arshan ML, Sivakumar V (2015) Comparative study on bioremediation of heavy metals by solitary ascidian, *Phallusia nigra*, between Thoothukudi and Vizhinjam ports of India. Ecotoxicol Environ Saf 121:93–99

376. Anastopoulos I, Kyzas GZ (2015) Progress in batch biosorption of heavy metals onto algae. J Mol Liq 209:77–86
377. Frutos FJG, Escolano O, García S, Babín M, Fernández MD (2010) Bioventing remediation and ecotoxicity evaluation of phenanthrene-contaminated soil. J Hazard Mater 183:806–813
378. Carvalho M, Vila MC, Soeiro de Carvalho JM, Domingues V, Oliva-Teles T, Fiúza A (2010) Bioventing tests in contaminated residual granitic soils. J Biotechnol 150 Suppl:S282
379. Sui H, Li S (2011) Modeling for volatilization and bioremediation of toluene-contaminated soil by bioventing. Chin J Chem Eng 19:340–348
380. Ryan CD, Jim L, Mehdi B (2012) Sustainable wind-driven bioventing at a petroleum hydrocarbon–impacted site. Remediation 22:65–78
381. Abid N, Chamkha M, Godon JJ, Sayadi S (2007) Involvement of microbial populations during the composting of olive mill wastewater sludge. Environ Technol 28:751–760
382. Ghaly A, Zhang B, Dave D (2011) Biodegradation of phenolic compounds in creosote treated wood waste by a composting microbial culture augmented with the fungus *Thermoascus aurantiacus*. Am J Biochem Biotechnol 7:90–103
383. Kharrazi SM, Younesi H, Abedini-Torghabeh J (2014) Microbial biodegradation of waste materials for nutrients enrichment and heavy metals removal: an integrated composting vermicomposting process. Int Biodeterior Biodegrad 92:41–48
384. Soobhany N, Mohee R, Garg VK (2015) Comparative assessment of heavy metals content during the composting and vermicomposting of municipal solid waste employing *Eudrilus eugeniae*. Waste Manage 39:130–145
385. Al-awadhi N, Al-Daher R, Elnawawy A, Balba MT (1996) Bioremediation of oil-contaminated soil in Kuwait. I. Landfarming to remediate oil-contaminated soil. J Soil Contam 5:243–260
386. Mancini O, Cuccu W, Molinari M (2005) Landfarming and phytoremediation in an urban area: a case study. In situ and on-site bioremediation. In: Proceedings of the 8th international in situ and on-site bioremediation symposium; Baltimore, 2005 June 6–9
387. Saadoun IMK, Al-Ghzawi ZD (2005) Bioremediation of petroleum contamination. Biorem Aquat Terr Ecosyst 173–212
388. Jacques RJS, Okeke BC, Bento FM, Peralba MCR, Camargo FAO (2007) Characterization of a polycyclic aromatic hydrocarbon-degrading microbial consortium from a petro-chemical sludge landfarming site. Biorem J 11:1–11
389. Paudyn K, Rutter A, Kerry Rowe R, Poland JS (2008) Remediation of hydrocarbon contaminated soils in the Canadian Arctic by landfarming. Cold Reg Sci Technol 53:102–114
390. Sanscartier D, Laing T, Reimer K, Zeeb B (2009) Bioremediation of weathered petroleum hydrocarbon soil contamination in the Canadian High Arctic: laboratory and field studies. Chemosphere 77:1121–1126
391. Mikkonen A, Hakala KP, Lappi K, Kondo E, Vaalama A, Suominen L (2012) Changes in hydrocarbon groups, soil ecotoxicity and microbiology along horizontal and vertical contamination gradients in an old landfarming field for oil refinery waste. Environ Pollut 162:374–380
392. Yee DC, Maynard JA, Wood TK (1998) Rhizoremediation of trichloroethylene by a recombinant, root-colonizing *Pseudomonas fluorescens* strain expressing toluene ortho-monooxygenase constitutively. Appl Environ Microbiol 64:112–118
393. Villacieros M, Whelan C, Mackova M, Molgaard J, Sánches-Contreras M, Lloret J et al (2005) Polychlorinated biphenyl rhizoremediation by *Pseudomonas fluorescens* F113 derivatives, using a Sinorhizobium meliloti nod system to drive bph gene expression. Appl Environ Microbiol 71:2687–2694
394. Johnson DL, Anderson DR, McGrath S (2005) Soil microbial response during the phytoremediation of a PAH contaminated soil. Soil Biol Biochem 37:2334–2336
395. Maila M, Randima P, Cloete TE (2005) Multispecies and monoculture rhizoremediation of polycyclic aromatic hydrocarbons (PAHs) from the soil. Int J Phytoremediation 7:87–98
396. Tovanabootr A, Semprini L, Dolan ME, Azizian M, Magar VS, Debacker D et al (2002) Cometabolic air sparging field demonstration with propane to remediate trichloroethene and

cis-dichloroethene. In: 6th international in situ and on site bioremediation symposium: 2001 Jun 4-7. Battelle Press, San Diego, pp 145–153

397. Urum K, Pekdemir T, Ross D, Grigson S (2005) Crude oil contaminated soil washing in air sparging assisted stirred tank reactor using biosurfactants. Chemosphere 60:334–343
398. Kabelitz N, Machackova J, Imfeld G, Brennerova M, Pieper DH, Heipieper HJ et al (2009) Enhancement of the microbial community biomass and diversity during air sparging bioremediation of a soil highly contaminated with kerosene and BTEX. Appl Microbiol Biotechnol 82:565–577
399. Gao S, Meegoda JN, Hu L (2013) A dynamic two-phase flow model for air sparging. Int J Numer Anal Meth Geomech 37:1801–1821
400. Kuyukina MS, Ivshina IB, Ritchkova MI, Philp JC (2003) Bioremediation of crude oil-contaminated soil using slurry-phase biological treatment and land farming techniques. Soil Sediment Contam 12:85–99
401. Mohan SV, Prasanna D, Purushotham Reddy B, Sarma PN (2008) Ex situ bioremediation of pyrene contaminated soil in bio-slurry phase reactor operated in periodic discontinuous batch mode: influence of bioaugmentation. Int Biodeterior Biodegrad 62:162–169
402. Nasseri S, Kalantary RR, Nourieh N, Naddafi K, Mahvi AH, Baradaran N (2010) Influence of bioaugmentation in biodegradation of PAHs-contaminated soil in bio-slurry phase reactor. J Environ Health Sci Eng 7:199–208
403. Tomei MC, Mosca Angelucci D, Annesini MC, Daugulis AJ (2013) *Ex situ* remediation of polluted soils by absorptive polymers, and a comparison of slurry and two-phase partitioning bioreactors for ultimate contaminant degradation. J Hazard Mater 262:31–37

Chapter 4
Biosorption of Heavy Metal from Aqueous Solutions

Prayad Pokethitiyook and Toemthip Poolpak

4.1 Introduction

Massive quantities of metals from anthropogenic source are both accidental and considered released into the environment throughout the year as the population number and industrialization increased. Toxic metals in concerned include emissions of heavy metals from industries, mine tailings, leaded gasoline, paints, land application of fertilizers, animal manures, sewage sludge, pesticides, wastewater irrigation, coal combustion residues, and atmospheric deposition can accumulate and persist in the ecosystem. Consequently, removing these substances from discharges before they enter the ecosystem has turned into a challenge for environmental engineering in our time. The conventional methods for treating metal-containing wastewaters are coagulation and flocculation, reverse osmosis, electrochemical, and activated carbon adsorption [1]. None of these technologies show both significant effectiveness and economic advantage in metal removal. However, activated carbon seems to be the most effective and widely used adsorbent but with a certain problem of its use. The higher prices of it correspond to higher levels of the quality. Furthermore, regeneration of activated carbon is almost impossible [2].

Biological materials, living or dead cell, have long been investigated in their capabilities to remove metal ions [1, 3]. Generally, there are such interactions between living things and metals, as the uptake and storage of essential and nonessential metals by the dead and living cells have been studied. Some organisms have developed mechanisms to uptake and store higher concentration of those metals and, hence, some species can also detoxify some toxic metals [4]. Both living and dead cell have a basic property to bind with not only inorganic but also organic chemicals and can concentrate the much diluted chemicals in solution [5, 6]. These

---

P. Pokethitiyook (✉) • T. Poolpak
Department of Biology, Faculty of Science, Mahidol University, Bangkok 10400, Thailand
e-mail: prayad.pok@mahidiol.ac.th; toemthip.poo@mahidol.ac.th

A.A. Ansari et al. (eds.), *Phytoremediation*, DOI 10.1007/978-3-319-40148-5_4

involved the consideration for an economy approach for metal-contaminated water cleanup. Bioremediation methods to clean up toxic metals from water discharge take account of biosorption, the use of biological materials to accumulate those metals through many metabolic or biochemical pathways and then concentrated them from solution [7, 8]. The biological materials used in biosorption to remove target substances from solution or biosorbents are derived from various types of origins including bacteria, yeast, fungi, algae, plant, and animal products such as chitosan [9] and crab shell [10]. Based on the metal uptake capacity, biosorbents used in practical application should hold some characteristics that improve its performance in engineering perspectives. These are: cost of biomass, selectivity of metal-binding sites, and resistance to wide range of physical and chemical conditions. Conventional techniques of biosorbent production and kinetics of biosorption from different species of organisms will be discussed as well as the appropriate immobilization techniques.

4.2 Biosorption of Metal

There are two general basic terms of sorption, absorption, and adsorption. Absorption is a process which atom or molecule of one phase interpenetrates practically uniformly within those of another phase to form a solution with it [11]. In the other way, adsorption is often used to designated the accumulation of substances that can take place at a surface or interface of any two phases, liquid–liquid, gas–liquid, gas–solid, or liquid–solid interface for example [11]. Accordingly, the absorption process does not count as a biosorption mechanism in this chapter. In addition, there are three major characteristics of adsorption. The first consists of electrical attraction or exchange adsorption which is the relation between microbial negatively charged ligands and positively charged ions. The second adsorption characteristic is physical or ideal adsorption which includes van der Waals force which the adsorbed molecule can have translational movement within the interface. The last one is chemical or activated adsorption which is chemical attraction between adsorbent and the adsorbate [12]. In general, it is difficult to separate physical and chemical characteristic from each other and these three usually collaborated in adsorption.

Biosorption, as defined by Naja and Volesky [11], is an operation that combines the use of biomaterials for sorbing, sequestering, and immobilizing both inorganic and organic compounds from aqueous solution. Biosorption is the capability of non-metabolizing biomass to bind and concentrate selected ions or other molecules from aqueous solutions based on the passive sequestration, and this mechanism is opposed to a much more complex phenomenon, bioaccumulation which is based on active metabolic transport [13]. The passive uptake of metal by biosorption may arise by one or a combination of different processes including complexation, coordination, chelation, ion exchange, microprecipitation, and entrapment [14]. Most of the passive uptake mechanisms are associated with either non-metabolizing or metabolizing cells with the exception of microprecipitation and entrapment which usually refer to immobilization of metal species in the solid form that located outside or inside the cells, such as the extracellular polymeric capsule or cytoplasmic compounds [11].

In immobilization or sequestration of metal, the use of non-metabolizing or nonliving cells offers some benefits over the metabolizing or living cells. In metabolizing cells, the active metabolic activities may influence biosorption by changing the environmental factors such as pH, Eh, and also the metabolites in cellular microenvironment. The other important reason of using the living cells in biosorption is practically that all biological macromolecule as well as cell walls and other associated biomolecules have some affinity for metal species. Additionally, there could be accurate control of the metal removal process using non-metabolizing biomass in specific removal system. For the purpose of removing dissolve metal from aqueous solution, metal immobilization or dissolve metal bonded to form a solid particle is easier to separate. The nonliving biomass deposited with metal ions can be removed together by solid–liquid suspension system including settling, flotation, centrifugation, and filtration.

4.2.1 Biosorbent

For economic reasons in metal biosorption, of particular interest are abundant biomass types either generated as a waste by-product of large-scale industrial fermentations or certain metal-binding algae found in large quantities in the sea or even microorganisms are fascinating within the past decades [15]. At least four broad areas of application for biosorbent materials have been considered which include detoxification of metal-bearing wastewaters, decontamination of radioactive wastewaters, recovery of metals from ore processing solutions, concentration or recovery of rare metals from seawater [16]. Biosorbents proposed for application need to be derived usually as granules of classified size ranges between 0.1 and 3 mm with a preferred rigidity to resist pressure in the column and water permeability. They may be chemically pretreated for better performance and/or suitability for process applications.

Biosorbents are biological materials capable of directly sorbing metal ionic species from aqueous solutions [11]. The key challenge for the biosorption was to select the most favorable types of biomass from available and inexpensive biomaterials [17]. Although many biological materials can bind metals, only those with appropriately high metal-binding capacity and selectivity for metals are suitable for a full-scale biosorption process. The biosorbent materials among easily available include three groups: bacteria, algae, and fungi, the latter two possibly giving broader choices [18].

4.2.2 Bacterial Biosorption

Bacteria are a main group of unicellular organisms belonging to the prokaryotes, which are abundant in environment especially in soil and water. They have simple morphology and present in three basic shapes: spherical or coccus, rod or bacillus, and spiral or spirillum. Bacteria vary both in size and shape. The typical size of bacteria cell is about 1.1–1.5 μm wide by 2.0–6.0 μm long. Cell size is an important characteristic for an organism as it affects a number of cell biological properties. Small size of bacteria ensures rapid metabolic processes.

The cell wall is the important for structure for bacterial cell and its main function including providing cell shape and protecting it from osmotic lysis; protecting cell from toxic substances; offers the site of action for several antibiotics; and the last one is the necessity for normal cell division. Bacterial classification by Gram staining technique, the Gram-positive bacteria stained purple, whereas Gram-negative were colored pink or red. The surface of Gram-negative cells is much more complex chemically and structurally than that of the other but the walls of Gram-positive cells are stronger because of the thicker peptidoglycan layer [18].

Gram negative bacterial cell wall has a 2–7 nm peptidoglycan layer surrounded by a 7–8 nm thick outer membrane. The peptidoglycan is covalently bound to the outer membrane by lipoproteins and sandwiched between the plasma membrane and the outer membrane, which is composed of phospholipids, lipopolysaccharides (LPSs), enzymes, and other proteins, including lipoproteins. The Gram-negative bacteria also have various types of complex macromolecular lipopolysaccharide and each LPS is held in the outer membrane by relatively weak cohesive forces, ionic and hydrophobic interactions and can be dissociated from the cell surface with surface-active agents. The net negative charge of LPSs attributes to the negative surface charge of Gram-negative bacteria. The phosphate groups within LPSs and phospholipids have been proved to be the primary sites for metal interaction. However, only one of the carboxyl groups in LPSs is free to interact with metals [19].

Using bacteria as biosorbent are promising because of their small size, ubiquity, and the ability to grow under controlled conditions. Additionally, they have capability to survive in a wide range of environmental situations. Bacteria may either retain the capacity for biosorption of many elements depending on the species, in some cases, may be element specific [18]. Many biosorption of metal are established such as cadmium biosorption by *Sphingomonas paucimobilis* biomass [20] *Arthrobacter* sp. [21], *Bacillus* sp. [22], and other [23]. Bacterial exopolysaccharide (EPS) consisting of extracellular DNA, lipids, polysaccharides, and proteins also studied their metal ion biosorption properties [24]. Immobilization of biosorbent seemed to work very efficiently. Rangsayatorn et al. [25] found that cadmium biosorption by immobilized cyanobacteria, *Spirulina platensis* TISTR 8217 on alginated gel and silica gel show the maximum capacities at 70.92 and 36.63 mg Cd/g biomass, respectively, and the immobilized cell could be repeatedly used up to five times (Fig. 4.1 and Table 4.1).

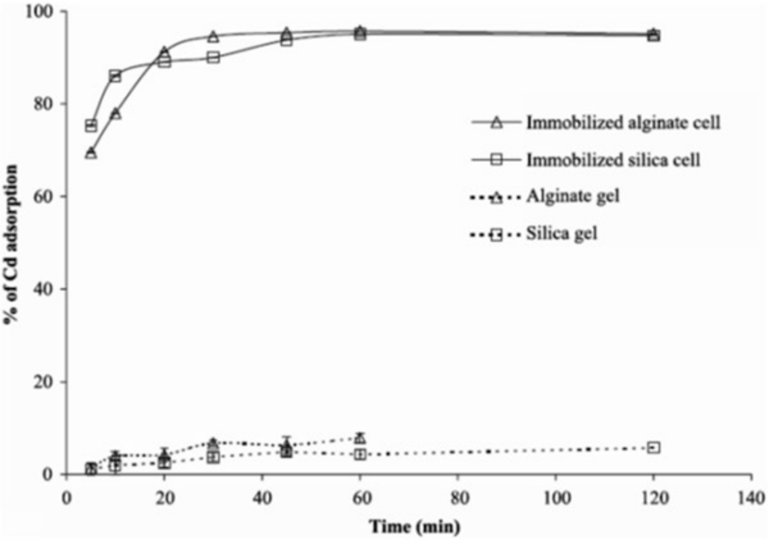


Fig. 4.1 Time course of cadmium removal by *S. platensis* immobilized on silica gel and alginate gel. Reproduced from Rangsayatorn N., Pokethitiyook P., Upatham E.S., Lanza G.R. 2004. Cadmium biosorption by cells of *Spirulina platensis* TISTR 8217 immobilized in alginate and silica gel. Environ. Inter. 30:57–63 [25], with permission of Elsevier

Table 4.1 Five cycles of cadmium adsorption–desorption using 0.1 M HCl as a desorbing agent

	% of cadmium adsorption[a]	
Cycle	Alginate-immobilized cell	Silica-immobilized cell
1	94.07 ± 0.06	92.67 ± 0.02
2	70.79 ± 0.15	66.99 ± 0.27
3	65.85 ± 0.34	78.31 ± 0.24
4	65.85 ± 0.11	78.47 ± 0.14
5	66.73 ± 0.71	63.21 ± 0.16

Reproduced from Rangsayatorn N., Pokethitiyook P., Upatham E.S., Lanza G.R. 2004. Cadmium biosorption by cells of *Spirulina platensis* TISTR 8217 immobilized in alginate and silica gel. Environ. Inter. 30:57–63 [25], with permission of Elsevier

[a] The values given are means ± SD

4.2.3 Fungal and Yeast Biosorption

Fungi can be clustered into molds or yeasts based on thallus development and most of them are filamentous. Yeasts are unicellular fungi that reproduce either asexually by budding and transverse division or sexually through spore formation. A mold such as *Penicillium* and *Aspergillus*, consists of long, branched, thread-like filaments of cells, hyphae, which form a tangled mass called a mycelium. The fungal hyphae are typically 5–10 μm wide but may vary depending on the species and a common cytoplasm exists throughout the hyphae. The hyphae walls are composed of cellulose or chitin or both of them.

In general, yeast colonies are much like those of bacteria but yeast cells are larger than bacteria with the size about 2.5–10 μm wide by 4.5–21 μm long. Yeast cell is commonly spherical to oval shaped depending on species, nutrition level, and also culture condition. The most important commercial yeasts are member of the genus Saccharomyces, the baker's and brewer's yeasts which are eukaryotic cells. They are excellent models for eukaryotic biology study especially for *S. cerevisiae*. Generally, yeast cells have a cell wall and most of the other eukaryotic organelles but lack of flagella.

The cell walls of the fungi are inflexible and provide structural support and shape, but they are different in chemical composition from prokaryotic cell walls with mainly 80–90 % polysaccharide, with proteins, lipids, polyphosphates, and inorganic ions. Chitin is a common constituent of fungal cell walls. Fungal cell wall consists of two layers, a thin outer layer consisting of mixed glycans, and a thick inner layer of microfibrillar polysaccharide fibers composed of chitin or cellulose. The cell membrane of eukaryotic cells is a thin, double-layered sheet composed of lipids, such as phospholipids and sterols and protein molecules. Cytoplasmic membranes served as selectively permeable barriers in transport. In contrast with prokaryotes, eukaryotic cells contain a number of individual membrane bound organelles that are extensive enough to account for 60–80 % in volume [18].

The metal ions are compartmentalized into different subcellular organelles, e.g., mitochondria and vacuole, after entering into the cell thus cytoplasm is important for living cells to interact with metal ions [26]. Limiting metal uptake by active excretion, storage in an inert form or stored metal excretion are the main approaches for essential metals. In contrast, excretion from the metal excess pool and internal storage without elimination are the major approaches for nonessential metals and the metal concentration in the cells will increase with elevating external concentration. Vijver et al. [26] pointed out that the cellular sequestration mechanisms mainly have two types, the formation of distinct inclusion bodies and the binding of metals to heat-stable proteins. The former the formation of distinct inclusion bodies includes three types of granules: (a) amorphous deposits of calcium phosphates, e.g., Zn, (b) mainly containing acid phosphatase, accumulating, e.g., Cd, Cu, Hg, and Ag; and (C) excess iron stored in granules as hemosiderin. The latter mechanism mainly relates to a specific metal-binding protein, metallothioneins (MT), which can be induced by many substances, including heavy metal ions, such as Cd, Cu, and Hg. The used of fungal group also extensively examined such as unmodified yeast cells of *S. cerevisiae* to remove Pb(II) and Cu(II) ions from aqueous solutions in continuous mode was studied [27] and Cu biosorption onto fungal *Rhizopus oligosporus* [28].

4.2.4 Algae and Plant Material Biosorption

Algal diversity can be defined based on phylogenetic relationships, life stage, morphological types, habitats occupied by different groups, or their chemical diversity. Their habitats range from open oceans which occupied by microalgal planktonic species to rocky shores which may be marine macroalgae or seaweeds, and benthic microalgae. Some algae occupy freshwater habitats including rivers, lakes, ditches, and ponds which are the group of conspicuous filamentous algae. Not as much observable are the benthic microalgae which populate bedrocks in various damp and temporarily damp marine, freshwater, and terrestrial habitats. The diversity of algal taxonomy with different habitats put forward that different species have evolved equivalent metabolic pathways, though not necessarily through a shared evolutionary pathway, to fulfill basic processes such as protection from biotic and abiotic stresses [29].

Microalgae are unicellular or colonial algae and can exist in filamentous form. Most of them contain chlorophyll but a few kinds of common algae are not green but appear brown or red because other pigments such as carotenoids are present in addition to chlorophyll. Algae cells contain one or more chloroplasts, membranous structures that house the photosynthetic pigments [18]. Algae demonstrate significant diversity in their cell wall structure and chemistry. In several cases, the cell wall is composed of a network of cellulose fibrils and usually modified by other polysaccharides adding such as pectin, alginic acids, or fucinic acid. In some algae, the wall is calcium carbonate deposition, where occasionally chitin is also present. Cell wall is absent in euglenoids whereas silica, protein and polysaccharide are added in cell wall of diatoms. Similar to the fungal cell wall in structure, the algal cell wall is made of multilayered microfibrillar framework containing cellulose which presented mostly about 90 % of the algal cell wall and interspersed with amorphous material consisting of glycoproteins.

In biosorption, various algae or seaweed were used and investigated as biosorbents for metal removal due to their high sorption capacity and their ready availability in practically unlimited quantities in the seas and oceans such as Brown Seaweed, Lobophora variegate [30, 31]. There are also a number of plant biosorption studies including fern biomass [32], duckweed [2], and many aquatic macrophytes such as batch and continuous packed column studies of cadmium biosorption by *Hydrilla verticillata* biomass [33] and other work [34].

4.2.5 Additional Biosorbent Extensively Used in Biosorption of Heavy Metals

Various kinds of adsorbents have been widely produced an applied for the removal of radionuclides and heavy metals such as chitin, a natural long chain polysaccharide polymer of *N*-acetyl-D-glucosamine. It is the main component of the exoskeletons of arthropods such as crustaceans. It is acid resistance and recognized as an excellent metal ligand, forming stable complexes with many metal ions [35]. Chitin can use as biosorbent in many forms such as acid wash crab shell [10], shrimp shell flakes [35], chitosan nanoparticles, and crab shell particles [9]. The alternative biomaterials that have high efficiency to remove metal ion from solution along with bark [36] and saw dust which produced in large quantities at sawmills as a solid waste and also contains primarily lignin and cellulose [37, 38] as well as many unusual biosorbent were examined including pollen pini [39] and human black hair as source of melanin granule [40].

4.3 Factors Affecting Metal Biosorption

Biosorption is affected by an amount diverse of physicochemical mechanisms, depending on many external environmental factors as well as on speciation of elements in solution.

4.3.1 pH

One of the most important environmental factors on metal ions biosorption is pH. It strongly impacts not only the binding site of the biomass, but also the chemistry of the metals including hydrolysis, complexation by organic and/or inorganic ligands, redox reactions, precipitation, speciation, and biosorption availability of the heavy metals [41]. Meanwhile ion exchange is the major mechanism-driven biosorption and protons compete with metal cations for the binding sites, pH is the key condition which powerfully affects the process [7]. The different metal species occurring at different pH values also have variable charges and adsorption ability at solid–liquid interfaces. The pH not only limits solubility of toxic metal ions but also affects the properties of biomass. Many metals are free hydrate species at acidic condition and after pH increases, hydroxides are formed and precipitation of metal may be occurring. In addition, pH effects negative charge level on biomass surface by either protonation or deprotonation of metal-binding sites.

As pH increase, metal ions in solution are likely to undergo hydrolysis, but their degree will be differing at different pH values with each metals. The typical series of hydrolysis involves the formation of hydroxylated monomeric species followed by the formation of polymeric species and then crystalline oxide precipitation after aged [42]. The different pH sorption capability for metal ions may possibly relate to the nature of chemical interaction of each metal with biomass. The uptake of heavy metal cation by most biomass types decreases as the pH decreases as most of the heavy metals precipitate at pH higher than 5.5 [25]. It is supposed that metals might accumulate inside the cell or cell wall at more alkaline condition by a combined sorption-microprecipitation mechanism [17].

The optimum pH for metal biosorption differs for each ion and both cations and anions express different sorption pattern on the same biomass in the same pH range. Principally, the negative charge of cell surface increase as pH increase until all significant functional groups are deprotonated, which favors electrochemical attraction and cation adsorption and cations may have more capability to compete for binding on cell surface with H+. In contrast, anions have a tendency to intensely interact with binding sites as their positive charge concentration increase due to the protonation of functional groups at acidic conditions [7]. Biomass can be noticed as natural ion-exchange materials that contain weakly acidic and basic groups which follow the theory of acid–base equilibria that, in the pH range 2.5–5, the binding of heavy metal cations is determined by the state of dissociation of the weakly acidic groups. Solution pH as well affects the surface properties of biomass because the adsorption capacity of biomass is as a result of anionic or polar chemical group on their surface [43].

There are diverse natural groups of chemical on biomass surface which influence the adsorption capacity including carboxyl, phosphate, amine, amino, hydroxyl, and sulfhydryl. The pH dependence of metal uptake pointed to the weak acidic carboxyl groups R–COOH of algal and fungal cell wall components and also R–COOH groups of peptidoglycan in Gram-positive bacteria as the probable sites of ion exchange (Fig. 4.2). Carboxyl groups in biological polymers have pK_a values ranging from 3.5 to 5.0 [44]. The metal binding to the carboxyl group increased with the pH up to 4.1, but slightly decreased over the optimum pH, because of competitive binding sites. With increasing pH, the carboxyl group free sites increased because most of the metal is present in the biomass phase, and only a low level is present in the solution.

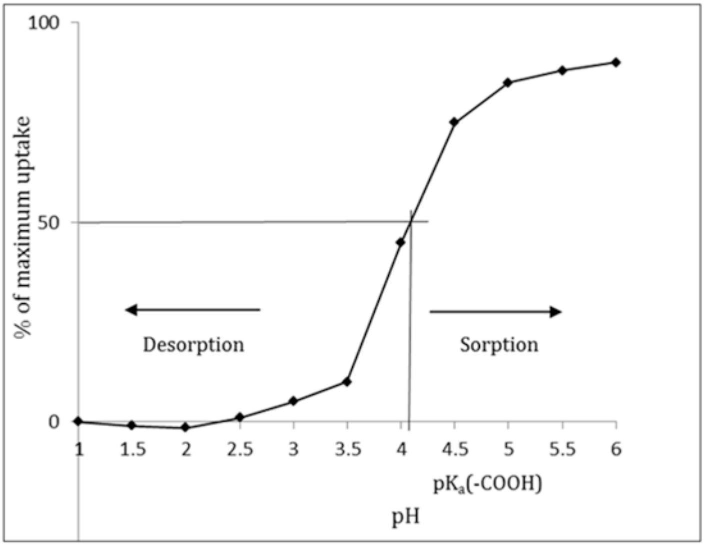


Fig. 4.2 Metal biosorption equilibrium as a function of pH in algal and fungal cell wall. Carboxyl groups (R–COOH, pK_a in the range of 3.5–5.5) of algal and fungal cell wall constituents as the probable sites of ion exchange. Reproduced from Kratochvil D., Volesky B. 1998. Advances in the biosorption of heavy metals. Trends. Biotechnol. 16:291–300 [17], with permission of Elsevier

The other such as phosphonate groups of plasma membrane phospholipids in brown seaweed have a similar range of the pK_a value whereas the amine group with pK_a values in various biomaterials ranging between 8 and 10. The positively charge, amide in crab shell chitin and chitosan, offered their binding sites for negative metal ion, increase as pH of aqueous solution decrease especially for anionic arsenate, $H_2AsO_4^-$ [10]. Uranium biosorption by shrimp flakes [35] increased with an increase in pH of the solution and when pH was over 3.6 the q_e values decreased. This could be explained that at slight acidic solution, amine groups in the flakes easily form protonation that induced an electrostatic repulsion of metal ions. Arsenate biosorption is not only determined by the acid–base properties of functional groups on the biomass but also by its chemical speciation in the solution which tends to hydrolyze depending on the solution pH.

4.3.2 Temperature

Dissimilarly with temperature independent non-metabolizing cells, the metabolizing cells are temperature dependent and the alteration of this factor will strongly affect the biosorption process. Naturally, adsorption and ion exchange are exothermic and therefore their reaction rate increase when temperature increases. On the other hand, the biosorption possibly will decrease at very high temperature due to cell walls damaged. Most of metal uptake increased when temperature increased in the range of 4–13 °C, while only a marginal decreased when temperature increased from 20 to 40 °C (Table 4.2) [7, 25].

If adsorption increases as temperature increases, it may be indicative of chemisorption; whereas decrease in adsorption with increasing temperature may be because of physical adsorption [36]. However, the biosorption evaluation at room temperature is still appropriate because it is fit to be replicated as demonstrated in uranium adsorption by shrimp shell flakes that the uranium adsorption efficiency decreases with the increase in the temperature and the highest adsorption efficiency occurs at room temperature after that the adsorption efficiency decreases to reach the lowest values at 70 °C [35].

Table 4.2 Effect of temperature on cadmium adsorption by immobilized *S. platensis*

	% of cadmium adsorption[a]	
Temperature (°C)	Alginate-immobilized cell	Silica-immobilized cell
20	96.20 ± 0.11	95.48 ± 0.06
26	92.32 ± 0.01	94.72 ± 0.02
30	94.72 ± 0.04	92.96 ± 0.09
40	94.88 ± 0.07	92.68 ± 0.10

Reproduced from Rangsayatorn N., Pokethitiyook P., Upatham E.S., Lanza G.R. 2004. Cadmium biosorption by cells of *Spirulina platensis* TISTR 8217 immobilized in alginate and silica gel. Environ. Inter. 30:57–63 [25], with permission of Elsevier

[a] The values given are means ± SD

4.3.3 Initial Metal Ion Concentration

Initial higher concentrations of metal ions have some effects on biosorption which resulting in a high metal uptake. The reason is at lower metal concentrations the ratio of ion to the available surface area declines; afterward the fractional sorption becomes independent of metal initial concentration. Conversely, at high metal concentrations, the availability of sorption sites decrease comparing to the ion numbers and then the metal removal is strongly rely on initial metal concentration [7, 41]. This occurrence also discovered in Srivastava et al. [36] which gradual decrease in percentage removal of Cr (VI) by native and chemically modified *Lagerstroemia speciosa* bark with an increase in initial ion concentration. This incidence occurred because of limited number of active sites on the biosorbent, which would have become saturated above a certain concentration. Increase of the initial Cr(VI) concentration results in a decrease in the initial rate of external diffusion and increase in the intraparticle diffusion rate.

4.3.4 Initial Biosorbent Concentration

The levels of metal biosorption strongly govern by biosorbent quantity. As biomass increases, metal biosorption also increases as a result of the biomass surface area increase, which in turn raises the binding sites number [7, 42]. In contrast, increasing biosorbent concentration decreases the quantity of metal sorption per unit of biomass weight due to the complex factors. The significant factor affecting biosorption at large amount of biosorbent is the deficiency of available metal to completely cover the available exchangeable sites resulting in low metal uptake. The interference between binding sites due to increasing biomass number cannot be taken precedence because low specific uptake may occur [7]. Ahmed et al. [35] reported that the adsorption efficiency increased with the initial metal concentration varying from 50 to 875 mg/L. They suggested that with more uranium content in a solution, larger fraction of the active sites is involved in the adsorption process then the increase in adsorption efficiency becomes less significant at 175.8 mg uranium/L, where 85 % from uranium was grafted on 1 g shrimp shell flake sand.

4.3.5 Effect of Contact Time

A passive physical adsorption of metal at the cell surface is very fast and takes place in a very short time after metal ions contact with the biosorbent. This behavior suggests that in the initial stage adsorption takes place rapidly on the external surface of the biosorbent followed by a slower internal diffusion process, which may be the rate limiting step. This is important because equilibrium time is one of the parameters for cost-effective wastewater treatment plant application [41]. The biosorption of Eu(III) using chitosan nanoparticle and crab shell particle significantly rapidly increased from within 15 min after metal ion contact with those two biosorbent, thereafter reached saturation in 1 h. After the equilibrium period, the amount of metal adsorbed did not change further with time [9, 33].

4.3.6 Speciation of Metal Ions

Metal ion in biosorption process can be separated into two phases, a solid and liquid. Metal first dissolved in the solution then sequestered on biosorbent, the solid phase, accordingly the properties and behavior of both metal ion and biosorbent in solution influence the biosorption performance. After dissolving, most of the common metals perform in the solution as positively charged cations including more toxic heavy metal group, Pb, Hg, Cd, Cu, Zn, Ni, U, and Th, for example [11]. More common negatively charged anionic metal species such as As, Se, V, and Mn are occurring in more complex forms. These differences in metal speciation are specific to positively and negatively charge of biosorbent biding sites and thus affect the biosorption process.

4.3.7 Presence of Co-ions

Wastewater commonly contain a number of metal species and this phenomenon is expected to cause cooperative effects as a function of many factors including co-ion competing for biding sites, metal concentration and biosorbent quantity. Lower metal uptake from mixed solution commonly observed comparing to those in a single species. In general, metal uptake increases as the ionic radius of metal cation increases, with metals having higher ionic charge presenting larger binding to biomass. Additionally, the reduction level of metal uptake in the presence of other cations is found to be dependent on concentration of the other cations, indicated that as the concentration of other cations increases, the metal uptake decreases.

To observe the ionic competition effects in solution during the biosorption, the concept of Pearson's classification of the elements is used [45]. According to the Pearson's, based on the chemical coordination characteristics of the elements, the elements are classified into three main groups: class A or hard ions, class B or soft ions, and class C or borderline ions. Class A elements tend to form ligands preferably with oxygen as a donor atom where B elements tend to coordinate better with ligands of decreasing electronegativity. The last group, borderline elements are characterized by intermediate coordination behavior. Each class of elements may possibly exhibit for different biosorbent-binding sites, depending on their structural chemistry [46]. The study by Bunluesin et al. [33] has found that the presence of Zn had an antagonistic effect on Cd biosorption (Fig. 4.3). The breakthrough point of the Cd-Zn mixed solution was 3900 mL, whereas, that of the Cd solution alone was

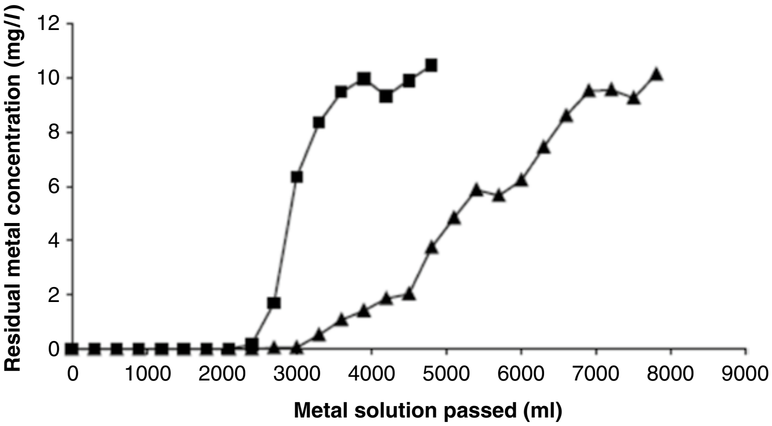


Fig. 4.3 Biosorption of Cd in mixed Cd and Zn solution in fixed bed column with continuous flow. Conditions: initial Cd and Zn concentration, 10 mg/L; pH5; actual flow rate 12.96 mL/min; 1 g of dry biomass; 25 ± 2 °C. Symbols: triangles, Cd; squares, Cd + Zn

at 7800 mL. This finding has shed some lights on the competition of different cations for biosorbent-binding sites.

4.4 Biosorption Isotherm and Kinetic Model

Biosorption can be defined as a cooperative term for several passive accumulation process including ion exchange, coordination, complexation, chelation, adsorption, and microprecipitation. At equilibrium, concentrations are a function of temperature, for that reason the adsorption equilibrium correlation at a specified temperature is mentioned as an adsorption isotherm [7, 47]. The solid–liquid sorption system assessment is typically based on two types of examinations: equilibrium batch sorption tests and dynamic continuous-flow sorption studies. Equilibrium isotherm model equations such as Langmuir and Freundlich are used in batch mode to describe experimental data, and it is important to find the best-fit isotherm to evaluate the efficacy of the prepared adsorbent to develop suitable industrial adsorption system designs [7, 13]. The Brunauer–Emmett–Teller (BET) model describes the multilayer adsorption at the biosorbent surface and assumes that the Langmuir isotherm applies to each layer [13].

4.4.1 Batch Biosorption Models

A typical biosorption batch design requires assessing the biosorbent quantity to process a given volume of a metal-containing solution. If sufficient time is allowed for equilibrium to be reached, the design of single stage batch systems is based on mass balances and thermodynamic equilibrium relationships.

The mass balance is given by:

$$V\left(c_o - c_e\right) = V_m\left(q_e - q_o\right)$$

where c_o and c_e are the initial and final metal concentration in the bulk solution, q_o and q_e are the initial and final metal concentration in the biosorbent, V is the volume of solution, and V_m is the volume of biosorbent. q_o is of course equal to zero when a biosorbent initially free from the metal contaminant is used. However, unlike gas-phase isotherms that generally function by temperature, liquid-phase isotherms are strongly affected by solution pH and ionic strength. In general, the equilibrium isotherm for a given metal–biosorbent system may not be expected from theory [48].

4.4.2 Equilibrium Isotherm

4.4.2.1 Freundlich Isotherm

Freundlich isotherm is an empirical equation that widely used for the adsorption equilibrium explanation. This isotherm can use for describing the heavy metals adsorption on diverse biosorbent types. This equation has the following form:

$$q_e = K_F C_e^{1/n}$$

It can also be expressed in the linearized logarithmic form:

$$\log q_e = \log K_F + \frac{1}{n}\log C_e$$

The plot of log q_e versus log C_e has a slope with the value of $1/n$ and an intercept magnitude of log K_F. Log K_F is equivalent to log q_e when C_e equals unity. However, in case when $1/n \neq 1$, the K_F value depends on the units upon which q_e and C_e are expressed. On average, a favorable adsorption tends to have Freundlich constant n between 1 and 10. Larger value of n or smaller value of $1/n$ implies stronger interaction between biosorbent and metal, whereas $1/n$ equal to 1 indicates linear adsorption leading to identical adsorption energies for all sites [13, 47].

4.4.2.2 Langmuir Isotherm

Langmuir equation is a well-known used model that relates the coverage of molecules on a solid surface to concentration of a medium above the solid surface at a fixed temperature. This isotherm use for describing heavy metal sorption onto biosorbent based on three assumptions, adsorption is limited to monolayer coverage, all surface sites are the same and only can provide accommodation to one adsorbed atom, and the ability of a molecule to be adsorbed on a given site is independent of its neighboring sites occupancy [47]. By applying these assumptions and a kinetic principle, in case that rate of adsorption and desorption from the surface is equal, the Langmuir equation can be written in the following form:

$$q_e = q_{\max} \frac{K_L C_e}{1 + K_L C_e}$$

where q_e is the amount adsorbed, C_e the equilibrium concentration, $q_{\max}$ the saturated monolayer adsorption capacity, and K_L the sorption equilibrium constant.

The linear form of this equation is often written as:

$$\frac{C_e}{q_e} = \frac{1}{q_{\max}} C_e + \frac{1}{K_L q_{\max}}$$

Within the Langmuir model, the saturation capacity $q_{\max}$ is supposed to correspond with saturation of a fixed number of identical surface sites, and it should reasonably be independent of temperature [8, 13, 47]. The decrease of K_L value with an increase in temperature indicates the exothermicity of the adsorption process or physical adsorption, while the opposite trend illustrates that the process needs thermal energy or endothermic which leads to chemical sorption. At higher temperature, the physical adsorption between metal and biosorbent active sites weakens; on the other hand, chemisorption becomes stronger. The exothermicity and endodermicity of biosorption can be determined by means of the heat of adsorption and commonly obtained through an integrated Van't Hoff equation, which relates the Langmuir constant, K_L, to the temperature:

$$K_L = K_o \exp\left(-\frac{E_a}{RT}\right)$$

where K_o is the adsorption equilibrium constant, E_a the activation energy of adsorption/heat of adsorption, R the gas constant (0.0083 kJ/(mol K)), and T the absolute temperature (K) [7].

4.4.2.3 Temkin Isotherm

The Temkin isotherm is based on the assumption that the decline of heat of sorption as a function of temperature is linear rather than logarithmic, as implied in the Freundlich equation [30, 49]. The Temkin isotherm has the form:

$$q_e = \frac{RT}{b} \ln\left(aC_e\right)$$

where *b* is the Temkin constant in relation to heat of sorption (kJ/mol) and a as the Temkin isotherm constant (*L*/*g*).

Temkin isotherms are not capable of predicting biosorption equilibria. The complex phenomenon involved in liquid-phase adsorption is not taken explanation by this equation as the basis of the Temkin equation involves simple assumptions. As a result, this equation is often not suitable for the representation of experimental data in complex systems [7].

4.4.2.4 Brunauer–Emmer–Teller (BET) Model

The BET model is removing the restriction in the Langmuir model which assumed that adsorption only occurs on the unoccupied adsorption sites. Assuming that the initial adsorbed layer can act as a substrate for additional adsorption, and then the isotherm, instead of leveling off to some saturated value at high concentrations, is able to increase indefinitely. The same kinetics concept proposed by Langmuir is applied to this multiple layering process, i.e., the rate of adsorption on any layer is equal to the rate of desorption from that layer [7]. The simplified form of BET equation can be written in the following form:

$$q_e = q_{\max} \frac{BC_e}{\left(C_e - C_s^*\right)[1 + \left(B - 1\right)\left(C_e / C_s^*\right)}$$

where B is a constant related to the energy of adsorption and C_s^* the saturation concentration of solute (mg/L).

Remarkably, other ideal assumptions within this model, that is all sites are energetically identical along with no horizontal interaction between adsorbed molecules, may be correct for heterogeneous material and simple nonpolar gases but not for complex systems involving heterogeneous adsorbent such as biosorbents and metals. For that reason, this equation is unpopular in the interpretation of liquid-phase adsorption data for complex solids [47].

4.4.2.5 Redlich–Paterson Isotherm

Redlich–Paterson is another empirical equation, designated as the “three parameter equation,” which is capable of representing adsorption equilibria over a wide concentration range [7]. This equation has the following form:

$$q_e = \frac{K_{\mathrm{RP}} C_e}{1 + a_{\mathrm{RP}} C_e^{\beta}}$$

where a_{RP}, K_{RP}, and β are Redlich–Paterson’s parameters.

This equation reduces to a linear isotherm at low surface coverage and to the Langmuir isotherm when b is equal to 1. Redlich and Paterson incorporated the characteristics of Langmuir and Freundlich isotherms into a single equation. Two limiting behaviors exist, i.e., the Langmuir form for $b=1$ and Henry's law form for $b=0$ [47].

4.4.3 *Kinetic Studies in Biosorption of Heavy Metals*

Adsorption equilibria studies are important to conclude the efficiency of adsorption. Despite of this, it is also necessary to identify the adsorption mechanism type in a given system. Kinetic models have been exploited to test the experimental data to examining the mechanism of biosorption and its potential rate-controlling steps that include mass transport and chemical reaction processes. Information on the kinetics of metal uptake is required to select the optimum condition for full-scale batch metal removal processes as well [7].

Several adsorption kinetic models have been established to understand the adsorption kinetics and rate-limiting step which include pseudo-first- and pseudo-second order rate models, Weber and Morris sorption kinetic model, Adam–Bohart–Thomas relation, first-order reversible reaction model, external mass transfer model, first-order equation of Bhattacharya and Venkobachar, Elovich's model, and Ritchie's equation. The pseudo-first- and pseudo-second-order kinetic models are the most well-liked models to study the biosorption kinetics of heavy metals and quantify the extent of uptake in biosorption kinetics.

4.4.3.1 The Pseudo-First-Order Kinetic

The Lagergren first-order rate expression based on solid capacity is generally expressed as follows:

$$\frac{\mathrm{d}q}{\mathrm{d}t} = k_1\left(q_e - q\right)$$

where q is the amount adsorbed at time t and k_1 the rate constant of first-order adsorption.

Integration of the above equation with the boundary conditions, $t=0$, $q=0$, and $t=t$, $q=q$, gives:

$$\ln\left(q_e - q\right) = \ln q_e - k_1 t$$

Theoretically, to determine the rate constants and equilibrium metal uptake, the straight-line plots of $\log(q_e - q)$ against t of above equation were made at different initial metal concentrations. The q_e value developed by this method is then compared with the experimental value. If large discrepancies are posed, the reaction cannot be classified as first-order although this plot has a high correlation coefficient from the fitting process.

This equation can be written in the nonlinear form:

$$q = q_e\left(1 - \exp\left(-k_1 t\right)\right)$$

Nonlinear fitting of this equation is another way to achieve the predicted value of q_e and k_1 although this is not a common application. The trend shows that the predicted q_e values seem to be lower than the experimental values. A time lag, probably caused by the presence of a boundary layer or external resistance controlling the beginning of the sorption process, was discussed to be the responsible factor behind the discrepancy [7, 13, 50].

4.4.3.2 The Pseudo-Second-Order Kinetic

Expecting the adsorption rate for a given system is among the most important factors in adsorption system design, as the system's kinetics determines adsorbate residence time and the reactor dimensions [7, 13, 51]. Although various factors play important rule on the adsorption capacity including initial heavy metals concentration, temperature, pH of solution, biosorbent size, and heavy metals nature, a kinetic model is only concerned with the effect of recognizable parameters on the overall rate.

Pseudo-second-order model is derived on the basis of the sorption capacity of the solid phase, expressed as:

$$\frac{\mathrm{d}q}{\mathrm{d}t} = k_2\left(q_e - q\right)^2$$

where k_2 is the rate constant for pseudo-second-order model. Integration of above equation with the boundary conditions $t=0$, $q=0$, and at $t=t$, $q=q$, results in:

$$\frac{1}{q_e - q} = \frac{1}{q_e} + k_2 t$$

This equation can be stated in the linear form as:

$$\frac{t}{q} = \frac{t}{q_e} + \frac{1}{k_2 q_e^2}$$

The pseudo-second-order rate constants can be determined experimentally by plotting t/q against t, this model is considered more appropriate to represent the kinetic data in biosorption systems, in comparison to pseudo-first-order kinetic intrinsically. Additionally, the pseudo-second-order model above has the highest coefficient of determination for the linear method. In contrast to the linear model, the subsequent kinetic parameters from the nonlinear model were almost identical among each other, as a result, the nonlinear method is considered as a better way to establish the preferred parameters [7, 13, 52]. Pseudo-first- and pseudo-second-order rate expressions have been and still in extensive use for the biosorption of heavy metals from aqueous solutions. In chemisorption process, the pseudo-second-order is superior to pseudo-first-order model as it takes into account the interaction of adsorbent–adsorbate through their valence forces [13, 51].

4.4.3.3 The Weber and Morris Sorption Kinetic Model

The Weber and Morris (WM) sorption kinetic model was initially employed by Pavasant et al. [53] to describe their biosorption experimental data. This model has the following form:

$$q = K_{\mathrm{WM}}\sqrt{t}$$

where K_{WM} is the Weber and Morris intra-particle diffusion rate. In their study, the Cu(II), Cd(II), Pb(II), and Zn(II) sorption process by *C. lentillifera* biomass was regulated by two main mechanisms, intra-particle diffusion and external mass transfer. The intra-particle diffusion (D) can be estimated with:

$$D = \frac{\pi}{8640}\left(\frac{\left(d_p K_{\mathrm{WM}}\right)}{q_e}\right)^2$$

where d_p is the mean particle diameter.

The external mass transfer process was determined by

$$\frac{\mathrm{d}q}{\mathrm{d}t} = K_L' A\left(C - C_s^i\right)$$

where K_L' is the liquid–solid mass transfer coefficient, A the specific surface area of biomass, C the liquid-phase concentration of sorbate in the bulk solution at t, and C_s^i the concentration of sorbate in the inner pore of sorbent. They observed that the external mass transfer coefficients can be ordered from high to low values as Cu(II)>Pb(II)>Zn(II)>Cd(II), while the intra-particle diffusion coefficients were as follows: Cd(II)>Zn(II)>Cu(II)>Pb(II) [13, 53]. All biosorbents, equilibrium isotherms, and kinetic modeling performed by several researchers have been summarized in Table 4.3.

Table 4.3 Summary of the biosorbents, equilibrium isotherms, and kinetic modeling performed by several researchers

Metal	Biosorbent	Equilibrium isotherm	Kinetic modeling	Reference
U	Shrimp shell flakes	Langmuir model (25.31 mg/g)	Pseudo-second order (8.196 mg/g)	Ahmed et al. [35]
	Human black hair	Langmuir model (62.5 mg/g)	Pseudo-second order (2.99 mg/g)	Saini and Melo [40]
	Pollen pini	Freundlich isotherm (281 L/kg at pH2.5 and 2336 L/kg at pH5)	Pseudo-second order (16.06 g/kg at pH2.5 and 67.23 g/kg at pH5)	Wang et al. [39]
	An aquatic macrophyte, *Eichhornia crassipes*	Langmuir model (142.85 mg/g)	Pseudo-second order	Yi et al. [34]
Eu	Chitosan nanoparticle	Langmuir model (3.23 mg/g)	Pseudo-second order (0.0408 mg/g)	Cadogan et al. [9]
	Crab shell particle	Langmuir model (114.9 mg/g)	Pseudo-second order (0.3963 mg/g)	
Cr (VI)	Natural plant bark	Freundlich isotherm (9.272 mg/g)	Pseudo-second order (10.4167 mg/g)	Srivastava et al. [36]
	Chemically modified plant bark	Temkin isotherm (1.3128 L/g)	Pseudo-second order (0.8039 mg/g)	
	Sal sawdust	Langmuir model (3.6 mg/g)	Pseudo-second order	Baral et al. [38]
Pb (II)	Fern (*Cyclosorus interruptus*)	Langmuir model (46.25 mg/g)	Pseudo-second order	Zhou et al. [32]
	Yeast (*S. cerevisiae*)	Langmuir isotherm	Pseudo-second-order	Amirnia et al. [17]
Methylene blue	Giant duckweed (*Spirodela polyrrhiza*)	Langmuir model	First order	Waranusantigul et al. [2]

4.4.4 Evaluation of Equilibrium Binding of Metals

The biosorption process involves a solid phase, biosorbent and a liquid phase, solvent, normally water containing a dissolved species to be sorbed. Due to the higher affinity of the sorbent for the metal species, the latter is attracted to the solid and bound by a number of different mechanisms. This process continues until equilibrium is established between dissolved and solid-bound sorbate (at a residual, final or equilibrium concentration C_f). The affinity of the biosorbent for the metal determines its distribution between the solid and liquid phases. The quality of the sorbent material is based on the number of metals it can attract and preserve in an immobilized form. The determination of the metal uptake (q) by the biosorbent is most often based on the material balance of the sorption system: metal that disappeared from the solution must be in the solid. The sorption uptake, q, can be expressed in different units depending on the purpose of the application: for example, milligrams of metal sorbed per gram of the (dry) sorbent material (the basis for engineering process-mass balance calculations), or mmol g21 or meq g21 (when stoichiometry and/or mechanism are considered). For biosorption process scale-up and applications, the uptake expressed per unit (reactor) volume is also important [17].

4.4.5 Comparison of Sorption Performance

To examine the performance of different biosorbent, the uptake of metal by those two must be compared only at the same equilibrium concentration as the illustration (Fig. 4.4) which one comparison at low C_f (e.g., 10 mg/L) and another at high C_f (e.g., 200 mg/L) [17]. The comparison of single metal sorption performance is best based on a complete single-sorbate sorption isotherm curve derived under the same environmental conditions, pH, temperature, ionic strength for example. Sorption

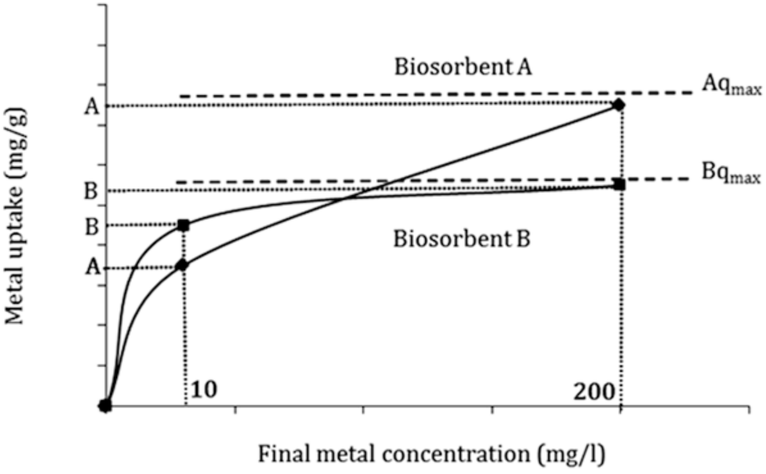


Fig. 4.4 Biosorption equilibrium-isotherm plots of metal uptake (q) against the residual (metal) concentration in the solution. Different biosorbents, A and B, are being compared. Biosorption performance in terms of uptake (q_{10}, q_{200}) has to be ruled on the same equilibrium (final) metal concentration, e.g., 10 and/or 200 mg/L. Reproduced from Kratochvil D., Volesky B. 1998. Advances in the biosorption of heavy metals. Trends. Biotechnol. 16:291–300 [17], with permission of Elsevier

isotherms are plots of the sorption uptake (q) and the final equilibrium concentration of the residual sorbate remaining in the solution (C_f).

$$q = q_{\max} \frac{bC_f}{1 + bC_f}$$

$$q = KC_f^{l/n}$$

These models do not reflect any mechanisms of metal uptake but reflecting the experimental curves. Ion exchange processes apparently play an important role in biosorption, and this is reflected in correspondingly different equilibrium models proposed for biosorption based on ion-exchange principles [17]. Langmuir isotherm is being widely used although it does not match up to the biosorption. It incorporates two easily interpretable constants: $q_{\max}$, which corresponds to the highest possible metal uptake and coefficient b, which is related to the affinity between the biosorbent and metal. Low values of b are reflected in the steep initial slope of a sorption isotherm, indicating an appropriate high affinity. Accordingly, the high quality biosorbent is one with a high $q_{\max}$ and a steep initial sorption isotherm slope (low b). Rangsayatorn et al. [25] have shown that alginate immobilized cell of *Spirulina platensis* was a better biosorbent than silica immobilized cells since $q_{\max}$ of alginate and silica immobilized cell was 70.92 and 36.63 mg Cd/g dry weight, respectively, while the binding constant (b) was 0.071 and 0.196, respectively (Fig. 4.5).

When the sorption equilibrium is established, the metal immobilized in the biosorbent will be in equilibrium with the residual concentration of metal in the liquid solution. As a consequence, the initial concentration of the metal (C_i) is of little significance to the batch sorption-equilibrium tests that can simply be seen. It can be used to identify the final concentration range which also depends on the amount of biosorbent (S) in the system. It should also be noted that in the result of the experiment there is very little control over the value of C_f–. This value is subsequently used for the uptake q calculation from the metal mass balance in the system with solution volume V:

$$q = \left(C_i - C_f\right)\frac{V}{S}$$

Therefore, it is necessary to consider both the uptake and removal efficiency when evaluating biosorbent potential. The uptake is an important parameter often used to characterize the performance of a biosorbent in a packed column. The comparison of biosorbent performance based on removal percentage is often used in the collected works which can be calculated as follows:

$$\text{Removal efficiency}\left(\%\right) = \frac{m_{\text{ad}}}{C_0 F t_e} \times 100$$

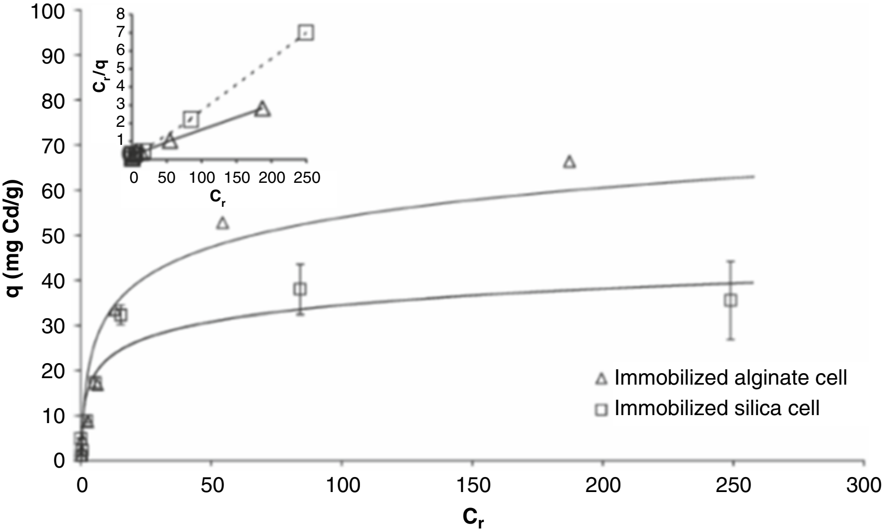


Fig. 4.5 Langmuir isotherm biosorption of *Spirulina platensis* immobilized on alginate gel and silica gel. To calculate $q_{\max}$, the Langmuir equation was rearranged as $\frac{C_f}{q} = \frac{C_f}{q_{\max}} + \frac{1}{b.q_{\max}}$, the linear transformation of which is shown in the inset. Reproduced from Rangsayatorn N., Pokethitiyook P., Upatham E.S., Lanza G.R. 2004. Cadmium biosorption by cells of *Spirulina platensis* TISTR 8217 immobilized in alginate and silica gel. Environ. Inter. 30:57–63 [25], with permission of Elsevier

where C_0 and F are the inlet solute concentration (mg/L) and flow rate (L/h), respectively. It is important to note that the removal efficiency in biosorption column is independent of the biosorbent mass, but exclusively dependent on the flow volume. The column uptake (Q_{col}) can be calculated by dividing the total mass of biosorbed sorbate (m_{ad}) by that of the biosorbent (M). The mass of biosorbed metal is calculated from the area above the breakthrough curve (C vs. t) multiplied by the flow rate.

The slope of the breakthrough curve from t_b to t_e ($\mathrm{d}c/\mathrm{d}t$) is often used to characterize the shape of the curve [54]. The overall performance of flow-through columns is strongly related to the length and shape of the ion exchange zone that develops during sorption and regeneration (Fig. 4.6).

This zone develops between the section of the column that is saturated with metal(s) and the section that still contains fresh biosorbent. The breakthrough point is the time (t_b) when the metal shows up in the effluent stream at some determined concentration. The time t_e is the time when the whole column sorption bed becomes totally saturated by the metal at its inflow concentration and the bed is no longer effective. The time interval between t_b and t_e relates to the length of the mass-transfer zone in the column bed. The fact that actual mass-transfer zones achieve S-shaped is attributable to adsorption mechanism and mass transport conditions. It is preferential with an extended breakthrough curve with a steep slope, by means of a shorter

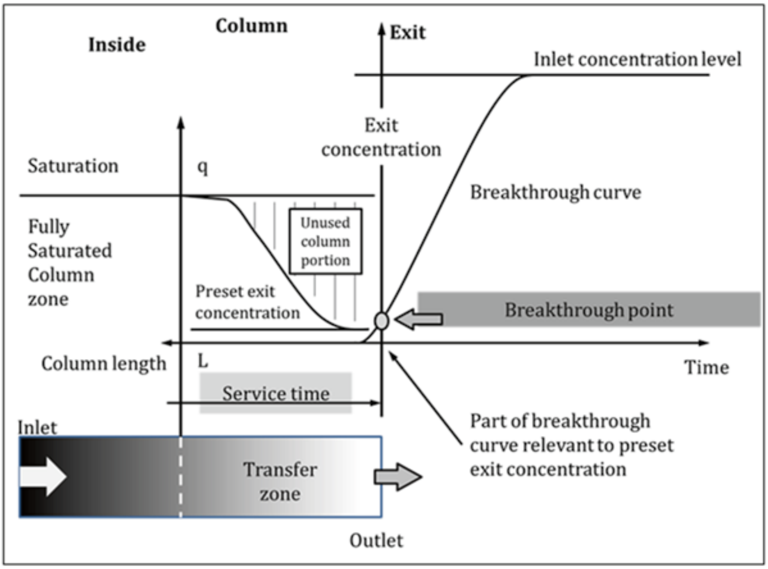


Fig. 4.6 Breakthrough point in a flow-through packed-bed biosorption column. When the metal "breaks through" and becomes detectable at the column exit at a given concentration, the column service time is over. Reproduced from Volesky B. 2003. Biosorption process simulation tools. Hydrometallurgy. 71:179–190 [47], with permission of Elsevier

mass transfer zone, which implies a longer column facility time and greater utilization of the biosorbent portion inside the column [17]. Thus, for effective biosorbents, a delayed breakthrough, earlier exhaustion, shortened mass transfer zone, high uptake, steep breakthrough curve, and high removal efficiency would be expected [7]. However, this is an estimation that could lead to complete misrepresentative conclusions on the relative sorption performance. It can only serve the purpose of basic coordination for the fast and fairly accurate screening of biosorbent materials [17].

A number of parameters can be used to characterize the performance of packed bed biosorption, including the length of the sorption zone, uptake, removal efficiency, and slope of the breakthrough curve [50, 54]. A mass transfer zone will develop between the gradually saturated section of the column and the fresh biosorbent section [55]. The length of this zone is important practically, which can be calculated from:

$$Z_m = Z\left(1 - \frac{t_b}{t_e}\right)$$

where Z denotes bed depth (cm), and t_b and t_e the column breakthrough and exhaustion times (h), respectively. A very important practical consideration arises from the affinity of different sorbates for the sorbent material. For the case of a

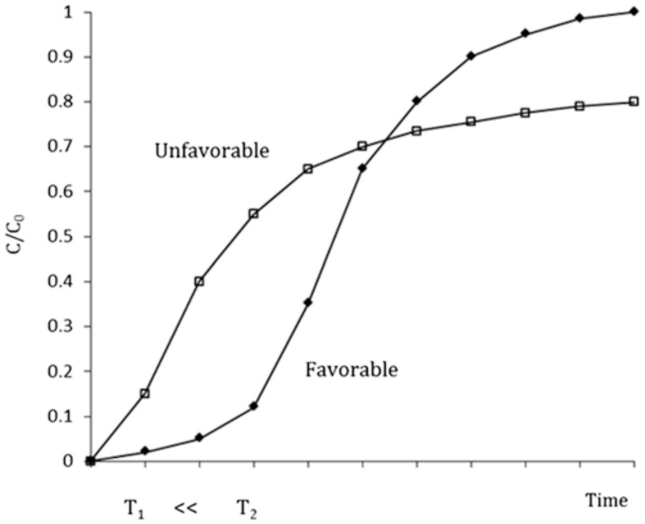


Fig. 4.7 Breakthrough curves obtained from operation of biosorption columns. An unfavorable breakthrough curve is flat and trailing, indicating a wasteful long transfer zone inside the column. A favorable breakthrough curve, on the other hand, is steep and sharp, showing the effective utilization of the biosorbent material inside the column. Reproduced from Kratochvil D., Volesky B. 1998. Advances in the biosorption of heavy metals. Trends. Biotechnol. 16:291–300 [17], with permission of Elsevier

species A sorbing onto B-saturated biosorbent, the theory of ion exchange separates between two different states of column performance, depending on the respective affinities of A and B for the sorbent material. These two states are reflected in the shapes of the breakthrough curves resulting from such column operations (Fig. 4.7).

If species A is more strongly bound to the biosorbent than species B, then the short zone develops in a column and maintains its shape as it moves down the column, on the other hand, if the affinity of B is greater than that of A, the zone extends across a large section of the column and is susceptible to extending as it moves along the column during the operation time. This indicates that a high degree of biosorbent utilization or regeneration is achieved only if the species sorbing onto a biosorbent has a higher affinity than the one used for presaturating the sorbent material. For that reason, the selection of both the ionic form of the biosorbent for the loading stage and the regenerant for the regeneration stage should follow the pattern of strongly binding A replacing weakly bound B [17].

4.5 Application of Biosorption: Packed Bed Column Continuous Flow Studies

Packed bed experiment was conducted at room temperature (25 ± 2 °C) in a 1-cm ID glass column, packed with 0.5 and 1 g of *Hydrilla verticillata* dry biomass to obtain bed volumes of 3 and 4.3 mL, respectively [33]. The results showed that a fixed-bed column packed with *H. verticillata* biomass designed to operate as a continuous liquid flow system for Cd biosorption was operational. Fixed-bed breakthrough curves at two different weights of dry biomass (0.5 and 1 g) were obtained to illustrate the capability of column operation (Fig. 4.7). The packed bed column could purify 10 mg/L Cd solution even below the detection limit of 0.02 mg/L before the breakthrough occurred at both weights used. The column containing 1 g of dry weight of the biomass removed 10 mg/L Cd solution to below the detection limit of 0.02 mg/L before the breakthrough occurred (Fig. 4.8). Regeneration of the biosorbents was also possible for at least three cycles as shown in Table 4.4.

Despite some existing limitations in biosorbent selection and usage. Biosorption of heavy metals from the aqueous solution is one of the most promising and alternative techniques for the removal of heavy metals from aqueous solutions.

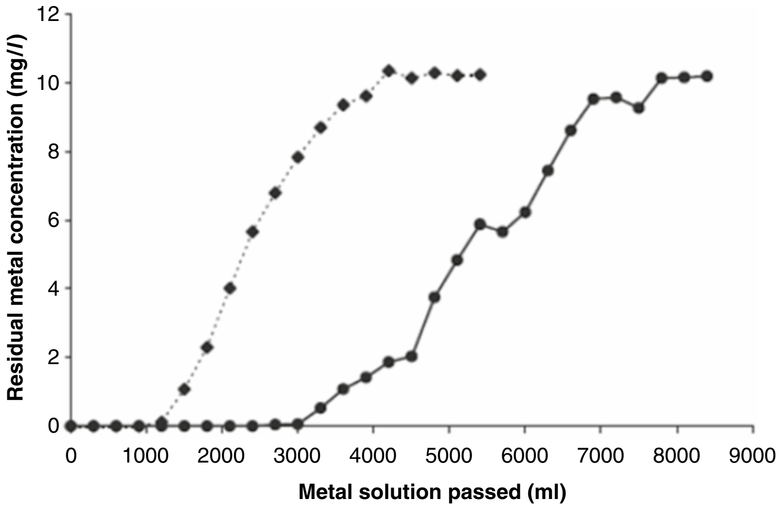


Fig. 4.8 Biosorption of Cd in fixed-bed column with continuous flow at different sorbent biomasses. Conditions: initial Cd concentration 10 mg/L; pH5; actual flow rates 10.91 mL/min for 0.5 g and 9.88 mL/min for 1 g; 25 ± 2 °C. *Symbols*: diamonds, 0.5 g of biomass; circles, 1 g of biomass

Table 4.4 Adsorption-desorption of Cd by *Hydrilla verticillata* dry biomass packed column using 0.1 M HCl as desorbing agent

Dry biomass (g)	Cycle	Cd adsorption (%)
1	1	100
	2	100
	3	95.62
2	1	100
	2	85.04
	3	98.32
3	1	100
	2	94.31
	3	80.29

References

1. O'Connell DW, Birkinshaw C, O'Dwyer TF (2008) Heavy metal adsorbents prepared from the modification of cellulose: a review. Bioresour Technol 99:6709–6724
2. Warangnusantigul P, Pokethitiyook P, Kruatrachue M, Upatham ES (2003) Kinetics of basic dye (methylene blue) biosorption by giant duckweed (*Spirodela polyrrhiza*). Environ Pollut 125:385–392
3. Farooq U, Kozinski JA, Khan MA, Athar M (2010) Biosorption of heavy metal ions using wheat based biosorbents—a review of the recent literature. Bioresour Technol 101:5043–5053
4. Zimmermann M, Wolf K (2010) Biosorption of metals. In: Hofrichter M (ed) Industrial applications: the Mycota X, 2nd edn. Springer, Berlin, pp 379–392
5. Volesky B, Holan ZR (1995) Biosorption of heavy metals. Biotechnol Pro 11:235–250
6. Kotrba P (2011) Microbial biosorption of metals—general introduction. In: Kotrba P et al (eds) Microbial biosorption of metals. Springer, New York, pp 1–6
7. Ansari MI, Masood F, Malik A (2011) Bacterial biosorption: a technique for remediation of heavy metals. In: Ahmad I, Ahmad F, Pichtel J (eds) Microbes and microbial technology: agricultural and environmental applications. Springer, New York, pp 283–291
8. Mudhoo A, Garg VK, Wang S (2012) Heavy metals: toxicity and removal by biosorption. In: Lichtfouse E et al (eds) Environmental chemistry for a sustainable world: volume 2: remediation of air and water pollution. Springer, New York, pp 379–442
9. Cadogan EI, Lee C-H, Popuri SR, Lin H-Y (2014) Efficiencies of chitosan nanoparticles and crab shell particles in europium uptake from aqueous solutions through biosorption: synthesis and characterization. Int Biodeterior Biodegrad 95:232–240
10. Nui CH, Volesky B, Cleiman D (2007) Biosorption of arsenic (V) with acid-washed crab shells. Water Res 41:2473–2478
11. Naja G, Volesky B (2011) The mechanism of metal cation and anion biosorption. In: Kotrba P et al (eds) Microbial biosorption of metals. Springer, New York, pp 19–58
12. Gadd GM (2001) Accumulation and transformation of metals by microorganisms. In: Rehm HJ, Reed G, Puhler A, Stadler P (eds) Biotechnology, 2nd edn. Wiley-VCH, Weinheim, pp 226–264
13. Gautam RK, Chattopadhyaya MC, Sharma SK (2013) Biosorption of heavy metals: recent trends and challenges. In: Sharma SK, Sanghi R (eds) Wastewater reuse and management. Springer, New York, pp 305–322
14. Srivastava S, Goyal P (2010) Biosorption: mechanistic aspects. Novel biomaterials, environmental science and engineering. Springer, Berlin, pp 47–50
15. Volesky B (1994) Advances in biosorption of metals: selection of biomass types. FEMS Microbiol Rev 14:291–302
16. Volesky B (1987) Biosorbents for metal recovery. Trend Biotechnol 5:96–101

17. Kratochvil D, Volesky B (1998) Advances in the biosorption of heavy metals. Trends Biotechnol 16:291–300
18. Wang J, Chen C (2009) Biosorbents for heavy metals removal and their future. Biotechnol Adv 27:195–226
19. Vijiyaraghavan K, Yun Y-S (2008) Bacterial biosorbents and biosorption. Biotechnol Adv 26:266–291
20. Tangaromsuk J, Pokethitiyook P, Kruatrachue M, Upatham ES (2002) Cadmium biosorption by Sphingomonas paucimobilis biomass. Biores Technol 85:103–105
21. Pagnanelli F, Panini MP, Toro L, Trifoni M, Veglio F (2000) Biosorption of metal ions on Arthrobacter sp.: biomass characterization and biosorption modeling. Environ Sci Technol 34:2773–2778
22. García R, Campos J, Cruz JA, Calderón ME, Raynal ME, Buitrón G (2016) Biosorption of Cd, Cr, Mn, and Pb from aqueous solution by Bacillus sp. strain isolated from industrial waste activate sludge. Rev Esp Cienc Quím Biol 19:5–14
23. Limcharoensuk T, Sooksawat N, Sumarnrote A, Awutpet T, Kruatrachue M, Pokethitiyook P, Auesukaree C (2015) Bioaccumulation and biosorption of Cd2+ and Zn2+ by bacteria isolated from a zinc mine in Thailand. Ecotox Environ Safety 122:322–330
24. Venkatesh P, Balraj M, Ayyanna R, Ankaiah D, Arul V (2016) Physicochemical and biosorption properties of novel exopolysaccharide produced by Enterococcus faecalis. LWT-Food Sci Technol 68:606–614
25. Rangsayatorn N, Pokethitiyook P, Upatham ES, Lanza GR (2004) Cadmium biosorption by cells of Spirulina platensis TISTR 8217 immobilized in alginate and silica gel. Environ Inter 30:57–63
26. Vijver MG, Van Gestel CAM, Lanno RP, Van Straalen NM, Peijnenburg W (2004) Internal metal sequestration and its ecotoxicological relevance: a review. Environ Sci Technol 38:4705–4712
27. Amirnia S, Ray MB, Margaritis A (2015) Heavy metals removal from aqueous solutions using Saccharomyces cerevisiae in a novel continuous bioreactor–biosorption system. Chem Eng J 264:863–872
28. Beolchini F, Pagnanelli F, Reverberi AP, Veglio F (2003) Copper biosorption onto Rhizopus Oligosporus: pH-edge tests and related kinetic and equilibrium modeling. Ind Eng Chem Res 42:4881–4887
29. Stengel DB, Connan S, Popper ZA (2011) Algal chemodiversity and bioactivity: sources of natural variability and implications for commercial application. Biotechnol Adv 29:483–501
30. Basha S, Jha B (2008) Estimation of isotherm parameters for biosorption of Cd(II) and Pb(II) onto brown seaweed, Lobophora wariegata. J Chem Eng Data 53:449–455
31. Vijayaraghavan K, Sathishkumar M, Balasubramanian R (2010) Biosorption of Lanthanum, Cerium, Europium, and Ytterbium by a brown marine alga, Turbinaria conoides. Ind Eng Chem Res 49:4405–4441
32. Zhou K, Yang Z, Liu Y, Kong X (2015) Kinetics and equilibrium studies on biosorption of Pb(II) from aqueous solution by a novel biosorbent: cyclosorus interruptus. J Environ Chem Eng 3:2219–2228
33. Bunluesin S, Kruatrachue M, Pokethitiyook P, Upatham S, Lanza GR (2007) Batch and continuous packed column studies of cadmium biosorption by Hydrilla verticillata biomass. J Biosci Bioeng 103:509–513
34. Yi Z-J, Chen H-L, Wang F, Yuan Z-M, Lui X (2016) Uranium biosorption from aqueous solution onto Eichhornia crassipes. J Environ Radioactiv 154:43–51
35. Ahmed SH, El Sheikh EM, Morsy AMA (2014) Potentiality of uranium biosorption from nitric acid solutions using shrimp shells. J Environ Radioact 134:120–127
36. Srivastava S, Agrawal SB, Mondal MK (2015) Biosorption isotherms and kinetics on removal of Cr(VI) using native and chemically modified *Lagerstroemia speciosa* bark. Ecol Eng 85:56–66
37. Argun ME, Dursun S, Ozdemir C, Karatas M (2007) Heavy metal adsorption by modified oak sawdust: thermodynamics and kinetics. J Harzad Mater 141:77–85

38. Baral SS, Das SN, Rath P (2006) Hexavalent chromium removal from aqueous solution by adsorption on treated sawdust. Biochem Eng J 31:216–222
39. Wang F, Tan L, Lui Q, Li R, Zhang H, Hu S, Liu L, Wang J (2015) Biosorption characteristics of Uranium (VI) from aqueous solution by pollen pini. J Environ Radioactiv 150:93–98
40. Saini A, Melo JS (2015) Biosorption of uranium by human black hair. J Environ Radioactiv 142:29–35
41. Sun J, Ji Y, Cai F, Li J (2012) Heavy metal removal trough biosorptive pathways. In: Sharma SK, Sanghi R (eds) Advances in water treatment and pollution prevention. Springer, New York, pp 95–145
42. Ziagova M, Dimitriadis G, Aslanidou D, Papaiannou X, Tzannetaki EL, Liakopoulou-Kyriakides M (2007) Comparative study of Cd(II) and Cr(VI) biosorption on Staphylococcus xylosus and Pseudomonas sp. in single and binary mixtures. Bioresour Technol 98:2859–2865
43. Antizar-Ladislao A, Galil NI (2004) Biosorption of phenol and chlorophenols by acclimated residential biomass under bioremediation conditions in sandy aquifer. Water Res 38:267–276
44. Yun Y-S, Volesky B (2003) Modeling of lithium interference in cadmium biosorption. Environ Sci Technol 37:3601–3608
45. Tsezos M, Remoudaki E, Angelatou V (1996) A study of the effects of competing ions on the biosorption of metals. Int Biodeterior Biodegradation 38:19–29
46. Tsezos M (2014) Biosorption: a mechanistic approach. Adv Biochem Eng Biotechnol 141:173–209
47. Volesky B (2003) Biosorption process simulation tools. Hydrometallurgy 71:179–190
48. Chu KH, Hung Y-T (2010) Modeling of biosorption processes. In: Wang LK, Tay J-H, Tay STL, Hung Y-T (eds) Handbook of environmental engineering, volume 11: environmental bioengineering. Springer, New York, pp 351–374
49. Isik M (2008) Biosorption of Ni(II) from aqueous solutions by living and non-living ureolytic mixed culture. Colloids Surf B Biointerfaces 62:97e104
50. Vijayaraghavan K, Palanivelu K, Velan M (2006) Biosorption of copper(II) and cobalt(II) from aqueous solutions by crab shell particles. Bioresour Technol 97:1411–1419
51. Febrianto J, Kosasih AN, Sunarso J, Ju YH, Indraswati N, Ismadji S (2009) Equilibrium and kinetic studies in adsorption of heavy metals using biosorbent: a summary of recent studies. J Hazard Mater 162:616–645
52. Pagnanelli F (2011) Equilibrium, kinetic and dynamic modelling of biosorption processes. In: Kotrba P et al (eds) Microbial biosorption of metals. Springer, New York, p 59
53. Pasavant P, Apiratikul R, Sungkhum V, Suthiparinyanont P, Wattanachira S, Marhaba TF (2006) Biosorption of Cu2+, Cd2+, Pb2+, and Zn2+ using dried marine green macroalga Caulerpa lentillifera. Bioresour Technol 97:2321–2329
54. Volesky B, Weber J, Park JM (2003) Continuous-flow metal biosorption in a regenerable Sargassum column. Water Res 37:297–306
55. Naja G, Volesky B (2006) Behavior of the mass transfer zone in a biosorption column. Environ Sci Technol 40:3996–4003

Chapter 5
Aquatic Macrophytes for the Removal of Heavy Metals from Coal Mining Effluent

Virendra Kumar Mishra and Reetika Shukla

5.1 Introduction

Pollution of our aquatic environment with heavy metals is currently of great concern to human being because of the health effects posed by them. Heavy metals are naturally occurring elements with atomic weights ranging between 63.5 and 200.6, and a specific gravity greater than 5.0. Living organisms require some heavy metals in trace amount such as cobalt, copper, iron, manganese, molybdenum, vanadium, strontium and zinc. Excessive levels of essential and non-essential metals, however, can be detrimental to the organism. Non-essential heavy metals of major concern to surface water systems are cadmium, chromium, mercury, lead, arsenic and antimony. Heavy metals are one of the most detrimental fractions of coal mining effluent, being persistent and accumulative in water, soil, sediment and living organism [1–4]. The threat of heavy metals to human and animal health is accelerated by their persistence in the environment. These heavy metals are bioaccumulative in nature due to which they may be transferred and accumulated in the bodies of animals or human beings through food chain.

5.1.1 Coal Mining and Water Pollution

The process of coal mining contaminates aquatic ecosystems by discharging huge amount of water on surface to facilitate mining operation. The discharged water often contains high load of TSS, TDS, hardness and heavy metals which contaminates surface and ground water [2]. Mining activity has accelerated the weathering

---

V.K. Mishra (✉) • R. Shukla
Department of Environmental Science, Indira Gandhi National Tribal University,
Amarkantak, MP 484887, India
e-mail: virendra78@gmail.com

A.A. Ansari et al. (eds.), *Phytoremediation*, DOI 10.1007/978-3-319-40148-5_5

of rocks and causes pollution of air, water, soil, degradation of forest and cropland [3]. The different mineralogical component of rocks chemically decomposes and under varying conditions would produce acidity or alkalinity. The surrounding water resources get affected when polluted mine water is discharged into aquatic system of the area which is maintaining the living being, flora and fauna [4]. Seepage of water from overburden dumps and coal seams along with discharge of industrial effluents from workshop, the mineral dressing and handling plant and domestic effluents from residential area [1, 5–7] are cause of pollution of waterbodies of surrounding areas. Acidic spoil contains toxic levels of soluble element such as Fe, Mn, Cu, Hg and Pb and inadequate supply of Mg, Ca and P. Due to seepage these elements are transported to the ground water table [8]. In mining area because of the explosion sometimes ground waters are exposed and contaminated by the toxic chemicals viz Pb and nitrogen oxides, so coal mines have a severe degree of effects, the severity of impact varies with the type, nature and the amount of sulphide minerals present in coal and associated with the rocks as well as geohydrological conditions at the mine site. Water pollution arising from coal mining is second to sewage as source of fresh water pollution [9]. Mine water discharges have harmful effect on the hydrochemistry and biological integrity of receiving waterbody. Many mine water contain seriously polluting concentration of iron yet have a neutral pH, an excess of alkalinity over acidity, such waters termed as net alkaline [10]. Coal mine drainage ranges widely in composition, from acidic to alkaline, typically with elevated concentrations of sulfate ($SO4^-$), iron (Fe), manganese (Mn) and aluminium (Al) as well as common elements such as calcium, sodium, potassium and magnesium.

5.1.2 Heavy Metal Sources for Aquatic Ecosystem in Coal Mining Environment

The following are major sources of heavy metals in coal mining area:

(a) *Drainage from Mine sites*:

Mine excavations usually have a water influx either due to rainfall or to interception of ground water. This water is usually an unwanted feature of mining though it can sometimes be used for processing and dust suppression and rest may have to be pumped out.

(b) *Sediment run-off from mining site*:

Run-off after rain can give rise to serious pollution problems. The disturbed land or active overburden dumps pile-up near the mine is usually very susceptive to erosion and silting is thus a widespread result. A variety of other pollutants may also be transported into watercourses by run-off.

(c) *Oil and fuel spills/workshop effluent*:

In case of opencast mines large number of mine machineries and vehicles are being used and thus every mine has its own workshop. Workshop effluents contain high amounts of oil and grease which are released during washing of the machineries and sometimes spillage of oils and other toxic reagents does occur in these areas which ultimately affects the water regime.

(d) *Leaching of pollutants from Overburden (OB) dumps*:

In OB dump, rainfall is likely to permeate into them and may dissolve some toxic metals from the heap and contaminate the watercourse.

5.1.3 Major Water Pollutants and Heavy Metals

Because of mixing of coal particles with water, it gets contaminated with different pollutants. In this way, huge amount of effluent is generated. Major water pollutants in coal mining effluent are as follows:

1. Total Suspended solids (TSS) and Total Dissolved solids (TDS)
2. Heavy metals (Fe, Cd, Cr, Cu, Zn, Ni, Mn)
3. Sulphate, Nitrate, Phosphate
4. Oil and Grease
5. BOD and COD
6. Oil and Grease

Mine water often contains high level of TDS ranging from 200–1000 mg L^{-1} and may have negative effect on water quality. The dissolve cation included Ca, Mg, Na and K. Major anions sulphates, chlorides, carbonate, bicarbonate, thiosulphates, sulphuric minerals might create problems due to their oxidation in acids.

Heavy metals are constant feature of mining effluent from where they may be transported to water, soil, sediments and living organism. Occurrence of toxic metals in plants and waterbodies adversely affects the lives of local people since they utilize this water for daily requirements. These heavy metals can be incorporated into food chain and their levels can increase through biological magnification [11]. Following table (Table 5.1) represents typical composition of coal mining effluent.

5.2 Phytoremediation of Heavy Metals by Aquatic Macrophytes

Phytoremediation refers to the use of plants and their associated microbes for the treatment of different pollutant by the use of green plants. It is a clean, efficient, inexpensive and non-environmentally disruptive option for the cleaning of environment. Green plants can be utilized to clean the various pollutants by stabilization, extraction, sequestration, transformation, degradation and volatilization. So far the green plants have been utilized for treatment of contaminated soils, water and air. The ability of the plant species to accumulate high concentrations of heavy metals in the roots or the leaves represents an important strategy in the remediation of sites

Table 5.1 Physico-chemical composition of coal mining effluent

	Parameter	Site 1	Site 2	Site 3
1.	pH	6.50–9.22	6.98–8.99	7.50–8.50
2.	Temp.	26.00–31.90	25.50–32.00	24.00–34.00
3.	TSS	700–1200	1000–1600	136.00–352.00
4.	TDS	136–860	192–772	453–765
5.	Oil and grease	0.03–0.05	0.08–4.78	0.001
6.	BOD	0.60–33.42	14–142	1.30–5.90
7.	COD	21.05–235.20	9.00–340.00	13.40–16.30
8.	Cu	0.2	0.34	0.27
9.	Cr	0.9	1.46	1.54
10.	Fe	1.30–3.10	0.28–4.20	4.3–12.25
11.	Cd	0.09	0.12	0.14
12.	Chloride	24.50–1009.00	27.00–73.00	15.60–14.70
13.	Ni	0.09	0.12	0.14
14.	Zn	0.08	0.12	0.11
15.	SO_4	206.00–401.00	14.00–379.40	10.20–25.80
16.	Hardness	600.50–711.40	650–781	171.00–276.00
17.	Nitrate	40.80–58.00	12.00–41.00	2.80–15.30
18.	Coliform (MPN 100 M L^{-1})	17.00–2400.00	920.00–1600.00	220.00–610.00

Source: Singh et al. [12] Used with permission of the Brahmavarta Geographical Society of India

polluted by heavy metals. Treatment of waste water employing biological means has assumed significance in recent years as it is economical and eco-friendly method of treatment. Many workers have worked on different aspects of biological treatment, such as uptake of nitrogen and phosphate by *Eichhornia crassipes* [13, 14] and use of duckweed for waste water treatment [15, 16]. Use of aquatic macrophytes for water pollution control has been very successful in various studies [17–24]. Some workers have demonstrated intracellular and extracellular uptake of cadmium by *Rhytidiadelphus squarrosus*, waste water treatment using floating aquatic macrophytes [22, 25, 26], accumulation of Cu, Zn, Cd and Pb by aquatic macrophytes [27] and accumulation of chromium in *Ceratophyllum demersum* [28].

Removal of heavy metals from waste water by aquatic macrophytes is of great interest. Many studies have been conducted on heavy metal removal by aquatic macrophytes [29], bioaccumulation of heavy metals by *Azolla filiculoides* [30], and accumulation of Hg and Cr by aquatic macrophytes [31]. According to Lytle et al. [32], some plants were accumulators of specific metals and others were found to be unspecific in the removal of diverse metals such as Pb, Cd, Cr, Mn, Fe and Cu. Many aquatic macrophytes found to accumulate higher amounts of Cu, Cr, Fe, Mn, Cd and Pb [11]. The ability of hydrophytes to remove and accumulate trace metals has been thoroughly demonstrated [33]. *Salvinia molesta* (Mitch) removed Cr and Ni more effectively than *Spirodela polyrhiza* (Schleid) when grown in a solution culture of Cr and Ni in a study. While the plant *Bacopa monnieri*, was able to accumulate higher amount of Cu than Cd in a study made by Chandra and Sinha [33].

Azolla pinnata was found to have higher uptake rate for both Pb and Zn [25]. *Lemna minor* was also found to be a good accumulator of Cd, Cu, Cr, Zn, Ni and a moderate accumulator of Cr and a poor accumulator of Ni and Pb [34]. Water hyacinth (*Eichhornia crassipes* L.) has been intensively studied as a bioindicator, and is reported to effectively accumulate a number of contaminants. Several aquatic macrophytes have been used for the removal of heavy metals from the water and waste water. Many workers have put their works on different aspects of removal [1, 35–42] and toxicity of these metals for the plants [43].

5.3 Aquatic Macrophytes for Phytoremediation

*5.3.1 Water Hyacinth (*Eichhornia crassipes*)*

The water hyacinth (*Eichhornia crassipes* Mart. Solms.) is a productive aquatic macrophyte which is found as a noxious weed in the most tropical and subtropical regions of the world. This is an angiosperm, large leaved, floating hydrophyte belonging to the family Pontederiaceae. This is a perennial, free floating weed and partly rooted in shallow water with numerous fibrous roots, short stem, broad glossy green leaves and light lavender flowers. It shows rapid vegetative reproduction and the water surface is rapidly covered by a thick mat. It is one of the most commonly used plants in constructed wetlands because of its fast growth rate and large uptake of nutrients and contaminants. Dried water hyacinth roots, ash derived from water hyacinth and the whole plant were used for the metal uptake by the roots of the plant [44–49]. *Eichhornia* has an outstanding pollutant removal capacity and many workers have studied the implications of growing *Eichhornia* and role of water hyacinths and their roots for reducing different pollutants from water and waste water including heavy metal [50–52].

5.3.2 Lemna minor

Lemna minor is an angiospermic aquatic plant; it is small leaved, free floating hydrophyte. It is a fast growing species and its biomass can be doubled in 4 days under optimum growth conditions. Many researches supported the use of duckweeds in heavy metal as well as organic matter removal study because of its fast growth and hyperaccumulation capacity. Axtell et al. (2003) have reported that duckweed (*Lemna minor*) can remove 82 % nickel and 76 % of lead [53]. It has been used for removal of Zn, Ni, Fe, Cu, Cr and Pb from electroplating waste water [54]. Heavy metal and all the other pollutants are removed through its leaves [55]. Leela Kaur et al. [56] showed the effect of pH on duckweed for the removal of Pd and Ni. They claimed the 99.99 % removal of Pb at pH5–6 and 99.3 % removal of Ni at pH 6 in 28 days time period. Effects of *Lemna minor* on the restoration of a polluted waterbody have also proved it an efficient cleaning agent [57].

5.3.3 Spirodela polyrrhiza

It is a floating or submerged perennial herb, a plant without roots or with the reduced roots to unbranched rhizoids. The whole plant body is reduced to a small or minute oval, oblong flat or globose thallus. *Spirodela polyrrhiza* belongs to the family lemnaceae and can be found worldwide on the surface of fresh and brackish waters [58]. The *Spirodela* is among the most standardized test organisms in aquatic ecotoxicology and used for large number of heavy metal removal studies [59]. *Spirodela* has been found successful in removing heavy metals from different matrices [1, 29].

5.3.4 Pistia stratiotes

It is a floating, stoloniferous aquatic plant found in ponds and streams, spread all over India up to a height of 1000 m. It is a perennial monocotyledon belonging to family araceae with thick, soft leaves that form a rosette. It floats on the surface of the water, its roots hanging submersed beneath floating leaves. The leaves can be up to 14 cm long and have no stem. *Pistia stratiotes L.* is a 'hyperaccumulator' and can remove, degrade or sequester heavy metals, organic compounds and radionuclides from water [60, 61]. Use of this macrophyte is increasing for remediation of contaminated aquatic system due to its low cost, and several other advantages over other methods [62]. Lower size of the plant for removal of heavy metals is a credit for this plant as compared to water hyacinth [63–65]. It has been used for Zn extraction from industrial wastes as it has strong affinity to Zn absorption in an eco-friendly manner [66]. The same is true about mercury too [67]. The accumulation of heavy metals like Fe, Zn, Cu, Cr and Cd does not cause any toxic effect on the plant which makes it more useful plant to be used for the phytoremediation of waste water for heavy metals on large scale. It has been considered a promising plant for the remediation of contaminated water [37, 68].

5.3.5 Azolla pinnata

Azolla is a small aquatic fern belonging to family azollaceae with a monotypic genus. *Azolla pinnata* is one of its important species of this family, native to Africa, Asia including India and Australia. It is an aquatic plant, floating upon the surface of the water which grows in slow-moving waterbodies [69, 70]. It is the only pteridophyte having symbiotic association with nitrogen-fixing cyanobacterium, and bacteria residing in leaf cavities [70]. Because of this ability the *Azolla* has been used as food and feed supplements, and weed suppressors in biogas and hydrogen production and waste water treatment [71, 72]. Some of its properties like fast growth rate, nitrogen fixing ability and hyperaccumulation capacity make it an excellent choice for phytoremediation [73–77].

5.4 Phytoremediation of Coal Mining Effluent

Coal is the most important fuel used for generating electricity worldwide; however, the process of coal mining involves the release of huge amount of effluent into the surface water. Effluent from coal mine contains high load of TSS, TDS, calcium carbonate and heavy metals, hence contaminates the aquatic regime [1, 2, 36, 37]. Heavy metals are the most damaging fractions of mining effluent, being persistent and accumulative in water, soil, sediment and living organism [1, 2]. Occurrence of toxic metals in plants and waterbodies adversely affects the lives of local people since they utilize this water for daily requirements. These heavy metals can be incorporated into food chain and their levels can increase through biological magnification [11]. Most of the coal mining effluent generated, ultimately find their way into the waterbodies i.e. rivers, ponds and lakes. The presence of these wastes in waterbodies becomes objectionable, as it hinders the sustenance of life in a proper way. In recent years, scientific community all over the world has shown great concern over the deteriorating state of waterbodies. Several techniques, all around the globe, have been developed to remove pollutants from the waterbodies. Most of these techniques though effective on one ground fell short on other grounds [1, 29, 37] and moreover, they are too costly to be adopted in a feasible manner. Most of the modern technologies used to treat the waste waters are quite costly and energy intensive. So, most of the developing countries may not be able to afford the huge expenditure required to treat the waste water.

Phytoremediation studies have been proven extremely useful for the removal of variety of pollutants including heavy metals from the coal mining effluent. However, only few studies are available to examine the pollutant removal capacity of different aquatic plants from coal mining effluent. A number of aquatic macrophytes have been used for phytoremediation of heavy metals from coal mining effluent. Some of the important studies are shown in Table 5.2.

Table 5.2 Phytoremediation studies for removal of heavy metals from coal mining effluent

Macrophytes	Heavy metals	Performance	Reference
Eichhornia crassipes	Cu, Cd, Cr, Zn and Ni	*E. crassipes* removed 70.5 %, 69.1 %,76.9 %,66.4 %,65.3 % and 55.4 % of Fe, Cr, Cu, Cd, Zn and Ni, respectively	Mishra et al. [1]
Spirodela polyrrhiza			
Lemna minor			
Eichhornia crassipes, Lemna minor	Hg, As	*E. crassipes* accumulated 0.45±0.02 and 0.34±0.012 µg g^{-1} mercury and arsenic	Mishra et al. [29]
Spirodela polyrrhiza			
Pistia stratiotes (*Azolla pinnata*)	Hg	*P. stratiotes* and *A. pinnata* removed 80 % and 68 % of Hg, respectively	Mishra et al. [37]

5.5 Studies on Phytoremediation of Heavy Metals from Coal Mining Effluent

5.5.1 *Removal of Five Heavy Metals from Mining Effluents by Three Macrophytes*

In a study performed by Mishra et al. [1] for removal of selected heavy metals from coal mining effluent, three aquatic macrophytes *Eichhornia crassipes, Spirodela polyrhiza* and *Lemna minor* were grown in monoculture as well as mixed culture under laboratory conditions. These macrophytes were grown in 100 lt coal mining effluent collected from the field in following seven sets.

Set 1—containing no aquatic plants (control)
Set 2—containing 100 % coverage of *E. crassipes*
Set 3—containing 100 % coverage of *Lemna minor*
Set 4—containing 100 % coverage of *Spirodela polyrhiza*
Set 5—containing 50 % coverage of *E. crassipes* and 50 % coverage of *Spirodela polyrhiza*
Set 6—Containing 50 % coverage of *Lemna minor* and 50 % coverage of *Spirodela polyrhiza*
Set 7—containing 50 % coverage of *E. crassipes* and 50 % coverage of *Lemna minor*

Results of this study revealed that *E. crassipes* has removed highest percentage of heavy metals among the selected aquatic macrophytes. *E. crassipes* removed 70.5, 69.1, 76.9, 66.4, 65.3 and 55.4 % Fe, Cr, Cu, Cd, Zn and Ni, respectively. The other aquatic macrophytes *L. minor* removed 60.6, 59.8, 58.4, 58.0, 57.0 and 47.4 % of Fe, Cr, Cu, Cd, Zn and Ni, respectively. Whereas *S. polyrrhiza* removed 63.8, 61.2, 60.5, 64, 65.6 and 57.9 % of Fe, Cr, Cu, Cd, Zn and Ni, respectively (Fig. 5.1).

5.5.2 *Removal of Mercury and Arsenic from Mining Effluent by Three Macrophytes*

In another study, phytoremediation of mercury and arsenic from a tropical opencast coal mine effluent was performed by Mishra et al. [29]. Three aquatic macrophytes *Eichhornia crassipes, Lemna minor* and *Spirodela polyrrhiza* removed mercury (Hg) and arsenic (As) from coal mining effluent. Highest removal was done by *E. crassipes* followed *L. minor* and *S. polyrrhiza*, respectively. Study suggested that there was a low transportation of metals from root to leaves leading to higher accumulation of metals in root. It was evident from plant tissue analysis that mercury and arsenic uptake by macrophytes had deteriorated the N, P, K, chlorophyll and protein content in these macrophytes. Based on these results, selected species can be used as promising accumulator of metals. Results shown in Fig. 5.2 revealed concentration of mercury as 0.007 ± 0.0001 and arsenic 0.05 ± 0.001 mg L^{-1},

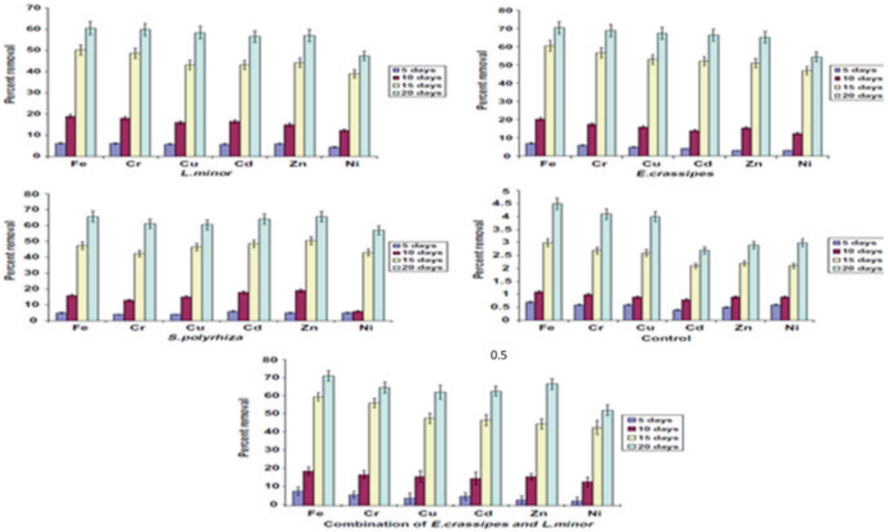


Fig. 5.1 Removal of heavy metals from coal mining effluent by three aquatic macrophytes (*Source*: Mishra et al. [1]. Used with permission of Elsevier.)

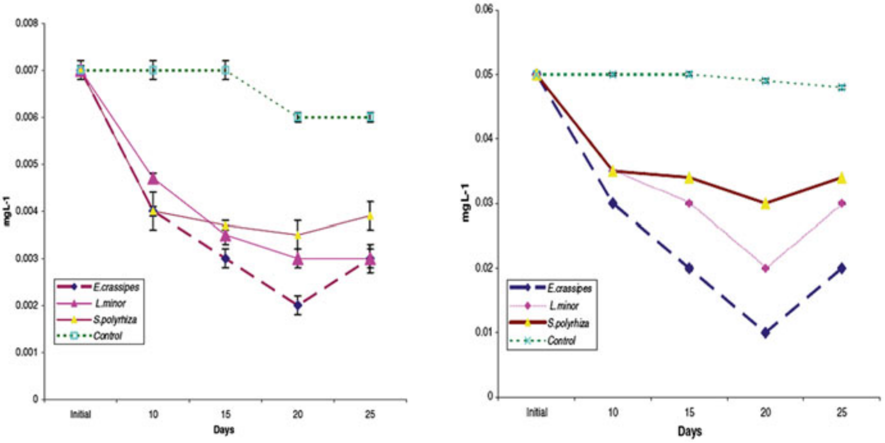


Fig. 5.2 Removal of Arsenic and Mercury from coal mining effluent (*Source*: Mishra et al. [29]. Copyright 2008, Springer Science+Business Media.)

respectively, in effluent at initial stage in all the experimental sets, which was found in decreasing order in 20-day experiment [29]. This indicates continuous absorption of metals by the plants. Highest removal of Hg and As from the effluent by E. crassipes (71 % and 80 % Hg and As, respectively) was shown. The high performance may be due to its fast growth, greater biomass accumulation and higher affinity towards uptake. Variations in the metal uptake may be associated with the difference in the rate of plant growth and efficiency towards metal absorption (Fig. 5.2).

5.5.3 Removal of Mercury from Coal Mining Effluent by Two Macrophytes

In this study, the mercury (Hg) removal capacities of two aquatic macrophytes, *Pistia stratiotes* and *Azolla pinnata,* were investigated against the coal mining effluent by Mishra et al. [37]. These plants reduced mercury from the effluent via rhizofiltration and subsequent accumulation in plant. The macrophytes *P. stratiotes* and *A. pinnata* removed 80 % and 68 %, respectively, after 21 days of exposure to the effluent containing 10 mg L^{-1} of Hg. As mercury from the effluent was accumulated in the root and shoot tissues of both aquatic macrophytes , they were proven to be a root accumulator with a translocation factor of less than one during the entire study. The decreasing Hg content in effluent (from 10 to 2.0 mg L^{-1}) was reflected by its accumulation in roots (0.57 ± 0.02 mg g^{-1} in *P. stratiotes)* and the leaves of the experimental plants (0.42 ± 0.01 mg g^{-1}, *P. stratiotes*) (Fig. 5.3). As a result, Hg concentrations in the coal mining effluent were tightly associated with those observed from macrophytes. Considering the high removal efficiencies of Hg by these aquatic macrophytes, these plants can be recommended for the actual treatment of Hg-containing waste waters.

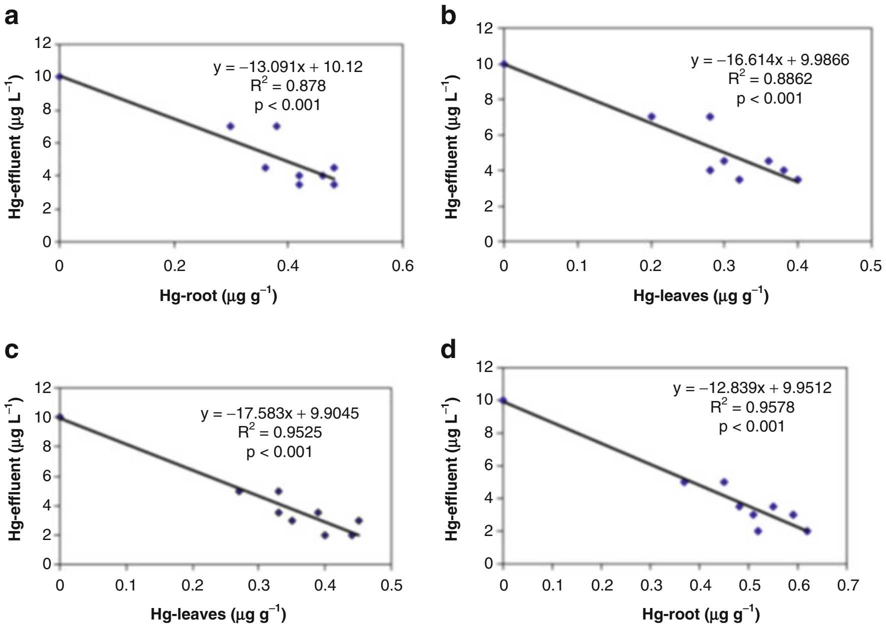


Fig. 5.3 Mercury accumulation in roots and leaves of *Pistia stratiotes* (*Source*: Mishra et al. 2009 [37]. Used with permission of Elsevier.)

5.6 Conclusion

Heavy metals are one of the most important pollutants of the aquatic environment. Coal mining activity is one such activity which is contributing towards the pollution of aquatic environment. Along with other pollutants heavy metals are the important component of coal mining effluent which needs to be treated in eco-friendly manner. Five aquatic macrophytes, *Eichhornia crassipes, Spirodela polyrhiza*, *Lemna minor*, *Pistia stratiotes* and *Azolla pinnata,* were used successfully for the removal of heavy metals from coal mining effluent. These macrophytes can be used for large-scale treatment of heavy metals from this source. The removal efficiency of the *Eichhornia crassipes* was found higher for heavy metals like iron (Fe), zinc (Zn), copper (Cu), chromium (Cr), cadmium (Cd), manganese (Mn), mercury (Hg) and arsenic (As) from coal mining effluent in laboratory experiment.

References

1. Mishra VK, Upadhyayay AR, Pandey SK, Tripathi BD (2008) Heavy metal pollution induced due to coal mining effluent on surrounding aquatic ecosystem and its management through naturally occurring aquatic macrophytes. Bioresour Technol 99:930–936
2. Dhar BB, Ratan S, Jamal A (1986) Impact of Opencast coal mining on water environment—a case study. J Mines Metals Fuels 34(12):596–601
3. Jamal A, Dhar BB, Ratan S (1991) Acid mine drainage control in an opencast coal mine. Int J Mine Water Environ 10:1–16
4. Chakraborty MK, Chaulya SK, Ahmad M, Singh KK, Singh RS, Tiwari BK, Gupta PK (2000) Hydro-geological conditions around an opencast mine. Minetech 22(1):41–53
5. Younger PL (1994) Minewater pollution: the revenge of old king coal. Geoscientist 4(5):6–8
6. Younger PL (1995) Hydrogeochemistry of mine waters flowing from abandoned coal workings in the Durham coal fields. Quart J Eng Geol 28(4):101–113
7. Younger PL (1995) Mine water pollution in Britain: past, present and future. Mineral Plann 65:38–41
8. Younger PL (1997) Mine water treatment using wetlands. In: Young PL (ed) Proceedings of national conference, University of Newcastle, London: Chartered Institution of Water and Environmental Management, 5 Sept 1997, 189p
9. Younger PL (1997) The longevity of mine water pollution: a basis for decision making. Sci Total Environ 1(94/195):457–466
10. Banks D, Younger PL, Arnesen RT, Iversen ER, Banks SD (1997) Mine water chemistry: the good, the bad and the ugly. Environ Geol 32:157–174
11. Cardwell A, Hawker D, Greenway M (2002) Metal accumulation in aquatic macrophytes from southeast Queensland, Australia. Chemosphere 48:653–663
12. Singh VK, Singh MB, Mishra VK (2009) Aquatic pollution and physic chemical characteristics of effluent in coal mining area, Sonebhadra (U.P.). Uttar Pradesh Geogr J 14:1–6
13. Ayoma I, Hisao N, Ma SY (1986) Uptake of nitrogen and phosphate, and water purification capacity of water hyacinth. Ber Ohara Inst Landw Biol 19:77–89
14. Ayoma I, Nishizaki H, Yogi M (1987) Uptake of nitrogen and phosphate and water purification capacity by water hyacinth (*Eichhornica crassipes* (Mart.) Solms.). Soil Fertility 50:345–349
15. Alnozaily F, Alaerts G, Veenstra S (2000) Performance of duckweed covered sewage lagoons-II. Nitrogen and phosphorus balance and plant productivity. Water Res 34(10):2734–2741

16. Smith MD, Moelyowati I (2001) Duckweed based wastewater treatment (DWWT): design guidelines for hot climates. Water Sci Technol 43(11):291–300
17. Pandey AK (2015) Waste water management through aquatic macrophytes. Int Res J Environ Sci 4(3):41–46
18. Brix H, Schierup HH (1989) The use of aquatic macrophytes in water pollution control. Ambio 18:100–107
19. Gersberg RM, Elkins BV, Lyon SR, Goldman CR (1986) Role of aquatic plants in waste water treatment by artificial wetlands. Water Res 20:363–368
20. Reddy KR, De Busk WF (1985) Nutrient removal potential of selected aquatic macrophytes. J Environ Qual 14:459–462
21. Reddy KR, De Busk WF (1987) State of the art utilization of aquatic plants in water pollution control. Water Sci Technol 18:61–79
22. Tripathi BD, Shukla SC (1991) Biological treatment of waste water by selected aquatic plants. Environ Pollut 69(1):69–78
23. Tripathi BD, Srivastava J, Mishra K (1991) Nitrogen and phosphorus removal capacity of four chosen aquatic macrophytes in tropical freshwater ponds. Environ Conserv 18(2):143–147
24. Tripathi BD, Mishra K, Pandey VS, Srivastava J (1990) Effect of tissue-N content on decomposition of water hyacinth (Eichhornia crassipes Mart) Solms. Geobios 17(2–3):67–69
25. Jain SK, Vasudevan P, Jha NK (1990) Azolla pinnata and Lemna minor for removal of lead and zinc from polluted water. Water Res 24(2):177–183
26. Maine M, Duarte MV (2001) Cadmium uptake by floating macrophytes. Water Res 35(11):2629–2634
27. Fayed SE, Hussaini AS (1985) Accumulation of Cu, Zn, Cd and Pb by aquatic macrophytes. Environ Int 11(1):77–87
28. Garg P, Chandra P (1990) Toxicity and accumulation of chromium in Ceratophyllum demersum L. Bull Environ Contam Toxicol 44(3):473–478
29. Mishra VK, Upadhyay AR, Pathak V, Tripathi BD (2008) Phytoremediation of Hg and As from tropical opencast coalmine effluents through naturally occurring aquatic macrophytes. Water Air Soil Pollut 192(1–4):303–314
30. De Wet LPD, Schoonbee HJ, Pretorius J (1990) Bioaccumulation of selected heavy metals by the water fern, Azolla filiculoides Lam, in a wetland ecosystem affected by sewage mine and industrial pollution. Water SA WASADV 16(14):281–286
31. Jana S (1988) Accumulation of Hg and Cr by three aquatic species and subsequent changes in several physiological and Biochemical parameters. Wat Air Soil Pollut 38(1–2):105–109
32. Lytle CM, Lytle FW, Yang N, Quian JH, Hansen D, Zayed A, Terry N (1998) Reduction of Cr(VI) to Cr(III) by wetland plants: potential for in situ heavy metal detoxification. Environ Sci Technol 32(20):3087–3093
33. Chandra P, Sinha S (1992) Assessment of heavy metal (Cu, Cd, Pb, Cr, Mn) uptake by wetland plant Bacopa monnieri from artificially contaminated sediments. In: Proc. IV INTECOL. International wetland conference, Columbus, p 118
34. Zayed A, Gowthaman S, Terry N (1998) Phytoaccumulation of trace elements by wetland plants: I. Duck weed. J Environ Qual 27:715–721
35. Hassan SH, Talat M, Rai S (2007) Sorption of cadmium and zinc from aqueous solutions by water hyacinth (Eichhornia crassipes). Bioresour Technol 98(4):918–928
36. Miretzky P, Saralegui A, Fernández Cirelli A (2004) Aquatic macrophytes potential for the simultaneous removal of heavy metals (Buenos Aires, Argentina). Chemosphere 57(8):997–1005
37. Mishra VK, Tripathi BD, Kim K (2009) Removal and accumulation of mercury by aquatic macrophytes from an open cast coal mine effluent. J Hazard Mater 172:749–754
38. Bala R, Thukral AK (2011) Phytoremediation of Cr (VI) by Spirodela polyrrhiza (L.) Schleiden employing reducing and chelating agents. Int J Phytoremediation 13(5):465–491
39. Rezania S, Ponraj M, Talaiekhozani A, Mohamad S, Din MFM, Taib SM, Sabbagh F, Sairan FM (2015) Perspectives of phytoremediation using water hyacinth for removal of heavy metals, organic and inorganic pollutants in wastewater. J Environ Manage 163:125–133

40. Tauqeer HM, Ali S, Rizwan M, Ali Q, Saeed R, Iftikar U, Ahmad R, Farid M, Abbasi H (2016) Phytoremediation of heavy metals by *Alternanthera bettzickiana*: growth and physiological response. Ecotoxicol Environ Saf 126:138–146
41. Harguinteguy CA, Pignata ML, Fernández-Cirelli A (2015) Nickel, lead and zinc accumulation and performance in relation to their use in phytoremediation by macrophytes *Myriophyllum aquaticum* and *Egeria densa*. Ecol Eng 82:512–516
42. Vymazal J, Březinová T (2016) Accumulation of heavy metals in aboveground biomass of Phragmites australis in horizontal flow constructed wetlands for wastewater treatment: a review. Chem Eng J 290:232–242
43. Drost W, Matzke M, Backhaus M (2007) Heavy metal toxicity to Lemna minor: studies on the time dependence of growth inhibition and the recovery after exposure. Chemosphere 67(1):36–43
44. Tiwari S, Dixit S, Verma N (2007) An effective means of biofiltration of heavy metal contaminated water bodies using aquatic weed Eichhornia crassipes. Environ Monit Assess 129:253–256
45. Hasan SH, Deeksha R, Talat M (2010) Water hyacinth biomass for the biosorption of hexavalent chromium: optimization of process parameters. Bioresources 5(2):563–575
46. Elangovan R, Philip L, Chandraraj K (2008) Biosorption of chromium species by aquatic weeds: kinetics and mechanism studies. J Hazard Mater 152:100–112
47. Mahmood T, Malik SA, Hussain ST (2010) Biosorption and recovery of heavy metals from aqueous solutions by Eichhornia crassipes (water hyacinth) ash. Bioresoures 5(2):1244–1256
48. Kadirvelu K, Kanmani P, Senthilkumar P, Subburam V (2004) Separation of mercury (II) from aqueous solution by adsorption onto an activated carbon prepared from Eichhornia crassipes. Adsorp Sci Technol 22:207–222
49. Jadia CD, Fulekar MH (2009) Review on phytoremediation of heavy metals: recent techniques. Afr J Biotechnol 8(6):921–930
50. Kim Y, Kim WJ (2000) Roles of water hyacinths and their roots for reducing algal concentration in the effluent from waste stabilization ponds. Water Res 34(13):3285–3294
51. Mishra VK, Tripathi BD (2008) Concurrent removal and accumulation of heavy metals by the three aquatic macrophytes. Biores Technol 99:7091–7097
52. Mishra VK, Tripathi BD (2009) Accumulation of chromium and zinc from aqueous solutions using water hyacinth (*Eichhornia crassipes*). J Hazard Mater 164:1059–1063
53. Axtell N, Sternberg S, Claussen K (2003) Lead and nickel removal using Microspora and Lemna minor. Biores Technol 89:41–48
54. Horvat T, Vidaković-Cifrek Z, Orescanin V, Tkalec M, Pevalek-Kozlina B (2007) Toxicity assessment of heavy metal mixtures by Lemna minor L. Sci Total Environ 384(1–3):229–238
55. Naumann B, Eberius M, Appenroth KJ (2007) Growth rate based dose–response relationships and EC-values of ten heavy metals using the Duckweed growth inhibition test (ISO 20079) with Lemna minor L. clone St. J Plant Physiol 164:1656–1664
56. Kaur L, Kasturi G, Sharma S (2012) Role of pH in the accumulation of lead and nickel by common Duckweed (Lemna minor). Int J Bioassays 1(12):191–195
57. Hou W, Chen X, Song G, Wang Q, Chang CC (2007) Effects of copper and cadmium on heavy metal polluted water body restoration by Duckweed (Lemna minor) physiology. Plant Physiol Biochem 45:62–69
58. Zimmo OR, van der Steen NP, Gijz HJ (2003) Comparison of ammonia volatilisation rates in algae and duckweed-based waste stabilisation ponds treating domestic wastewater. Water Res 37:4587–4594
59. Aquatic plant toxicity test using LemnaSpp (1996) Tiers I and II-OPPTS 850.4400. United states Environmental protection Agency Prevention, pesticides and Toxic substances Unit, Newyork, 9p
60. Sinha S, Basant A, Malik A, Singh KP (2009) Multivariate modeling of chromium induced oxidative stress and biochemical changes in plants of Pistia stratiotes L. Ecotoxicology 18:555–566

61. Sinha S, Saxena R, Singh S (2002) Comparative studies on accumulation of Cr from metal solution and Tannery effluent under repeated metal exposure by aquatic plants: its toxic effects environ. Monitor Assess 80:17–31
62. Prasad MNV, Freitas HMD (2003) Metal hyperaccumulation in plants-biodiversity prospecting for phytoremediation technology. Electr J Biotech 6:285–321
63. Quinones E, Silva EA, Palacio SM, Modenes AN, Rizzutto MA, Rossi FL, Szymanski N, Costa Jr. IL, Thome LP, Castro JKD (2003) Removal of chromium ions by three aquatic macrophytes from an aqueous solution. Brazilian Synchrotron Light Lab. LNLS, Activity Report, pp 1–2
64. Mufarrege MM, Hadad HR, Maine MA (2010) Response of Pistia stratiotes to heavy metals (Cr, Ni, and Zn) and P. Arch Environ Contam Toxicol 58:53–61
65. Singh G, Sinha A (2011) Phytoremediation of chromium (VI)-laden waste by Eichhornia crassipes. Int J Environ Tech Manag 14:33–42
66. Nurhayati P, Abimanyu S, Kaswati S, Fajr IR (2012) Water lettuce (P. stratiotes L.) potency as one of ecofriendly phytoextraction absorbers of zinc heavy metal to solve industrial waste problem in Indonesia. Inter Conf Environ Biomed Biotech 41:151–156
67. Skinner K (2007) Mercury uptake and accumulation by four species of aquatic plants. Environ Pollut 145:234–237
68. Maine MA, Duarte MV, Sue NL (2001) Cadmium uptake by floating macrophytes. Water Res 35(11):2629–2634
69. Pabby A, Ahluwalia AS, Dua S (2003) Current status of Azolla taxonomy. In: Ahluwalia AS (ed) Phycology: principles, processes and applications. Daya Publishers, India, pp 48–63
70. Sood A, Ahluwalia AS (2009) Cyanobacterial–plant symbioses with emphasis on Azolla-Anabaena symbiotic system. Indian Fern J 26:166–178
71. Ahluwalia AS, Pabby A, Dua S (2002) Azolla: a green gold mine with diversified applications. Indian Fern J 19:1–9
72. Pabby A, Prasanna R, Singh PK (2004) Biological significance of Azolla and its utilization in agriculture. Proc Indian Natl Sci Acad B 70:301–335
73. Padmesh TVN, Vijayraghavan K, Sekaran G, Velan M (2006) Application of Azolla rongpong on biosorption of acid red 88, acid green 3, acid orange 7 and acid blue 15 from synthetic solutions. Chem Eng J 122(1–2):55–63
74. Rai PK, Tripathi BD (2009) Comparative assessment of Azolla pinnata and Vallisneria spiralis in Hg removal from G.B. Pant Sagar of Singrauli Industrial region, India. Environ Monit Assess 148(1):75–84
75. Mashkani SG, Ghazvini PTM (2009) Biotechnological potential of Azolla filiculoides for biosorption of Cs and Sr: application of micro-PIXE for measurement of Biosorption. Bioresour Technol 100(6):1915–1921
76. Sood A, Pabbi S, Uniyal PL (2011) Effect of paraquat on lipid peroxidation and antioxidant enzymes in aquatic fern Azolla microphylla Kual. Russian J Plant Physiol 58:667–673
77. Sood A, Uniyal PL, Prasanna R, Ahluwalia AS (2012) Phytoremediation potential of aquatic macrophyte, Azolla. Ambio 41:122–137

Chapter 6
Heavy Metals Accumulation Ability of Wild Grass Species from Industrial Areas of Kazakhstan

Saule Atabayeva

6.1 Introduction

Heavy metals are prevalent man-made pollutants that cause a set of human diseases, entering a body through the food chain as a consequence of contamination of soils and vegetation. The search for effective methods of remediation of technogenic contaminated soils is an important environmental task in Kazakhstan. The urgency of the problem is associated with soil heavy metals contamination, in particular, in the area around the steel mills and tailings. Heavy metals, which come into the soil in a variety of ways as a result of human activity, are dangerous environmental pollutants. The amount of heavy metals accumulated in this way can be many times greater than its natural content in soil. Dispersion of man-made heavy metals pollution into the atmosphere has become global. The main sources of contamination of the environment by copper, lead, zinc, and cadmium are mining, metallurgical and chemical industries, heat power engineering, transport and chemical pesticides, as well as household waste. Pollution of air, soil, plants, and water by heavy metals in the vicinity of large industrial centers has become one of the most pressing environmental problems. In soils near industrial areas, heavy metal content is tens or hundreds of times higher than background levels in similar soils [1].

High concentrations of some heavy metals in the soil are adequately reflected in the yield and quality of vegetable products grown within the boundaries of the industrial centers in the garden plots. In vegetation, heavy metals content exceeds the allowable concentrations in 2–3.5 times [2, 3]. The excessive concentrations of heavy metals in plants violate the physiological and biochemical processes, inhibit the growth and development of plant organisms, and reduce the quality of the agricultural products obtained. Thus, the increasing of anthropogenic pollution by

S. Atabayeva (✉)
Department of Biology and Biotechnology, Al-Farabi Kazakh National University,
Research Institute of Ecology, Almaty, Kazakhstan
e-mail: sauleat@yandex.ru

A.A. Ansari et al. (eds.), *Phytoremediation*, DOI 10.1007/978-3-319-40148-5_6

heavy metals, the migrating of heavy metals along the trophic relations, lead to various adverse effects on living organisms. It is well-known that heavy metals negatively affect the biochemical and physiological processes of plants, change membrane properties [4–6], the activity of the enzymes, cause oxidative stress [7–9], resulting in inhibited growth processes, in changing the time required for the phenological phases, in morphological changes occurring in plant organs, in reduced yields. In response to heavy metals contamination in plants, a number of protective mechanisms are activated, such as the increased synthesis of metallothioneins (phytochelatins), organic acids, polyamines, and antioxidant enzymes [10–13] to reduce the toxic effects of heavy metals and to maintain homeostasis.

But in various kinds of plants, defense mechanisms against stress develop in varying degrees. Different species and even populations within a species may differ in sensitivity to heavy metals and the degree of their accumulation in their organs, which can be the basis of the formation of metallophytic flora [14]. The use of characteristics of plants like a resistance to heavy metals and a high metal-accumulating ability are the basis of the technology of phytoremediation of contaminated soil, which is defined as a cleaning technology of the environment from chemical contaminants using plants [15–17]. One of the necessary steps towards preventing the toxic effect of heavy metals on animal and human being is to clean soil. Currently, the most effective method is soil phytoremediation, i.e., cleaning soil using plants-hyperaccumulators of heavy metals. Compared to physical and chemical methods of soil cleaning, this method is less expensive, more efficient and safe [15, 18]. According to literature, the cost of conservative methods (chemical and physical methods) of cleaning the soil ranges from $30–$350 per hectare, and the cost of cleaning the soil using plants is about $160 per hectare [15]. According to other estimates, depending on the soil conditions and concentration of heavy metals, the cost of treatment with the help of plants (using only the sun energy) may be only 5 % of the costs required for other methods of ecosystem restoration, contaminated by metals [19]. It is necessary to use various plant species which are adapted to native environment, tolerant to heavy metals, and able to accumulate them in their organs and to implement these plants for different types of phytoremediation to reduce the risk of further spread of environment pollution.

6.1.1 Phytoremediation Technology

There are different types of phytoremediation. The technology includes phytoextraction, phytovolatilization, phytostabilization, and rhizofiltration [19, 20]. Phytoextraction — the use of plants that accumulate metals mainly in the aerial organs and the further using of the aerial part for the incineration and recovery of metals from plant's ash (Figs. 6.1 and 6.2). This type of phytoremediation is used to extract metals out of the soils. The advantage of this method is the ability to extract a large amount of metals from the soil by aerial organs of plants-hyperaccumulators.

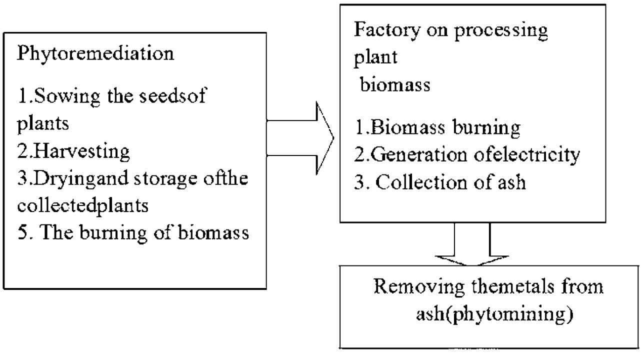


Fig. 6.1 The scheme of phytoremediation of soils contaminated by heavy metals

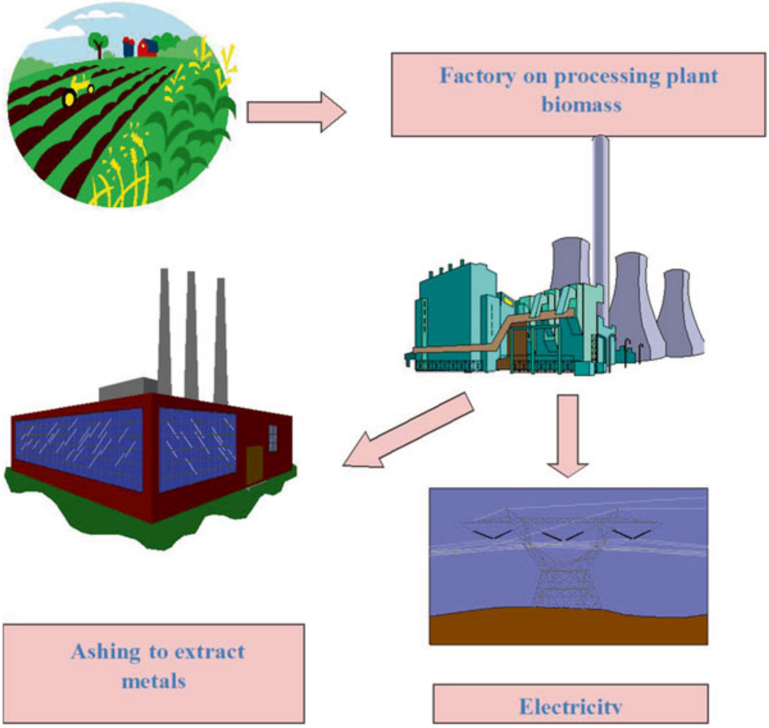


Fig. 6.2 Scheme of phytomining

The disadvantage of the method is that the plant-hyperaccumulators suitable for this type of phytoremediation usually have a small size, low biomass that could affect the efficiency of the method. So now researchers are working on the application of conventional breeding methods and bioengineering of plants to create new forms of plants that have high capacity to accumulate metals and large biomass [20].

6.1.1.1 Phytovolatilization

This includes using plants to volatilize the chemical elements. Some plant species are able to volatilize such heavy metals as Hg, Se, As [21]. The modified gene Hg-reductase (mercury-reductase) was transferred from bacteria to plants *Arabidopsis thaliana* L. to obtain species which can be used for phytovolatilization. Research is being conducted towards the production of higher plants, in which bacterial genes responsible for the hydrolysis of methyl and dimethyl-Hg-Hg are expressed. Organic mercury compounds are the main source of danger as lipophilic components are accumulated in the body of predatory birds and animals [22, 23]. The disadvantage of this method is a possible pollution of the atmosphere with toxic volatile compounds. Therefore, this method can be applied to areas that are far from crops and settlements.

6.1.1.2 Phytostabilization (Phytoimmobilization)

This is the use of plants to convert metals into less toxic forms but without removing metals from the soil [24]. This kind of phytoremediation technology is possible to use towards lead (Pb) and chromium (Cr). Plants with long and strong root system are very effective for this type of phytoremediation. The roots of *Agrostis capillaris* L. (bentgrass hairlike) grown in heavily Pb/Zn-polluted soil form pyromorphites of Pb and phosphorus (P). The deposits in the roots have pyromorphites ($Pb_5(PO_4)_3C1$)-type structure and some of Cl-atoms may be substituted by OH, but the mechanism of their formation is still unknown [25]. The formation of heavy metals P-precipitates is a tolerance mechanism to heavy metals, which passively sequester Pb in a metabolically inactive form. Although it is believed that *Thlaspi rotund folium* L. is a hyperaccumulator of Pb, Zea mays (corn) can accumulate a large amount of lead (Pb) at low pH and low phosphorus concentration [26]. The addition of chelating agents (NEDTA, EDTA) increases the solubility of lead (Pb) and its mobility within the plant. Lead (Pb) content in the above ground organs can reach 1 %; this allows to extract a sufficient amount of Pb.

"Immobilization" method can be used on Pb-contaminated soils [27]. Plants that accumulate Pb in the roots could keep it from leaching down the soil profile. Therefore, inactivation of Pb-contaminated soil with soil additives (hydroxide Fe, oxides of Mn, phosphates, limestone) and the use of plants to prevent erosion is one of the ways of phytoremediation of Pb-contaminated soils [28]. The disadvantage of this method is that the metal is not completely recovered from the soil surface and it remains bound in the roots of plants. This method is not suitable for the soil, which in the future will be used for growing crops. This method can be applied to chromium (Cr) too. Soils containing 10,000 mgCr/kg of Cr^{3+} are not a potential hazard, while the soil containing chromium in the form of Cr^{6+} is toxic to plants and other organisms. The roots of the plants could play an important role in restoring Cr^{6+} to Cr^{3+} in the soil, allowing the toxic form of chromium to immobilize to an inert form, which does not represent a potential risk [29].

The disadvantage of this method is the need in periodic cleaning of the contaminated plant parts and recycling them.

6.1.1.3 Rhizofiltration

This is using plant roots to extract the metal from flowing water. This technology uses plants that absorb heavy metals or radioactive elements in the aqueous medium through its root system. The plants are grown in hydroponic system and the contaminants are “filtered” through root system (rhizofiltration), which absorbs and concentrates the pollutants [30, 31]. The use of plants with a long root system or a large absorption surface with greater storage capacity (water hyperaccumulators) and tolerant to contaminants brings the best results. For effective development of phytoremediation, each element should be considered separately. We need an agronomic approach based on genetic properties of plants. Some elements can be absorbed by plant roots and turn into a volatile form as dimethylselenid [20, 21] and mercury [20]. While many plants are able to volatilize dimethylselenid, the concomitant pollution by sulfates and salinity of Se-contaminated soils inhibits this process. Therefore, it is necessary to improve soil conditions by using additives to achieve the best effect of phytoremediation.

6.1.2 Plants-Hyperaccumulators of Heavy Metals

For phytoextraction of heavy metals from the soil, the most beneficial is the use of plants hyperaccumulators of heavy metals. The term “hyperaccumulators” refers to species of plants, which accumulate 10–100 times more metals than conventional plants. Plants-hyperaccumulators cause considerable interest from the point of view of phytoremediation [26], phytomining [32], and biofortification of crops [33, 34]. These plants can be used to extract toxins from the soil and thus may help restore a fertility of contaminated soils. Plants-hyperaccumulators are endemic to the soil, which is contaminated with heavy metals and they do not compete with other species in uncontaminated soils. An accumulation of heavy metals in nontoxic form is one of the strategies used by plants to survive in conditions of strong pollution. The best known plants hyperaccumulators are *Ambrosia artemisiifolia* L. (ragweed), *Thlaspi rotundufolium* L., and *Thlaspi caerulescens* L., absorbing a significant amount of Zn, Cd, and Pb. Hyperaccumulators of Ni are *Alyssum* L. and *Arabidopsis* L. The latter is considered as a convenient object for research because it has a short life cycle and the small number of chromosomes. Typically, plant hyperaccumulators of heavy metals are mostly scrubby weeds with low yields. At present, the improved by genetic bioengineering plant forms of *Alpine penycress* L. with a high yield can absorb about 500 kg/ha of zinc and 6–8 kg of cadmium per year [35]. *T.caerulescens* can accumulate 2.5 and 0.2 % of dry weight cadmium (Cd) and zinc (Zn), respectively, from contaminated soils. With these plants, there can be removed

125 kg and 10 kg Zn and Cd per hectare [36]. According to the earlier estimation, the cost of these metals extracted from 1 ha of plants will be \$200 at market price of zinc — \$1.33, cadmium — \$4.6 per kilogram [36]. Researchers have identified some of the most specific signs of hyperaccumulators:

1. The plants must be resistant to high concentrations of metals in roots and aerial parts. Hyperaccumulation ability is the key feature that makes possible the hyperaccumulation. Hypertolerance is the result of chelation and vacuolar compartmentation of metals [35]. This was demonstrated in vacuoles of isolated protoplasts of tobacco cells, which accumulated high levels of Cd and Zn. Electron microscopic analysis of leaves of *Thlaspi caerulescens* [37] also indicates the vacuolar compartmentation of Zn.
2. The plants must be able to translocate elements from roots to aerial organs. Normally, the content of Zn, Cd, or Ni in roots is ten or more times higher than that in aerial organs. The ratio of metal content in the aerial parts to its content in the roots should be greater than one, indicating that hyperaccumulators can redistribute heavy metal ions in the aerial organs [37].

   Krameret al. [38] found that the ions of Ni, detected in leaf extracts of hyperaccumulator plant *Alyssum bertolonii* L., were chelated with citrate and malate, and in xylem exudate histidine chelates 40 % of nickel (Ni). The addition of histidine to the nutrient medium increased resistance to Ni and its translocation to the aerial organs in non-accumulator *A. montanum* L. [38].
3. Plants must absorb metals in large quantities. Plants *T. caerulescens* in natural conditions contain up to 1–4 % Zn, while the other species — less than 0.05 % of Zn. It was shown that Zn-hypertolerant genotypes of *T. caerulescens* require much more Zn in the nutrient solution (10^4 times more) for normal growth than non-accumulators. A high effective compartmentation of heavy metals to reduce the toxicity of Cd and Zn as it requires the plants to accumulate a large amount of metals [39]. To understand the technology of phytoremediation in detail, it is necessary to turn to classical works in this area. Currently, the definition of R. Brooks is generally accepted [40], and according to which hyperaccumulators of heavy metals are those plants that accumulate zinc (Zn) >10,000, lead (Pb) >1000, cadmium (Cd) >100 μg/g in the aerial parts (Table 6.1). Plants -non-accumulators of heavy metals should accumulate Zn <100, Pb <10, and Cd <1 on the uncontaminated soils (μg/g); on the contaminated soil — Zn <1000, Pb <100, Cd <10 μg/g.

Table 6.1 Thresholds of metal concentrations in organs of plants hyperaccumulators

Metals	% DW	Concentration, ppm
Cd	0.01	100.0
Co	0.1	1000.0
Cu	0.1	1000.0
Pb	0.1	1000.0
Ni	0.1	1000.0
Mn	1.0	10,000.0
Zn	1.0	10,000.0

Different populations of the same species may differ in the degree of metal-accumulating ability. V. Bert et al. [41] studied several tens of populations of *Arabidopsis halleri* L. growing in polluted and unpolluted soils. Plants were tested for the ability to accumulate Zn, Cd, and Pb. It was found that all populations of *A. halleri* exhibit properties to accumulate metals regardless of the place of growth. Populations of plants from uncontaminated areas contain these metals below the threshold concentration for hyperaccumulators and higher than for non-accumulators of heavy metals. When these plants were transferred to hydroponic conditions with a high concentration of heavy metals, their hyperaccumulation status confirmed for Zn and Cd. These data indicate that in cases where the plants have relatively high metal accumulation capacity, but the value of the metal concentration in the tissue is below the commonly accepted thresholds for hyperaccumulators, additional studies with the use of hydroponic conditions to identify the real plants hyperaccumulators of heavy metals are required [37].

It was found that there is a threshold of saturation of plants hyperaccumulators by metals above which the concentration of this metal in the plant does not rise [41]. The curve has a plateau. The similar fact has also been found for Cd [42]. The authors explain this fact by blocking the flow of metals from the roots to the aerial organs. In this case, the protection mechanism is triggered, which limits the toxicity of the metal to the plants at a high concentration of metal in the medium [42]. The authors are paying attention of researchers to some important points in the study of plant-hyperaccumulators. McGrath [43] considers that while comparing hyperaccumulation ability of various kinds of plants, it is necessary to consider not only the metal concentration in plants (metal content per unit weight of the plant), but the amount of metal recovered by this plant species from a certain area. Thus, if one species is strongly suppressed in the biomass accumulation of above-ground organs and in other species biomass accumulation is reduced to a lesser extent, the concentration of the metal in the above-ground organs of the latter may be lower than the first due to the dilution effect. The absolute value of the metal content in plants, based on a certain area, will give a more accurate picture to assess hyperaccumulation activity of plants in a comparative analysis [43].

Another important point is the ratio of the metals in above-ground plant organs to its content in the soil. Typically, for plants-hyperaccumulators, this value is high (up to 40 or more) [44]. According to the authors, the most accurate definition of the hyperaccumulator status can be found only in hydroponic environment where the ability of plants to tolerate high concentrations of metals is evident [45]. The question “What is more important for the development of phytoremediation: an accumulation of metals in a large amount or a significant accumulation of biomass of above-ground organs?” is under the discussion. If high-yielding species as *Zea mays* L. and *Brassica juncea* L. grow on Zn-contaminated soils with low pH, the yield is reduced by 50 %. Under normal conditions, the yield of these plants is equal to 20 tonnes of dry biomass per hectare.

When soils are polluted by Zn and Cd (100 mg Zn : 1 mg Cd), plants suffer greatly and reduce crop yields; when the content of Zn in aerial parts is up to 500 mg/kg. The Zn toxicity of the soil is a determining factor which controls productivity of plants. At reducing the yield by 50 % (10 t per hectare), the biomass will comprise 500 mg/kg of Zn (Zn 500 g per tonne). This will result in extraction of only 5 kg Zn per ha per year [39]. *T. caerulescens* initially has lower yield in comparison with the above-mentioned species, but can accumulate up to 25,000 mg of Zn per kg (25 kg/t) without reducing yield. Even at low yield of 5 t per hectare, zinc extraction will be 125 kg/ha [39]. Therefore, Chaney et al. [39] believes that the ability to hyperaccumulate heavy metals and a hypertolerance to high concentrations of metals are more important properties of plants for phytoremediation than the ability to accumulate a large biomass.

6.1.3 Phytosiderophores

The low availability of iron (Fe) for plants due to the high pH of the soil is one of the most common abiotic stresses in the world for agricultural plants. Most crop plants get not enough iron from the soil, which leads to chlorosis, low yields, and reduces the quality of agricultural products. It is known that 30 % of the arable land are the land are the alkaline soils and they are not optimal for crop production [46]. An extremely limited bioavailability of iron from the soil plants led to the development of two established extraction strategies. Strategy I used by dicotyledonous and non-graminaceous monocotyledonous species, releasing protons by roots to decrease soil pH, induce the expression of Fe(III)-reductase to reduce Fe(III), and take up Fe(II) through Fe(II) transporters [47, 48]. Although there is a large amount of iron in the soil, Fe-deficiency in plants growing at high pH in calcareous soils is developed. Under these adverse conditions, grass species have an ability to secrete low-molecular weight secondary imino acids (mugineic acids) known as *phytosiderophores* that form soluble iron chelates.

The strategy of chelation involves the secretion of mugineic acid family phytosiderophores (MAs) [49] to uptake soluble Fe in the form Fe(III)-MAs. This way of Fe uptake was termed by Römheld and Marschner [50] as a strategy II of Fe acquisition. There are numerous data that the phytosiderophores synthes is induced by Fe-deficiency [51]. A secretion of phytosiderophores family mugineic acid increases in response to Fe-deficiency and shows an accurate circadian rhythm of intake of iron in the plant organism [48]. Among the *Poaceae* family, barley (*Hordeum vulgare*) is a most tolerant plant to iron deficiency and secretes a greatest amount of mugineic acids [52, 53]. Since phytosiderophores have the ability to form thermodynamically stable complexes with other metal cations present in the growth medium, they are also involved in the transport and bioavailability of these metals in the environment [51]. It has previously been shown that phytosiderophores promote the absorption by gramineous plants, not only iron, but also zinc [54]. It was found that phytosiderophores are capable of forming complexes and accelerate the absorption by plants not only iron, but heavy metals that can compete with the iron. Phytosiderophores, iron chelators, are allocated in cereal plants under conditions of iron restriction, but they also form complexes with other metals, including cadmium [55, 56].

It is assumed that phytosiderophores play an universal role in the consumption of other trace metals such as Zn, Mn, and Cu, which have a low solubility in alkaline soils [57]. In support of this hypothesis, it was shown that phytosiderophores form stable chelates with Zn, Mn, and Cu [58] and are effective in the extraction of these elements from the calcareous soils [59].

Phytosiderophores are strictly specific and have a high affinity for iron ligands [60]. This particular system is governed solely by the plant absorption of iron from nutrient medium, so the term *phytosiderophores* more suited to these compounds than *phytochelatins*.

Phytosiderophores is a family of linear hydroxy- and amino-substituted imino-hydroxylic acids, several members of which include 4-membered azetidine ring. It was also established that phytosiderophores can be secreted in response to a shortage of zinc in the environment [61]. Phytosiderophores are allocated in species *Aegilops tauschii* and *Triticum* species under Zn- and Fe-deficiency conditions [62]. Cadmium is not essential trace metal and frequent soil contaminant. A constant anthropogenic release of cadmium into the environment leads to permanent accumulation of Cd in the soil. Extraction and Cd accumulation in plant tissues and seeds can cause them to transfer the food chain to man. The use of synthetic chelates is proposed to increase the mobilization of metals and facilitate phytoextraction as a means for cleaning metal-contaminated soils [63].

However, most of the chelate-extractable complexes can be destroyed rather than mobilized by plant roots. Unlike synthetic chelates added to the soil, the plants produce phytosiderophores that are released in the rhizosphere [49, 64–66].

Cadmium increases the release of 2′-dioximugineic acid (DMA) under Fe-sufficient and Fe-deficient conditions [67]. It was found that the presence of cadmium in the soil causes the symptoms of Fe-deficiency [65, 67]. Symptoms of Fe-deficiency lead to more production of DMA, which bound to cadmium ions and reduces the availability and uptake of metal by plant roots [63, 67].

Since phytosiderophores exept iron bind to other heavy metals and transfer them into plants, this mechanism of phytosiderophores synthesis, available in cereals, can be used to clean soils contaminated by heavy metals. These compounds are able to form complexes and accelerate the absorption of iron and heavy metals that can compete with iron. It is expected that the release of phytosiderophores is the main adaptive response to accumulation of trace elements. When iron deficiency increases the activity of nicotineaminesinthase (NAS), catalyzing the formation of nicotineamine - a phytosiderophores precursor, particularly mugineic acid, in cereals increases too [50]. According to the ability to release phytosiderophores, Fe-deficiency plants are located in the following order: barley > wheat > oats > rye > corn > sorghum [68, 69]. The chemical structures of selected components differ among species and even varieties. Study of biosynthesis in vivo and in vitro of mugineic, dioxymugineic and avenic acids showed that L-methionine is a precursor of mugineic acid as well as 2-dioxymugineic and avenic acids [21, 68, 69].

At present the study of genetic regulation of phytosiderophores synthesis is worked out. The research to identify the gene-encoding enzymes that catalyze synthesis of mugineic acid is being conducted [21, 70]. It is contemplated that transgenic plants could secrete phytosiderophores in the rhizosphere and absorb heavy metals in large quantities. It was found that in the roots of a hyperaccumulator *Arabidopsis hallieri*, the gene AhNAS2 is highly and constitutively expressed and this could play a definite role in Zn tolerance and accumulation [71]. In the roots of *Arabidopsis halleri,* a twofold increased content of NA, probably, is linked to the constitutive expression of AhNAS2 gene. At the expression of AhNAS2 cDNA in a zinc-sensitive *Schizosaccharomyces pombe* strain the tolerance to zinc is increased [71]. It was reported that the overexpression of TcNAS in *A. thaliana* transgenic plants also confers Ni resistance [72], supporting the idea that NA could play some role in metal tolerance and hyperaccumulation [56].

6.1.4 Possible Mechanisms of Heavy Metals Hypertolerance and Hyperaccumulation in Plants

To identify mechanisms of hypertolerance and hyperaccumulation of heavy metals in plants is a necessary step in the development of phytoremediation. The researchers suggest that the increase in concentration of metal-binding proteins or peptides in plant cells can increase the ability to bind metals and plant tolerance. Detoxification processes can be specific or nonspecific. It depends on whether the synthesis of binding heavy metals compounds is induced or they are formed in a cell constitutively. To specific mechanisms belong for metal binding the sinthesis of cysteine-rich proteins - metallothioneins, synthesized in animal cells and plant organisms in response to heavy metals. Metallothioneins, metal-binding proteins, got their name due to the high metal content, which can reach 20 % of the molecular weight [73]. Metal-binding proteins are commonly synthesized in small quantities. Their content in the cell is increased rapidly if they are affected by heavy metals and is decreased in the case of reducing their concentration in the nutrient substrate [74, 75]. Sulfur is present in metallothioneins usually in the form of thiolate and its content generally is about 10–13 %[76].

Moreover, the increased concentrations of heavy metals in living organisms not only stimulate the synthesis of metallothioneins, but assist to bind these proteins to metals. Nevertheless, both the processes of synthesis of metal-binding proteins and the synthesis of heat shock proteins are integral responses of a cell to the effect of many cell's stressful agents. According to recommendations of the Committee on the Nomenclature of Metallothionein in the 2nd International Congress on Metallothionein and other low molecular weight metal-binding proteins (Zurich 1985), any polypeptide which is similar in structure and function to the mammalian metallothionein may be considered to be in the group of these compounds. Metallothioneins are divided into three classes based on the chemical structures of molecules [77]. The first class (MT1) — metal-binding proteins in vertebrates. The molecule of MT1 in a metal-binding domain contains 20 cysteine residues, the location of which is always constant for this class.

The second class (MT2) — polypeptides that are similar in structure to the MT1, but do not have such a conservative position of cysteine residues. They are common for invertebrates, plants, fungi, cyanobacteria, and other prokaryotes, algae, and yeast. The third class (MT3)—phytochelatins (PC), kadastins, glutamylpeptides, i.e., polypeptides of some algae, higher plants and fungi containing-γ-glutamyl cystein residues, and differ from other metallothioneins by enzymatic method of their synthesis [74]. All MT I (mammals) and MT II are composed of one polypeptide chain containing 60 amino acid residues (sometimes 25 and 70). The number of Cys therein is up to 30 %. Often there is such sequence as Cys-X-Cys, where X is anyone different from Cys the amino acid. The metallothioneins often contain thw basic amino acid like Lys, rarely Arg. Metallothioneins of higher plants and algae (MT 3) are composed of two or more amino- and polypeptides, including primarily cysteine, γ-glutamic acid, and glycine. The most common sequence is Cys-γ-Glu-Cys.

In most metallothioneins, all Cys residues are deprotonated and are able to bind heavy metals in the ratio 3 ligands to one metal ion. Glutamine (Glu) performs a definite role in the mechanism of resistance. This compound restores the metallothionein molecule, oxidized by superoxide anion radicals [78]. Glutamine is involved in the synthesis of phytochelatins in the cells of higher plants [79]. The specificity of this group of plant metallothioneins is a large gap, the length of which is about 40 amino acid residues including the aromatic acid residues, separating it into two metal-binding domains. The length of the gap of other groups of metallothioneins is less than ten amino acid residues and contains no aromatic amino acids [80]. Stimulation of metallothionein synthesis of the corresponding class is dependent on many factors. The main ones are the properties of heavy metal and its concentration, ionic environment, and specific features of the plant.

Various metal ions stimulate the synthesis of metallothionein not to the same extent. Metals such as Ca, Al, Na, Mg, and U do not induce MT [13, 81]. Elevated levels of metal-binding peptides in the cell under the effect of Fe and Cs are obviously observed in certain cases, which depend on the type of a plant and the concentration of heavy metals [82]. Predominantly, Cd, Zn, Cu, Hg, Au, Ag, Co, Ni, Pb induce a metallothionein synthesis [83]. However, the effectiveness of the activation of the MT synthesis is different. For example, according to some data, the synthesis of MT1 and MT2 is already stimulated by Cd at concentration of about 10^{-7} M, whereas to obtain the same effect the concentration of Zn exceeded 3×10^{-4} M [74, 76, 84]. The same metal also affects the formation of metal-binding proteins differently. For example, Cd induces the MT2 synthesis some time after the beginning of the MT3 synthesis [80]. The formation of metallothioneins shows the dependence on the ionic environment. It was established, that the synthesis of CUP1 in *Saccharomyces cerevisia*e, inducible by Cu, when cultured yeast, in a medium contained ions of other heavy metals is largely reduced. At the same time, to inhibit the synthesis of CUP1, the content of Co^{2+}, Ni^{2+}, Zn^{2+} should be higher than that of Cd^{2+} and Mn^{2+} [85].

The plants can form metallothioneins of several classes and the synthesis of metallothioneins is carried out in different organs. For example, in *Arabidopsis* were found metal binding proteins of all known types [86], and in *Silene vulgaris*

L. - MT2 only [80, 87]. Under the effect of Cu in *Arabidopsis,* MT2 is synthesized in trichomes and MT1 — in leaves, roots, and flowers [86]. Differences in the expression level of metal-binding proteins in *Silene vulgaris* populations were associated with not similar tolerance to copper [87]. The most widespread plants metallothioneins are metallothioneins of the third class (MT3) —phytochelatins (PC) found in almost all species of plants, as well as some fungi and invertebrates [75, 77, 78, 88]. Therefore, the investigation of genetic and molecular basis of metal detoxification in plants by this group of metal-binding peptides is of greatest interest. First, PCs were found by researchers in the *Schizosaccharomyces pombe* [85], in cell culture of *Rauvolfia serpentina* [89]. Nowadays, there is a considerable progress in understanding the molecular mechanisms of synthesis and functioning of MT3 [74].

Structure of phytochelatins (*PC*). Phytochelatins are compounds of general formula (γ-Glu-Cys)*n*-Gly, where *n* is equal to 11 maximum, but usually varies from two to five [90]. On this basis, MT3 are divided into two groups — with low and high molecular weights [88]. The terminal amino acid in the MT3 structure can vary: in addition to glycine (PC), it can be serine (hydroxymethyl-PC), β-alanine (homo-PC), and also glutamine (Glu) and cysteine (Cys) [90]. The ratio of PC and their derivatives is dependent on the plant species as well as on the ratio of metals in the soil or nutrient solution [75]. For example, the resistance to Cd of *Oriza sativa* L. is provided by hydroxymethyl-PC [91]. Thiol-peptide level and proteomic changes in response to cadmium toxicity in *Oryza sativa* L. roots [92], in *Vigna angularis,* are only provided by homo-phytochelatins [76].

Biosynthesis of PC. There are many mechanisms that regulate the synthesis of phytochelatins, which includes several stages [74, 75, 77, 93]. For example, in *Brassica juncea* L., the synthesis of phytochelatins is preceded by a series of stages, the initial one is the reaction between cysteine and glutathione. This process is regulated by genes involved in the transport and sulfur assimilation and the biosynthesis of glutathione. In *Arabidopsis thaliana* L., biosynthesis of PC begins with metal-activating transcription of genes encoding glutathione reductase (GR), and the enzymes involved in biosynthesis of glutathione are γ-glutamylcysteine synthetase (γ-Glu-Cys-synthetase) and glutathione sinthetase (GS) [77]. Glutathione is the main substrate for PC formation and the key enzyme with crucial activity in the process of biosynthesis is PC-synthase. In *Triticum aestivum* L., the synthesis of PC from glutathione can be carried out without intermediate steps. This process is catalyzed by PC-synthase [74, 90].

The specific activator of this enzyme is mainly Cd, but some other heavy metals can provide this role as well. In descending order of their specificity, they can be arranged as the following: Ag, Bi, Pb, Zn, Co, Hg, Au [84]. Previously, it was thought that only free metal ions are able to activate PC-synthase and subsequent PC synthesis. Now it is known that anions $(AsO_4)^{3-}$, AsO^{2-} [88], phosphate anions [94], and jasmonic acid can participate in this process [95]. It is also has been shown [96] that heavy metal-thiolates, glutathionates, and heavy metal complexes with low molecular weight PC are active substrates for the synthesis of MT3, being either a catalyst or a substrate. Thus, the formation of MT3 is activated when exposed to a large number of heavy metals. However, only the Cd detoxification mechanism can be considered universal: more than 90 % of the Cd^{2+} ions penetrating into the cells of studied 200 species of three taxa (*Bryophyta*, *Pteridophyta*, *Spermatophyta*) are associated with phytochelatins synthesis [76, 87, 97].

The role of PC in the detoxification of metal ions. A number of researches clearly demonstrated that MT3 are involved in the detoxification of heavy metals in plants, although there are some other hypotheses about the role of PC [76]. Metal detoxification mechanism by PC includes a number of steps: (1) PC-synthase activation by metals; (2) the complex formation of MT3 with metals; (3) the complex transfer to the vacuole [97]. Moreover, it is considered that low molecular weight MT3 transport Cd to the vacuole where it accumulates in the form of a complex with high molecular weight MT3 or organic acids [98]. Any violation of at least one stage of detoxification leads to lowering the plant tolerance to heavy metals [76]. For example, either damage of PS synthase gene or glutathione synthase gene leads to hypersensitivity of organisms to Cd [76, 79]. Conversely, overexpression of these genes increases a metal plant tolerance, as it was demonstrated in cell cultures of *Lycopersicon esculentum* [99] and *Saccharomyces cerevisiae* [100]. Changes in the activity of enzymes involved in the metabolism of sulfur [88] and the reduce of sulfur containing amino acid - Cys [101], necessary for the synthesis of glutathione also affect the tolerance of plants to Cd.

With the growth of polymerization degree, the affinity of MT3 to metal ion [102] is increased and the efficiency of detoxification is raised. It is proved that the tolerance to heavy metals increases with the increase of the degree of MT3 polymerization and with saturation of the coordination valence with sulfide anions, i.e., with the formation of additional bonds to S^{2-} [76]. In turn, the increase in the proportion of molecules with high degree of polymerization occurs with the increase of Cd concentration and the time of the exposure [98]. The formation of the complexes is largely dependent on the pH of the solution. In the acidic medium, metal is replaced by hydrogen [102] and hence the efficiency of detoxification of heavy metals decreases. It is important to mention that the activation of MT3 synthesis is already observed within a few minutes after the treatment of plants with heavy metals [103], but the highest concentration of peptides in the tissues is revealed only after a certain time after the start of exposure [99].

Some authors suggest that phytochelatins play no significant role in the hypertolerance of plants to heavy metals. Although in cell culture the expression of metallothioneins [89] or phytochelatins [77] increased plant tolerance to Cd, the transfer of genes responsible for the synthesis of metallothioneins in higher plants had no effect on the accumulation of metal ions. While testing plants hyperaccumulators, there were no changes in the concentration of phytochelatins which suggests that hypertolerance to Cd and Zn is provided not by phytochelatin synthesis [104]. The evidence for the certain role of phytochelatins is that there was found correlation between their presence and normal rate of tolerance to the metals. The mutation leading to inability to produce phytochelatins resulted *Arabidopsis thaliana* L. in being hypersensitive to Cd [105]. It was found that high phytochelatins content correlates with the ability of plants to transport Cd to aerial organs. The alternative point of view is that phytochelatins supply plants with normal resistance to metal excess in the environment. For plants with normal resistance (*A. thaliana*), the synthesis of phytochelatins is definitely necessary if there is an excess of metals in the environment, while for hypertolerance of plants-hyperaccumulators PCs unlikely play any role [106].

To determine the limiting factors of heavy metals accumulation and to obtain tolerant transgenic plants with enhanced ability to accumulate heavy metals, the gene of *Escherichia coligshii*, encoding the synthesis of γ-glutathione synthetase (GS), was activated in the cytosol of Indian mustard [107]. Transgenic plants significantly more than wild species accumulated metal: the concentration of Cd in the above ground organs was higher by 25 %. However, these plants showed an enhanced tolerance to Cd in the phase of seedlings and mature stage. The accumulation of Cd and the tolerance to Cd was correlated with the level of *gshii* gene expression. Cadmium-treated plants contained a larger amount of glutathione, phytochelatins, thiols, S, and Ca as compared to the wild type. It was assumed that the enzyme (GS) at Cd presence is a limiting factor for the biosynthesis of glutathione and phytochelatins. The use of the method of overexpression of GS is a promising strategy to obtain plants with superabilities necessary to phytoremediation.

The reduced glutathione GSH plays an important role in the protection of plants against various stresses. Glutathione is not only a substrate for glutathione-*S*-transferase, neutralizing the potentially toxic xenobiotics [108], but also a reducing agent of dehydro ascorbate [109]. Moreover, GSH is a precursor of phytochelatins. Phytochelatins contain a high percentage of Cys-sulfhydryl residues that bind and isolate ions into stable complexes and are induced by metals such as Cd in all tested plants [110]. Glutathione is synthesized from its constituent amino acids in two consequences. ATP-dependent reaction is catalyzed by *γ-Glutamyl-Cys-Syntheta*se (GCS) and by *γ-glutathione synthetas*e (GS), respectively. *Phytochelatine synthase* sequentially catalyzes an elongation of (γ-Glu-Cys)*n* by the transfer of γ-Glu-Cys-group to glutathione or phytochelatins [111]. A manipulation by the expression of enzymes involved in the synthesis of glutathione and phytochelatins can be an excellent approach for the improvement of plant resistance to heavy metals. *Phytochelatin synthase* enzyme can not be a limiting factor for the synthesis of phytochelatins due to their constitutive expression in plants [112] and the activation at the presence of metals. The genes encoding enzymes involved in the synthesis of glutathione are more promising in this regard. The limiting step for the synthesis of glutathione in the absence of metal is a reaction catalyzed by γ-Glutamyl-Cys-Synthetase since the activity of this enzyme is regulated by glutathione feedback and depends on the availability of Cys. Overexpression of the gene *gshi* of *E. coli* encoding γ-Glutamyl-Cys-Synthetase increased glutathione levels in poplar [109]. Furthermore, the expression in tomato of γ-GCS can restore the tolerance of glutathione-deficient mutant of *Arabidopsis cad 2*. However, the overexpression of this gene did not increase tolerance to Cd in wild-type of *Arabidopsi*s.

Normally, the GS is not a limiting factor as a glutathione content does not change much due to the low concentration of phytochelatins. Overexpression of the gene *gshii* of *E. coli*, coding GS, did not increase the level of glutathione in poplars [113]. Nevertheless, the presence of heavy metals affects the regulation of the biosynthesis of glutathione which is substantially altered. Heavy metals activate phytochelatins synthase and thereby induce biosynthesis of phytochelatins resulting in reduced glutathione levels [114]. Successively as a feedback, glutathione removes the inhibition of γ-Glutamyl-Cys-Glutathione synthetase. Moreover, the expression of γ-glutamyl-cys-synthetase can be increased by heavy metals. It was demonstrated that Cd increases the gene transcription of γ-Glutamyl-Cys-synthetase and deactivates the GS. There is a decrease of glutathione and its accumulation is inhibited by γ-Glutamyl-Cys by the reducing of GS activity [115]. The corn roots exposure in the presence of Cd caused the decrease of glutathione and γ-glutamyl accumulation of cysteine by reducing the activity of GS. Therefore, GS can become a limiting factor for the biosynthesis of glutathione and phytochelatins [116]. The overexpression of *gshii* gene can increase the content of glutathione and phytochelatins synthesis (Fig. 6.3).

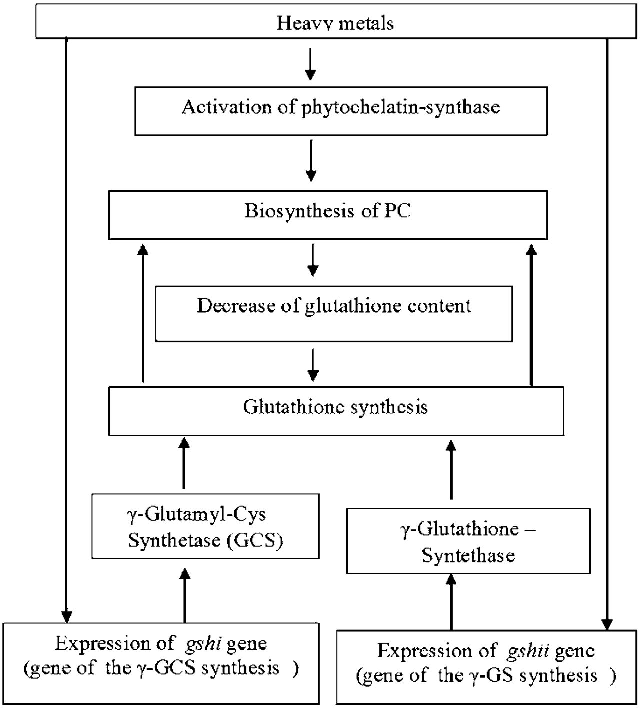


Fig. 6.3 The scheme of regulation of phytochelatines synthesis in plants

As a result of overexpression of *E. coli* gene *gshii* in Indian mustard, there was an increase of glutathione and phytochelatins content. Cd accumulation and the tolerance of plants also increased. In the Cd-treated roots of wild-type plants glutathione content was three times lower than in the control plants by increasing the synthesis of phytochelatins in transgenic plants compared to wild type. In the tissues of transgenic plants, the glutathione content was similar in the treated and untreated plants, while the level of phytochelatins in roots and aerial parts of transgenic plants was two times higher than that in the wild type. Since the roots are the main organ of PS synthesis, there was a decrease of glutathione in the wild type in the roots, but not in aboveground organs. Cadmium increased the thiol groups content in the roots of transgenic plants by ten times, and only three times in the aerial parts, which is the best explanation that the tolerance of transgenic plants is a result of increased PS synthesis.

The high levels of glutathione in the roots of transgenic plants produce a greater resistance to cadmium. Cd-PC form complexes with sulfide groups in vacuoles. It is believed that the plant tolerance to metal can be limited by the availability of sulfur for Cys and by sulfides synthesis [117]. The level of total sulfur was higher in aboveground organs. Cadmium significantly reduced the concentration of calcium in wild and transgenic plants, but overexpression of the GS gene decreased the rate of decline in Ca in the aerial parts. In the roots Ca content are not much different in the transgenic plants and in the wild type. Cd is a calcium channel blocker, cadmium interferes with binding of Ca to calmodulin, the protein which regulates the activity of many enzymes and cellular processes [118]. The increased levels of Cd-bound peptides in transgenic plants may reduce the effect of cadmium on interaction with calcium.

Transgenic plants has accumulated more Cd in the aboveground organs than wild type plants. The Cd translocation from the roots to the aerial parts of the xylem transpiration stream was provided by transpiration flow [119]. The more Cd binds phytochelatins stored in vacuoles in transgenic plants, the smaller are destroyed vital biochemical and physiological processes. This leads to the leaf surface increase, thus to greater accumulation of Cd (as a result of increasing the transpiration). Transgenic plants absorbed more cadmium due to the less damage of the root surface. The water absorption is the primary mechanism for increasing the movement of Cd in the plant [119]. High level of phytochelatins in the roots of transgenic plants reduces the negative effect of Cd on water absorption. Thus, regulation of gluthatione synthesis promotes an accumulation of heavy metals and increases the tolerance of transgenic plants. Transgenic plants allow to increase the efficiency of the phytoextraction of heavy metals from contaminated soils. The manipulation of gene expression of glutathione synthesis, may be one of the promising approaches to increase phytoextraction of heavy metals and metal tolerans of plants.

6.1.5 The "Induced" Phytoremediation

For toxic metals, such as lead, main restricting factor is a limited solubility and absorption by plant roots. One of the ways to induce a solubility of lead is the lowering of pH. A strong acidification of soils mobilizes Pb below the root zone. To improve a phytoextraction of heavy metals can be used synthetic chelators. These components are associated with the lead and remain soluble in the metal chelate complexes available for plants and transport within them. The chelating agents, such as EDTA HEDTA EDDS, DTPA, EDDHA, EGTA, and others, were tested for their ability to dissolve the metal components and to increase the absorption by plants [26, 120, 121]. EDTA is a powerful relatively biostable and extractable chemical reagent that is widely used for phytoremediation of soils [122]. The addition of EDTA (ethylene diamine tetra acetic acid) is a technique of extraction and is intended for immobilization of heavy metals as an important aspect of managing leaching of metals from soils [123–126]. This method is a relatively cheap and environmentally friendly technology [127].

Indian mustard exposed to lead (Pb) and EDTA in a hydroponic medium accumulated up to 1 % of the dry biomass. Other synthetic chelators as HEDTA (*N*-(2-hydroxyethyl)ethylendiaminetriacetic acid) applied at a concentration of 2.0 g/kg in soil containing 2.5 g/kg Pb increased accumulation of lead by aerial organs of Indian mustard from 40 to 10,600 g/kg. The accumulation of increasing amounts of Pb is toxic and can cause death of plants. Therefore, the authors recommended the use of chelates after maximum accumulation of biomass by plants. Immediately in the optimum time at the maximum phytoextraction level (after 1 week treatment) the plants should be removed to minimize the loss of biomass from the toxic effect of the metal [128]. Blaylock et al. [129] suggests that the addition of chelates is possible for the other metals too. EDTA also stimulated Cd-, Ni-, Cu-, and Zn-phytoextraction. The ability of these compounds to chelate metals facilitates a phytoextraction due to the high affinity to metals. For example, EGTA (ethylene glycol-bis(2-aminoethylether)-*N,N,N′,N′*-tetraacetic acid) has high affinity to Cd, but does not bind Zn. EDTA, HEDTA, DTPA (diethylenetriaminepentaacetate) are selective for Zn.

The addition of EDTA at concentration of 100 mM into the cultivation medium of *Sesbania drummondii* increased the amount of Pb by 16 % in comparison to the control. The use of EDTA increased an extraction of Cd up to 1140 mg/kg, and the use of ammonium sulfate did not affect the phytoextraction [130]. Application of NTA (nitrilotriacetate) and an elemental sulfur S increased the mobility of Zn, Cd, Cu in the soil that caused the increase of its accumulation in the aerial plant organs in 2–3 times. The addition of chelating agents (0.5 and 2 g/kg EDTA), 0.5 g/kg DTPA (diethylene triaminopentaacetate) and 0.5 g/kg of NTA (nitrilotriacetate)), to the soils, where poplar trees were grown, caused the increase of Cd absorption. The authors note that it was necessary to select the optimal concentration of chelating agents and the optimal time of removing of plants [131]. The chelating agents such as EDTA and metal cations increase the solubility and thus bioavailability of metals for plants. The positive effect of EDTA on phytoextraction of metals is accompanied by negative effects. The disadvantage of the use of the synthetic chelating agents is their non-selectivity. They extract a large number of metals including Ca and Mg, which are essential for plant growth [126].

The natural organic chelators are more suitable for phytoremediation. There are the widespread natural organic chelators in the soils, physiologically active compounds that the soil organic matter consists of and which determine its fertility. Humic acids are the dark-colored high molecular substances whose structure is not fully established. The maximum amount of humic acids is contained in the black earth (10 %). The structure of humic acids is defined by the presence of low condensed and substituted aromatic rings bound with sites of non-aromatic character. The molecules of humic acids include carboxyl and carbonyl groups, alcoholic and phenolic hydroxyls, sometimes methoxyl groups [24]. The most important function of humic acids in biosphere is their ability to protect and preserve soils and vegetation biotics, better to resist drought and water logging, and to bind firmly many radionuclides, detergents, pesticides, heavy metals. Humic acids are able to convert them into inactive forms, which over time break down into non-toxic compounds and thus remove them from the sphere of direct contact with living organisms, soil and groundwater, and atmosphere. The different chemical nature of the functional groups in the humic acids determine their high sorption capacity for radionuclides, pesticides, and heavy metals [132].

It has been found that Cd are mainly associated with low molecular weight fractions of humic acids (<1000 D), whereas Pb binds to high molecular weight fraction of humic acids (10,000 D). Their complexes with low molecular weight compounds have higher value of stability constant, more easily transported across cellular membranes than the complexes with high molecular substances that can cause a greater bioavailability of cadmium in the presence of humic acids [133]. Thus, to improve processes of phytoextraction, an optimization of agronomic practices used in phytoremediation technologies is required. The use of fertilizers is necessary for the greater accumulation of plants biomass, thus for the increase of extractable metals amount. Uptake of metals by plants may be limited by the low solubility of metals in the soil. This requires the use of synthetic chelators, which increase the mobility of metals in the soil.

It was shown that the humic acids increased the bioavailability of cadmium and concentration of Cd in the shoots of *Nicotiana tabacum* [134]. The authors suggested that the reason for this reduction of pH results from greater availability of cadmium. Another idea is to consider that plants can take cadmium complexes with fragments of humic acids, which are the result of microbiological degradation or self-dissociation. Humic acids have a positive effect on phytoextraction of other heavy metals such as Zn, Cu, Ni, and Pb [135]. The application of humic acids increased significantly the concentrations of metals in the shoots and plant uptake of metals, but the plant growth was declined. Thus, humic acids can be used in phytoremediation as an alternative way to increase a phytoextraction of heavy metals and remove them from the soils. Hence, to improve the processes of phytoextraction, the optimization of the agronomic practices used in phytoremediation technologies is required. The use of fertilizers is necessary for the greatest accumulation of plant biomass to increase the amount of extractable metals. Uptake of metals by plants may be limited by the low solubility of metals in the soil. Therefore, the application of synthetic and natural organic chelating agents is the best way for successful phytoremediation technology.

6.1.6 The Use of Energy Crops for Phytoremediation of Contaminated Soils

Phytoextraction is a kind of phytoremediation technology which has a long duration. Therefore, for the productive use of the contaminated soils, the economically viable and socially acceptable method of purification of contaminated land must be implemented. Industrial crops and “energy” crops which have a phytoextraction potential may be the candidates for biofuel production [120]. The use of the “energy” crops for phytoextraction of heavy metals is possible, on the one hand, to reduce the level of pollution and, on the other hand, to increase the productive value of the contaminated soil. The best candidates are sunflower plants (*Helianthus annus* L.), castor bean (*Ricinus communis* L.) [136], and white mustard (*Sinapis alba* L.) [137]. High-yielding plant species as *Helianthus annuus* L. have the potential to extract large amounts of trace metals by the aboveground biomass if they reach a sufficient concentration in the tissues. Nevertheless, a low bioavailability of some metals limits translocation of them to aerial parts. The addition of EDTA or citric acid increased the concentration of heavy metals in the tissues of plants. When these plants were grown on calcareous soils contaminated by Cu, Pb, Zn, and Cd and treated with EDTA (0.1,1, 3,5, 7 and 10 mM/kg) and citric acid (0.01, 0.05, 0.25, 0.442, and 0.5 M kg), the concentration of metals in the tissues and the removal of heavy metals from the soils were increased [138].

Sunflower can be used for phytoextraction of arsenic (As). Oxidative status of As may be different. Pentavalentarsenate (AsO^{4-}) is the most persistent and prevalent in well-aerated soils, that is why arsenic contamination of soils is a big problem. Arsenates and phosphates (PO^{4-}) are chemically similar and therefore can compete for the binding sites in the soil. Thus, the addition of phosphates can increase the content in the soil solution of arsenate as a consequence of phosphate substitution of arsenate on specific anion exchange sites in soil. This will increase the bioavailability of arsenic to plant roots. Phosphate fertilizers directly increase the accumulation of As in plant by stimulation of phosphate-absorbing mechanism. The preliminary study showed that sunflower (*Helianthus annuus* L.) may be a candidate for the phytoextraction of arsenic by the addition of phosphorus as a mobilizing agent [139].

In the comparative study of the activity of metal-accumulating plants *Helianthus annuus*, *Nicotiana tabacum,* and *Vetiveria zizanioides* on hydroponic medium containing Pb $(NO_3)_2$ in concentrations of 0.25 and 2.5 mM Pb with and without chelating agents (EDTA or DTPA), it was found that the addition of chelators increased the absorption of Pb. Lead accumulated in the leaves and stems with the highest content in the leaves. After 4 weeks, the lead content was increased in

23-fold in aerial organs of *H. annuus* and *N. tabacum*. Higher concentration of Pb (2.5 m MEDTA) leads to its increase in tissues during 4 weeks compared to the medium containing DTPA. Pb-accumulation potential of *H. annuus* was higher than that of *N. tabacum* and *V. zizanioides*, which was determined by bioconcentration potential (171, 70, and 88 kg/ha, respectively). The largest amount of Pb was found in the roots, stems, and leaves of *H. annuus* (2668, 843, and 3611 mg/g, respectively) grown at 2.5 m MEDTA [140].

Among the species *Brassica juncea* (L.), *Brassica nigra* (L.), *Raphanus sativ*us L., *Helianthus annuus* L, and *Ipomea triloba* L., it was found that sunflower accumulates Pb in most amounts [141]. In the studies on the accumulation of metals in plants *Echinochloa crusgalli*, *Helianthus annuus*, *Abutilon avicennae,* and *Aeschynomene indica*, grown on soils contaminated by cadmium (Cd), lead (Pb), and 2,4,6-trinitrotoluenom (TNT), it was found that the concentration of Pb was high in *A. avicennae*and *H. annuus.* The total removal of Cd from the soil was the highest in *E. crusgalli* (50.1 %), than in *H. annuus* (41.3 %) [142].

The study of phytoextraction potential of rape seed (*Brassica napus* L.) and sunflower (*Helianthus annuus* L.) in the presence of 3 mM DTPA/kg of soil showed that maximum concentration of Pb and Zn in above the ground parts were 234.6 and 1364.4 mg/kg, respectively. Sunflower showed the greatest phytoextraction potential compared to rape [143]. These results proved that *H. annuus* is the best candidate for its use as a hyperaccumulator and that it has the potential to be used for remediation of contaminated soils. These data suggest the use of chelating agents to enhance the phytoextraction by plants with high biomass of poorly soluble complexes of heavy metals.

Another kind of oil-bearing plants, from which castor oil is extracted, is known as a castor bean (*Ricinus communis* L.), researchers also determined as hyperaccumulator. Plants of castor beans (*Ricinus communis* L.), growing in hydroponic medium at concentrations of Pb 0, 100, 200, and 400 μM/L, showed their hyperaccumulation ability. According to I. Raskin et al. [144], castor beans may be considered as hyperaccumulators of Pb plants accumulating 1.0 g/kg [21]. Castor plants accumulated in hydroponic environment — from 10.54 to 24.61 g Pb/kg of soil [145]. The use of chelating agents such as EDTA might enhance the translocation of lead to the shoots.

Other researchers in the study of cadmium and lead phytoextraction by sunflower plants (*Helianthus annuus* L.) and castor bean (*Ricinus communis* L.) found that these species are capable of accumulating metals in a large amount [146]. Experiments with *Ricinus communis* L., which were grown on soil contaminated with lubricants (1–6 % oil/soil) containing Ni and Mn, showed the highest concentration of metals in the leaves. At concentration of lubricating oils of 2 % and more, the content of Pb was the highest in the leaves. In that study Mn, Ni and Pb were most strongly accumulated in leaves and vanadium—in roots of *R. communis* [147]. According to the literatute white mustard also has the potential to accumulate heavy metals. In the study of phytoextraction potential of 14 plant species in the presence of 5 mM Pb/kg of soil EDTA increased the proportions of phytoavailable Pb, Zn and Cd. Their absorption was increased in 48 times in white mustard (*Sinapis alba*), 4.6

times in radish (*Raphanus sativus oleiformis*), and 3.3-fold in amaranth (*Amaranthus* spp.). In mustard, the concentration of Pb was equal to 479.71; Zn— 524.68; Cd — 7.93 mg/kg, respectively, and phytoextraction potential were 1.32; 1.44 and 0.022 kg/ha, respectively [148]. Thus, the need to develop phytoremediation technologies using "energy" crops is due to the following: (a) these species accumulate large biomass; (b) the use of chelating agents enhances phytoextraction of metals which have low bioavailability and intensifies their translocation to their aerial parts.

6.1.7 Transgenic Plants for Phytoremediation

The efficiency of phytoremediation processes is largely determined by the ability of plants to absorb and accumulate toxicants in the cell structures. Progress related to phytoremediation of environment polluted by organic toxicants by its scale far exceeds similar processes associated with the assimilation of inorganic toxicants and radionuclides. This is due to long-term selection of suitable plants for organic toxicants, adapted to the specific soil and climatic zone, high productivity, ability to accumulate large biomass, availability of relevant physiological (capacity for transpiration) and morphological (developed root system) performance, adaptation to field conditions, the presence of the relevant enzyme systems, and others. The above-mentioned features and possibly some others cause accumulation and sequestration of heavy metals, i.e., it is determined by their phytoremediation potential of plants [149].

The ideal plant species for phytoremediation is a plant with a high biomass and with high phytoextraction ability. There are already exist a number of plant-derived transformants with enhanced ability to accumulate heavy metals in intracellular structures (predominantly in vacuoles), in the intercellular space, and to conjugate endogenous compounds with toxicants. In this direction, the study is being intensively developed in many countries of the world. More suitable for genetic engineering are plants like Indian mustard (*Brassica juncea*), poplar (*Popul*us spp.), yellow poplar (*Liriodendron tulipifera*), and cordgrass (*Spartina* spp.). The gene construct of the large biomass phytoremediation species may be transformed to the model plant species, like *A. thaliana*. This plant has a short life cycle and high seed production and it is very suitable to test it in a short time [150]. There are two possible strategies in genetic engineering to create the plants with high phytoremediation potential. One of them is to increase the biomass productivity of species that are good accumulators; another strategy is to enhance tolerance to heavy metals and metal accumulation capacity.

It is possible to introduce the genes responsible for the above-mentioned features into the plant from any other organism. The first large-scale field trials were conducted in USA. The most significant work on the production of recombinant plants were carried out in different directions. It is believed that phytoremediation can be commercialized very quickly if the absorption capacity of the plants hyperaccumulators as *T. caerulescens* is transformed into a highly productive plant as Indian mustard (*Brassica juncea*) and corn (*Zea mays*). Biotechnology methods has been successfully applied to manipulate the processes of absorption and tolerance to heavy metals in several species. For example, in tobacco plants (*Nicotiana tabacum*) the tolerance to heavy metals was increased after expression of genes of metallothioneins and other metal-binding proteins [151].

An effective application of biotechnology methods for environmental restoration is a bioengineering of plants capable of volatilizing mercury from soils contaminated by methylmercury. Methylmercury is a powerful neotoxin synthesized on mercury-contaminated soils. For detoxifying the toxin were used transgenic plants of *Arabidopsis* or tobacco where the genes *mer B* and *mer A* were transformed. In these modified plants, *mer B* catalyzed the protonolysis of carbon-mercury associated with the generation of Hg^{2+} in less mobile mercury. Consistently, *mer A* turns Hg (II) to Hg^0, a volatile element, which is released into the atmosphere. *Mer A-Mer B* double-transgenics were obtained by crossing *Mer A* and *Mer B* transgenics [152]. *Mer B* plants were tenfold more tolerant to organic mercury than wild-type plants; *Mer A-Mer B* plants were 50-fold more tolerant. When supplied with organic mercury, *Mer A-Mer B* double transgenics volatilized elemental mercury, whereas single transgenics and wild-type plants did not. Thus, *Mer* A-Mer B plants were able to convert organic mercury to elemental mercury, which was released in volatile form. The same *Mer A/Mer B* gene constructs were used to create mercury-volatilizing plants from tobacco plants and yellow poplar [153, 154]. They showed enhanced tolerance to mercury [155]. These experiments demonstrated that plant-expressed with *Mer B* (*organo mercurial lyase* under plant promoter control) can be used for the degradation of methyl mercury and subsequently for removing mercury through extraction. Despite the benefits of biotechnology, little is known about the genetics of plants hyperaccumulators. In particular, the heritability of such mechanisms as the mechanisms of metal transport and accumulation by plants and plant resistance to metals are to be better understood.

R. Chaney [39] suggested using conventional breeding approaches for improving phytoremediation processes and the possibility of combining field tests on metal tolerance and uptake of heavy metals in a highly biomass productive plant. For example, E.R. Brewer et al. [156] generated somatic hybrids between *T. caerulescens* (Zn-hyperaccumulators) and *Brassica napus* (canola), obtaining by hybrid selection for Zn-tolerance. The obtained hybrids have a large biomass and were hypertolerant to zinc. Among the large variety of plants, poplar plants (*Populus*), perspective for phytoremediation deserves a special attention because of their strong root system, which has a large absorption capacity. Multiple genetic engineering modifications of this plant convince the appropriateness of practical use of a number of obtained transgenic forms. One of such work is dedicated to the enrichment of the poplar genome of a bacterial gene coding the synthesis of γ-glutamylcysteine-synthetase (γ-ESC), which is a key enzyme in the biosynthesis of glutathione. The authors obtained several transgenic clones. Glutathione-*S*-transferase is widely distributed in plants, an enzyme which participates in normal metabolic processes in plant cells and in plant protection from stressful situations. In creating of transgenic plants for phytoremediation the target is a gene of this enzyme [157]. Overview of genetically engineering work conducted in this area indicates that in many cases in transgenic plants there is a significant increase the detoxification ability of plants.

This is evidenced by the fact that some transgenic plants have an increased ability to assimilate organic toxic compounds and heavy metals absorption capacity.

6.1.8 Utilization of Biomass

Phytoextraction is a multiple process of planting of hyperaccumulators on contaminated soil, after which the concentration of metal in soil falls to an acceptable level. The ability of the plant to reduce the concentration of metals in soil, depending on the metal absorption, the biomass production plays an important role in the phytoremediation process. Theoretically, the level of removal of metals from the soil can be calculated by determining the concentration of the metal in plants multiplied by the value of the biomass and it is necessary to compare this value with the decrease in the concentration of metals in the soil. Many factors impede this process. One of the obstacles to the commercialization of phytoextraction is the removal of contaminated plant material. After the cycle of plant development, the plant biomass is removed from the field, which leads to the accumulation of large quantities of hazardous waste. This contaminated biomass should be a certain way buried or disposed of properly so that it represents no risk to the environment.

Biomass contains carbon, hydrogen, and oxygen. The basic components of any biomass is lignin, hemicellulose, cellulose, mineral substances, and ashes. They have high moisture and volatile components. The percentage of these components varies in the plants species. The dry weight of *Brassica juncea* at induced phytoextraction of lead reach up to 6 t 1 ha with 10,000–15,000 mg/kg of metal in dry weight [158]. The processing of huge amounts of this kind of waste is a problem and therefore there is a strong need to reduce the volume of the contaminated biomass [159]. Therefore, after removing, a composting and sealing of biomass are necessary [27]. During composting, the formation of soluble organic compounds occurs, which increases the solubility of the metals (Pb). Studies of some researchers [160] have shown that composting can significantly reduce the amount of harvested biomass, but plant biomass contaminated by metals should be treated. One of the traditional and necessary ways to use biomass in phytoremediation is a thermochemical conversion process.

If phytoextraction is accompanied by high biomass production, it is advantageous to use it commercially as a source of energy and ash formed after combustion can be used as bio-ore [161]. It is a basic principle of phytomining [162]. The process of phytomining can bring huge profits by extracting heavy metals from the ash. The process of combustion and gasification are the most important components for production of electricity and heat. Energy production from biomass during combustion or gasification can help to make the process of phytoextraction more cost-effective. The thermochemical conversion of energy promotes the use of the biomass by the best way because it cannot be used as animal feed and fertilizer. The biomass combustion process must take place under controlled conditions; the volume at the same time should be reduced up to 2–5 %, and the ash can be disposed off properly

[161]. *Gasification* is the process by which biomass is subjected to a number of chemical changes to produce clean fuel and gas. This mixture of gases is called *pyrogas* that can be burned to produce heat and electricity.

The gasification process of biomass includes *drying*, *burning*, *thermal decomposition* (pyrolysis), and *gasification* [163]. It is possible to use the co-firing of biomass with coal plant [160]. Such incineration reduces the weight of lead-contaminated plant biomass for more than 90 %. This makes it possible to recover lead from fly [16]. Further experiments should focus on the development of combustion systems and methods for processing of various metals from the ash. This process destroys organic matter. Metals are extracted in the form of oxides. Considering other technologies for the utilization, this method is environmentally friendly. *Pyrolysis* is a new method for processing municipal waste [164], which can also be used for the contaminated plant material. *Pyrolysis* decomposes material under anaerobic conditions with no emissions into the atmosphere. The final product is the liquid pyrolytic oil and coke; heavy metals remain in the coke from which the metals can be recovered.

6.1.9 Advantages and Disadvantages of Technology Phytoremediation

According to estimates in the literature, the cost of simply removing 50 cm of contaminated soil and dumping of conventional methods is $960,000 per hectare. This does not include the cost of transportation, sorting revegetation of excavated layer. In contrast, for the cleaning of the same soil, biological methods will cost from

Table 6.2 Main factors, limiting the success of phytoremediation of contaminated soils

Biological restrictions	Adjustable and other restrictions
1. The weak resistance of plants to contaminants	1. The lack of data on the cost and implementation of phytoremediation in a certain area
2. Low translocation of contaminants from the roots to the aerial organs	2. Lack of the technology knowledge
3. The small size of the plants used for phytoremediation. The main problem of phytoremediation is that hyperaccumulators are small biomasses of leaves and small dimensions The level of recovery of heavy metals from the soil depends on the biomass and concentration of metals in the aerial part	3. Distribution of contaminated plant waste
	4. The risk of contamination of the food chain
	5. The pollutants are below the root zone
	6. Processing time is long. Removal rate may take 15–20 years, depending on the initial concentration of the metal in soil and the depth of the contaminated soil
	7. Pollutants are in biologically unavailable form. Low bioavailability of some metals (Pb). Metals are associated with inorganic components and soluble metal complexes in the soils are available for plants
	8. Lack of plant species suitable for phytoremediation

Adapted from Lasat MM, The use of plants for the removal of toxic metals from contaminated soils. Environmental protection agency, New York, 2001 [165]

$144,000 to $240,000 per hectare [165]. But phytoremediation technology has both advantages and disadvantages (Table 6.2).

Thus, phytoremediation technology is the safest and most effective and economically advantageous method for purifying contaminated soil. However, to develop the technology for phytoremediation of specific areas, there is a strong need in individual approach associated with the peculiarities of the soil, plants and species specificity, and type of pollution. For effective development of phytoremediation, each element should be considered separately. It is necessary to apply agronomic approach taking into account the physical and chemical properties of the metal, soil, and genetic properties of plants.

6.2 The Possibility of Using Grass Species of Kazakhstan Flora for Phytoremediation

East Kazakhstan region is the largest mining and metallurgical industry center. The territory of the East Kazakhstan region stores about one billion tons of solid waste as a result of strong pressure of human activity [166]. The wastes of industrial production form new man-made landscapes. They become a source of intense dust, polluting the environment and posing a threat to human health and biodiversity of the region [167]. Considerable part of the land in Eastern Kazakhstan is contaminated with trace metals, mainly lead (Pb) and zinc (Zn), since metal smelters and metallurgical enterprises are located in this area of the country. Smelting and mining processes are the point sources of pollution and contamination causing environmental problems. Heavy metals from the air, soils, and water affect plants and ecosystems. The soil of Eastern Kazakhstan region is most polluted by Zn, Cd, Pb, Cu as a result of prolonged activity of metallurgic plants of East Kazakhstan [168]. An important feature of heavy metals is that they belong to a class of non-specific substances which are “normally” present in the biosphere, in contrast to specific pollutants such as pesticides. Another difference from other contaminants is that heavy metals do not apply the concept of “self-cleaning”.

At present in Kazakhstan, the ability of wild species to accumulate heavy metals in plants growing in contaminated areas is poorly studied. In this regard, the study of metal accumulation activity of natural species in Kazakhstan is particularly relevant and timely and the use of suitable species for phytoremediation of contaminated soils is the most promising direction. Grasses are tolerant to heavy metals and have played a considerable role in the use of phytostabilization [169–173]. There are a lot of mine soils and estuarine sediments that are successfully phytostabilized against erosion by grasses [174, 175]. The thick adventitious roots, unique root morphology [171], and high bioproductivity make grasses [171] suitable for using in phytostabilization. Moreover, grasses are often associated with mycorrhizal and endophytic fungi [176, 177]. Application of grasses together with legume plants improves in situ stabilization of chemical waste [178, 179]. The study of accumulation of heavy metals by plants widely spread in the contaminated areas in Kazakhstan and the selection of tolerant and capable to accumulate heavy metals plant species are necessary steps for the development of phytoremediation technology in Kazakhstan. The aim of this work was the screening of wild plant species, growing on the contaminated area around the metallurgical plants of Eastern Kazakhstan, of their metal-accumulating ability, and test these grass species on artificially contaminated soils and hydroponic conditions.

6.2.1 Materials and Methods

The wild grass species of the family *Poaceae*: cocksfoot (*Dactylis glomerata* L.), a fire inermis (Bromus inermis L.), white bent (*Agrostis alba* L.), timothy grass (*Phleum pratense* L.), couch (*Agropyron repens* L.) green foxtail widely spread around metallurgic plants in East Kazakhstan, were identified. Samples of soils and plants were collected from the territory of Zinc and Lead Plants (Center) and 500 m from the plants to the North, South, East, and West of the enterprises and analyzed on heavy metals content. Soil from roots was removed gently and mechanically without washing. Washing roots may result in uneven loss of certain trace metals [180]. All samples were air-dried for 4 days and divided into roots and shoots. Then they were dried at 105 °C for 3 h, ground, and analyzed.

6.2.1.1 Experiments with Artificial Contamination of Soils

Grass species (*Agropyron repens*, *Dactylis glomerata*, *Phleum pratense,* and *Setaria viridis*) widely spread around metallurgic plants in Eastern Kazakhstan were identified by local floras. The seeds of *Agrostis alba* did not germinate on these soils. The seeds were sown in test pots 1 m × 1 m in size spiked with the following salts: $ZnSO_4 \cdot 7H_2O$, $Pb(NO_3)_2$, $(CuSO_4)_2 \cdot 5H_2O$, $CdSO_4$ in May. The final concentrations of the spiked soils were (in mg/kg): Zn — 1000, Pb — 1000, Cu — 100, Cd — 100. Grass species (*Agropyron repens*, *Dactylis glomerata*, *Phleum pratense,* and *Setaria viridis*) widely spread around metallurgic plants in Eastern Kazakhstan were identified by local floras. The seeds of *Agrostis alba* were sown, but they did not germinate on these soils. Seeds were collected in August from 25 to 30 plants. Seeds were sown in test pots 1 m × 1 m in size spiked with the following salts: $ZnSO_4 \cdot 7H_2O$, $Pb(NO_3)_2$, $(CuSO_4)_2 \cdot 5H_2O$, $CdSO_4$ in May. The final concentrations of the spiked soils were (in mg/kg): Zn — 1000, Pb — 1000, Cu — 100, Cd — 100.

There were three replicates for each treatment. The seeds were placed at a depth of 2–3 cm, with an inter-row distance of 5–7 cm. Grass seedlings were collected in August with roots. The contents of trace metals in shoots and roots were determined as described next. Plant samples (0.5 g) were digested in a mixture of 5 mL of 50 % HNO_3 and 0.5 mL HCl at 95 ± 5 °C according to standards for operation procedures [181]. Samples were transferred to digestion block (section) at temperature 90 ± 5 °C, closed by glass and heated without bringing to a boil for 10–15 min. Then they were cooled and added 5 mL of concentrated HNO_3, moved in digestion block with

90 ± 5 °C, closed by glass, and heated without bringing to a boil for 30 min before the disappearance of brown fumes. Then the samples were cooled and added 2 mL of water and 3 mL of H_2O_2, continued heating up until the volume has been reduced to about 5 mL, removed from digestion blocks, allowed to cool, filtered, washed filter, and added deionized water up to final volume to 50 mL. The process proceeded to the analysis of samples, using the appropriate SOP.

Hydroponic experiments: Seeds of *Agropyron repens* (*A. repens*), *Agrostis alba* (*A. alba*), *Bromus inermis* (*B. inermis*), *Setaria viridis* (*S. viridis*), and *Pleum pratense* (*P. pratense*) were collected in August from fields of the Altay Botanic Garden. The seeds of *Dactylis glomerata* (*D. glomerata*) were not viable and they were not used in hydroponic experiments. Seeds were stored in a dark room at 22–24 °C; before sowing, the seeds were stored for 20 days at 4–6 °C [182]. The seeds were sterilized with 16 % H_2O_2 followed by three rinses in distilled water, 5 min for each rinse. Seeds were germinated on water-moistened filter paper at 25 °C in a dark room for 7 days. Afterwards, the seedlings were placed in plastic containers (20 × 30 cm) filled with Hoagland's 1/4 strength (macro- and microelements) medium [183]. After 7 days, 30 seedlings were transferred to medium containing various concentrations of Pb (450, 900 mg/L) and Zn (350, 700 mg/L) in the forms of $Pb(NO_3)_2$ and $ZnSO_4$. Control plants were grown on 1/4-strength Hoagland's without metals. The experiment was carried out in a controlled environment room under the following conditions: 14-h photoperiod with a light intensity of 400 μmol photons m^{-2} s^{-1}; 22 °C: 18 °C day: night temperature; relative humidity, 60 %. There were three replicates for each treatment. Plants were harvested 6 days after treatments. Shoots and roots were separated, oven dried at 80 °C for 48 h, and dry weights were recorded. The contents of trace metals in shoots and roots were determined as described next.

6.2.1.2 Analysis on Heavy Metals (Pb, Zn) Content

Plant samples (0.5 g) were digested in a mixture of 5 mL of 50 % HNO_3 and 0.5 mL HCl at 95 ± 5C according to standards for operation procedures [181]. Samples were transferred to a digestion block (section) at ±5 °C, closed within glass and heated without bringing to the boil for 10–15 min. Then they were cooled and 5 mL of concentrated HNO_3 was added, placed into a digestion block at 95 ± 5 °C, closed within glass, and heated without bringing to the boil for 30 min until brown fumes disappeared. Then the samples were cooled and 2 mL of water and 3 mL of H_2O_2 were added; heating was continued until the volume was reduced to about 5 mL, removed from digestion blocks, allowed to cool, then filtered; the filter was washed and deionized water was added up to final volume to 50 mL. Samples were analyzed using the appropriate SOP. The concentration of metals in plants and soils was measured by atomic absorption spectrophotometry using an installed Winlab A Analyst 300 (Perkin Elmer, Germany) [184] with an installed and aligned HCL/EDL lamp. HCL lamps were stabilized/aligned for 25-min, EDL lamps for 45 min; operating pressure ~0.7 kgf/cm^2 for acetylene, and 2.8–3.0 kgf/cm^2 for compressed air. Following calibration, samples were analyzed.

6.2.1.3 Statistical Analysis of Data

In field experiments, the samples for measurement of trace metal contents were taken from three test pots for each treatment. The data of pot experiments were analyzed statistically using two-way ANOVA with species and treatments as main effects for shoot and root biomass and concentration of metals in plant parts. LSD was calculated using the following equation: LSD 0.05 = t0.05 √(2.MSError/n) was used to differentiate the means. All values were expressed as the mean of three measurements for each treatment. Values represent means ± standard error (SE). In hydroponic experiments, the samples for measurement of trace metal contents were taken separately from three plastic containers (three replicates) for each treatment. All values are expressed as the mean of three measurements for each treatment. The data were analyzed statistically using two-way ANOVA with species and treatments as main effects for shoot root biomass and concentration of metals in plant parts. LSD was calculated as above to differentiate the means. Values represent means ± SE.

6.2.2 *Results and Discussion*

6.2.2.1 Contamination of Soils Around Metallurgic Plants in East Kazakhstan

The first step of the study was determination of heavy metal content in soils around metallurgic plants of East Kazakhstan. Soil samples were taken at a distance of 500 m to the North, South, East, and West from the territory of enterprises. The soils around Zinc plant (ZP) and Lead plant (LP) differed on the content of heavy metals.

Table 6.3 Soil content of heavy metals around metallurgic plants

Regions	Cd	Pb	Cu	Zn
Zinc Plant, South	539.1 ± 17.8	5990.4 ± 215.6	5616.0 ± 179.7	129,792.3 ± 467.2
Zinc Plant, West	11.3 ± 0.4	12.3 ± 0.5	43.2 ± 1.5	355.7 ± 13.9
Zinc Plant, North	120.0 ± 4.5	1645.3 ± 64.2	1749.3 ± 69.9	22,990.8 ± 804.6
Zinc Plant, East	9.3 ± 0.29	195.8 ± 7.0	373.8 ± 14.6	2898.3 ± 101.4
Zinc Plant, Center	83.5 ± 3.0	932.8 ± 30.8	645.7 ± 23.2	17,881.9 ± 679.5
Lead Plant, South	35.8 ± 1.1	3046,0 ± 109,6	449.2 ± 14.8	3893.0 ± 120.6
Lead Plant, West	11.0 ± 0.3	1308.9 ± 45.8	159.8 ± 5.3	1598.7 ± 57.6
Lead Plant, North	22,4 ± 0,7	4769.2 ± 175.4	1073.7 ± 38.1	22,972.4 ± 748.8
Lead Plant, East	20.0 ± 0.5	1723.9 ± 65.5	1079.3 ± 41.0	5796.5 ± 226.0
Lead Plant, Center	134.9 ± 4.7	12,672.4 ± 430.7	1519.1 ± 51.6	22,986.2 ± 750.1

Soils around metallurgical plants had high concentrations of lead (Pb) (12.3–12,672.4 mg/kg) and zinc (Zn) (355.7–129,792.3 mg/kg) [185] (Table 6.3).

The content of Zn and Pb was much higher than that of Cd and Cu in the soil. The territory of LP (Center) was the most polluted by Pb; the soil to the West of the ZP was the least polluted site by Pb. On the content of Pb, the sites adjacent to the metallurgic plants can be arranged in the following order: LP, "Center" > ZP, South > LP, North > LP, South > LP, East > ZP, North > LP, West > ZP, "Center" > ZP, East > ZP, West. In soils to the South of the ZP was found the highest content of Zn. The least Zn content was found in the soils to the West of the ZP. The sites with the content of Zn can be arranged in the following order: ZP, South > ZP, North > LP, "center" > LP, North > ZP, "Center" > LP, East > LP, South > ZP, East > LP, West > ZP, West. Thus, the most polluted by heavy metals areas were located to the South and to the North of the ZP and in the territory of Zinc and Lead Plants (center). Minimal contaminated by heavy metals areas were found to the West and East from the ZP and LP. A Zn hyperaccumulator is defined as a plant that contains >10,000 mg/kg Zn dry wt, whereas a Pb hyperaccumulator contains >1000 mg/kg Pb dry wt. The mean of Zn and Pb concentrations in non-accumulating plants growing on contaminated soils is expected to be <1000 for Zn and <100 for Pb [43].

6.2.2.2 Contamination of Plants, Growing Around Metallurgic Factories, by Lead and Zinc

These were collected and identified plant species, growing around Zinc and Lead Plants in East Kazakhstan (Ridder) at a distance of 500 m from the metallurgic factories. *Dactylis glomerata* L., *Bromus inermis* L., *Agropyron repens* L., *Agrostis alba* L., and *Phleum pratense* L. are the most common plant species growing in this area. The study of heavy metals content in the parts of plants growing around the metallurgical plants showed that all collected and identified grass species growing around Zn and Pb manufacturing plants in Eastern Kazakhstan accumulated Zn and Pb in great amounts, mainly in the roots [185]. Our data showed that the content of Pb and Zn in these species was much higher than the means defined for non-accumulators growing on contaminated soils. These plant species which were growing on highly contaminated soils (total soil Pb—12,672.4 mg/kg) around metallurgic plants in Eastern Kazakhstan accumulated Pb: *D. glomerata*—up to 3760.0 mg/kg in shoots and 6715.9 mg/kg in roots, *B. inermis*—up to 709.1 mg/kg in shoots and 6787.8 mg/kg in roots, *A. repens*—up to 287.0 3 mg/kg in shoots and 3982.8 mg/kg in roots, *A. alba*—up to 339.7 mg/kg in shoots and 2496.0 mg/kg in roots, and *P. pretense*—up to 419.6 mg/kg in shoots and 4789.4 mg/kg in roots (Table 6.4).

In the shoots of all species, content of Pb was relatively low compared with that in the roots. *Agropyron repens* L. had a low level of Pb accumulation in the aboveground parts at low- and medium-polluted soils (Table 6.4). In the shoots of *Agropyron repens,* concentration of Pb increased on soils with high content of metal in the soil, but it was lower than in other species.

Table 6.4 Lead concentration in plants, growing around metallurgical enterprises in East Kazakhstan

Plant, parts	*Dactylis glomerata*	*Bromus inermis*	*Agropyron repens*	*Agrostis alba*	*Phleum pratense*
Zinc Plant, West. Total Soil Pb—12.3 mg/kg					
Shoots	26.2 ± 0.9	249.0 ± 8.9	44.9 ± 1.67	26.2 ± 1.1	10.80 ± 0.39
Roots	109.8 ± 4.2	164.7 ± 6.9	36.7 ± 1.4	171.8 ± 6.5	119.8 ± 4.3
Zinc Plant, East. Total soil Pb—195.8 mg/kg					
Shoots	92.4 ± 3.2	27.90 ± 0.89	56.3 ± 2.2	146.7 ± 5.4	–
Roots	159.7 ± 6.1	99.8 ± 3.7	69.3 ± 2.5	870.0 ± 36.5	–
Zinc Plant, Center Total soil Pb—932.8 mg/kg					
Shoots	256.9 ± 9.2	190.0 ± 5.7	179.8 ± 5.8	191.7 ± 6.0	68.3 ± 2.7
Roots	269.7 ± 8.36	140.0 ± 4.9	270.5 ± 8.4	121.7 ± 3.8	83.6 ± 2.8
Lead Plant, West Total soil Pb—1308.9 mg/kg					
Shoots	372.6 ± 13.4	–	107.7 ± 3.9	243.8 ± 8.8	51.8 ± 1.7
Roots	3719.2 ± 141.3	–	309.8 ± 11.8	254.0 ± 9.1	239.7 ± 8.9
Zinc Plant, North Total soil Pb—1645.3 mg/kg					
Shoots	176.0 ± 5.0	581.0 ± 20.9	83.1 ± 2.8	118.8 ± 4.3	69.9 ± 2.9
Roots	384.0 ± 15.7	1897.0 ± 75.8	149.8 ± 5.8	87.5 ± 3.4	149.0 ± 5.4
Lead Plant, East Total soil Pb—1723.9 mg/kg					
Shoots	146.0 ± 5.5	96.1 ± 3.8	101.8 ± 3.6	69.9 ± 2.3	8.2 ± 0.3
Roots	567.7 ± 117.6	1545.9 ± 19.7	2735.0 ± 15.1	525.0 ± 11.9	399.2 ± 40.7
Lead Plant, South Total soil Pb—3046.0 mg/kg					
Shoots	387.7 ± 13.9	162.8 ± 6.1	233.5 ± 9.8	99.9 ± 3.7	354.5 ± 12.1
Roots	2164.3 ± 69.2	1339.1 ± 52.2	1331.8 ± 59.0	1527.8 ± 58.0	3137.5 ± 11.9
Lead Plant, North Total soil Pb—4769.2 mg/kg					
Shoots	279.8 ± 9.8	98.8 ± 3.6	100.1 ± 3.7	304.6 ± 9.7	539.7 ± 22.7
Roots	2800.0 ± 117.6	546.3 ± 19.7	399.1 ± 15.1	2776.6 ± 119.3	2917.6 ± 113.7
Zinc Plant, South Total soil Pb—5990.4 mg/kg					
Shoots	85.1 ± 0.3	291.6 ± 8.0	125.0 ± 4.4	452.0 ± 15.8	8.5 ± 0.4
Roots	97.5 ± 3.12	101.8 ± 3.6	2125.3 ± 72.2	988.0 ± 30.5	308.1 ± 9.9
Lead Plant, Center Total soil Pb—12,672.4 mg/kg					
Shoots	3760.0 ± 146.8	709.1 ± 7.1	287.6 ± 9.2	339.7 ± 12.5	419.6 ± 14.2
Roots	6715.9 ± 255.0	6787.8 ± 237.5	3982.8 ± 151.0	2496.0 ± 82.0	4789.4 ± 181.9

– The species are not found in this area

It should be noted that in the aerial parts of this species were found relatively low levels of Pb as compared to roots even at the highest metal concentrations in the soil, indicating that there is an active protective function of the distribution of root system. With the increase of concentration of Pb in the soil, metal content in the plants roots steadily climbs up, which indicates that at low concentrations in the environment the selective permeability of cell membranes of roots prevents free penetration of metals into root cells, and at high concentrations, there occurs the breach in the barrier function of membrane permeability which drastically increases

Table 6.5 Shoot/root ratio for Pb

Soil content of Pb, mg/kg	*Agropyron repens*	*Dactylis glomerata*	*Bromus inermis*	*Agrostis alba*	*Phleum pratense*
ZP, West—12.3	1.2	0.24	1.5	0.15	0.09
ZP, East—195.8	0.81	0.58	0.3	0.17	–
ZP, Center—932.8	0.66	0.95	1.36	1.58	0.82
LP, West—1308.9	0.35	0.1	–	0.96	0.22
ZP, North—1645.3	0.55	0.46	0.3	0.60	0.47
LP, East—1723.9	0.04	0.26	0.06	0.13	0.02
LP, south—3046.0	0.18	0.18	0.05	0.07	0.11
LP, North—4769.2	0.25	0,1	0.18	0.11	0.18
ZP, South—5990.4	0.06	0.87	2.86	0.46	0.03
LP, Center—12,672.4	0.07	0.56	0.10	0.14	0.09

– The species are not found in this area

the concentration of metal in the roots. For example, when the concentration in soil was 932.8 mg/kg (ZP, “Center”), the concentration of this metal in the roots of the species remained relatively low (270.5 mg/kg). With increasing Pb concentration in the soil in the roots of this plant, lead content increased, reaching 982.8 mg/kg on the soils with maximum content of Pb (12,672.4 mg/kg, LP, “Center”). To assess the degree of removal of heavy metal by shoots, it is necessary to calculate the ratio of heavy metal content in the shoots and in the roots (shoot/root ratio), i.e., rate of translocation of the metal. Usually for hyperaccumulators used for phytoextraction of heavy metals, the mean of ratio is greater than one.

The ratio of the metal content in aerial parts and its content in the roots, i.e., translocation rate of the metal, was calculated. The coefficient of Pb translocation (shoot/root ratio) of *A. repens* was <1 in almost all studied areas (Table 6.5).

For *D. glomerata,* the concentration of Pb in the roots sharply increases up to 3179.2 mg/kg (soil concentration Pb—1308.9 mg/kg) in the site to the West of the LP This is a manifestation of pronounced protective function of roots, which concentrate heavy metals and limit their translocation to the photosynthetic and reproductive plant parts. Only in the most contaminated site in the center of LP, where concentration of Pb in the soil equals to 12,672.4 mg/kg, the content of lead in the shoots rises sharply (Table 6.4), probably due to strong air pollution.

In *B. inermis,* Pb content in the roots begin to rise sharply at concentrations in the soil above 1645.3 mg/kg to the North of the ZP and reaches more than 6000 mg/kg at a maximum soil concentration (12,672.4 mg/kg of soil) (Table 6.4). To the South of the LP in the roots of *A. alba* and P. *pratense,* Pb content increased significantly compared to areas with relatively low lead content in the soil. In all investigated species, lead is accumulated predominantly in the roots. In these species, shoot/root ratio of Pb was lower than that in all studied pots (Table 6.5). For *D. glomerata,* this value was close to 1 (0.95) in the center of ZP. In the center of LP, *D. glomerata* accumulated up to 3760.0 mg/kg, which exceeds the threshold concentration for

Pb-hyperaccumulators (1000 mg/kg) in the shoots, but the shoot/root ratio of Pb was less than one. For *B. inermis* and *A. alba,* the factor of translocation in the center of ZP was >1, and for other species, this value was close to one, probably due to strong air pollution.

In highly contaminated soils (LP, Center), the content of Pb of the studied species was equal to 0.03–0.4 %, in roots—0.2–0.7 % of dry weight. The highest percentage of Pb was in the aboveground parts of *D. glomerata* (0.4 %) and in the roots of *D. glomerata* and *B. inermis* (0.7 %). Lead content in the roots of plants in highly contaminated soils in the center of LP (12,672.4 mg/kg Pb in the soil) can be

Table 6.6 Zinc concentration in plants, growing around metallurgical enterprises in Eastern Kazakhstan, mg/kg

Plant parts	*Dactylis glomerata*	*Bromus inermis*	*Agropyron repens*	*Agrostis alba*	*Phleum pratense*
Zinc Plant, West. Total Soil Zn—355.7 mg/kg					
Shoots	181.8±6.4	249.5±9.0	226.7±7.5	195.9±6.9	162.7±5.7
Roots	1547.2±49.5	3095.0±117.6	522.6±19.9	1688.3±60.7	1028.7±36.0
Lead Plant, West Total Soil Zn—1598,7 mg/kg					
Shoots	299.7±10.4	–	126.7±4.6	319.7±11.8	265.4±7.7
Roots	6300.0±239.4	–	499.7±19.5	909.6±34.5	359.6±14.0
Zinc Plant, East. Total soil—2898.3 mg/kg					
Shoots	510.7±18.3	199.7±6.9	247.6±8.9	828.6±29.8	–
Roots	1647.0±59.2	948.8±33.2	1166.9±45.5	4789.5±167.6	–
Lead Plant, South Total soil Zn—3893.0 mg/kg					
Shoots	729.4±25.5	379.7±14.4	409.3±14.3	138.8±4.9	355.6±12.8
Roots	5126.6±179.4	2578.4±92.8	1815.6±74.7	2516.5±95.6	2697.8±105.2
Lead Plant, East Total soil Zn—5796.5 mg/kg					
Shoots	169.7±6.1	303.7±12.4	161.7±4.14	679.7±25.8	192.9±7.3
Roots	1139.3±36.4	2942.3±105.9	1647.0±67.5	1989.6±65.6	968.0±34.8
Zinc Plant, Center Total soil Zn—17,881.9 mg/kg					
Shoots	1499.4±63.2	1300.0±49.4	1298.9±46.7	1498.2±47.9	1399.4±47.6
Roots	2697.3±105.1	1600.0±57.6	3194.2±108.6	2195.3±79.1	1747.5±102.3
Lead Plant, North Total soil Zn—22,972.4 mg/kg					
Shoots	519.8±20.7	309.4±11.8	379.9±14.1	595.3±23.8	1049.6±37.8
Roots	3539.3±120.3	2197.4±79.1	1147.5±43.6	9288.8±325.1	5095.9±43.8
Lead Plant, Center Total soil Zn—22,986.2 mg/kg					
Shoots	1350.0±48.6	271.7±9.8	709.1±23.4	439.6±15.4	599.5±22.7
Roots	1948.8±70.2	2894.8±104.2	3992.8±139.7	4238.9±135.6	5936.9±189.9
Zinc Plant, North Total soil Zn—22,990.8 mg/kg					
Shoots	101.0±3.6	4472.8±147.6	698.7±25.1	1068.9±38.4	453.5±15.4
Roots	4588.9±165.2	6981.8±251.3	2177.4±82.7	1516.0±57.6	2160.0±84.2
Zinc Plant, South Total soil Zn—129,792.3 mg/kg					
Shoots	1498.2±56.9	1498.2±53.9	2743.9±104.2	4900.0±186.2	997.0±35.9
Roots	2300.0±89.7	2896.5±107.2	29,934.1±1047.6	14,820.3±533.5	10,383.3±394.6

– The species are not found in this area arranged as follows: *B. inermis*≥*D. glomerata*>*P. pratense*>*A. repens*>*A. alba*. The content of lead in the aerial parts decreased in the following order: *D. glomerata*>*B. inermis*>*Ph. pretense*>*Ag. alba*>*A. repens*.

These species accumulated Zn in great amounts too: *D. glomerata*—up to 1498.2 mg/kg in shoots and 2300.0 mg/kg in roots, *B. inermis*—up to 1498.2 mg/kg in shoots and 2896.5 mg/kg in roots, *A. repens*—up to 2743.9 mg/kg in shoots and 29,934.1 mg/kg in roots, *Ag. alba*—up to 4900.0 mg/kg in shoots and 14,820.3 mg/kg in roots, *P. pratense*—up to 997.6 mg/kg in shoots and 10,383.3 mg/kg in roots (Table 6.6). The tested plant species have accumulated Zn predominantly in the roots (Tables 6.5 and 6.6). Coefficient of Zn translocation from roots to shoots of plant species was lower than one (Table 6.6). *A. repens* accumulated the highest amount of this metal in the roots as compared to other species on the soil with high concentration of Zn (ZP, South). The concentrations of Zn in the roots of *A. repens*, *A. alba,* and *P. pratense* to the South of ZP (29,934.1, 14,820.3, and 10,383.3 mg/kg, respectively) were significantly higher than those of other species.

Bioconcentration factor of Zn in less contaminated soil was more than one; in highly contaminated soils—less than one. In highly contaminated soils to the South of ZP (129,792.3 mg/kg), concentrations of zinc in grass species can be arranged as follows: in the roots—*A. repens*>*A. alba*>*P. pretense* > *B. inermis*>*D. glomerata*; in the shoots—*A. alba*>*A. repens*>*B. inermis*>*D. glomerata*>*P. pratense*. In this area, the percent of Zn for *P. pratense* was equal to 1 %, *A. alba*—1.4 %, *A. repens*—3 %. The analysis of the experimental data showed that wild grass species, growing around metallurgical plants of East Kazakhstan, accumulate significant amounts of lead and zinc mainly in the roots. It is known that for hyperaccumulators, the thresholds of concentrations of metals absorption have the following values (mg/kg): Zn—10,000, Pb and Cu—1000, Cd—100; the ratio of the metal content in aerial parts of its content in the roots must be greater than one [40]. The studied plant species do not belong to hyperaccumulators because the concentration of heavy metals in plant parts is lower than the thresholds for hyperaccumulators.

Table 6.7 Shoot/root ratio for Zn

Soil content of Zn, mg/kg	*Agropyron repens*	*Dactylis glomerata*	*Bromus inermis*	*Agrostis alba*	*Phleum pratense*
ZP, West—355.7	0.43	0.12	0.08	0.12	0.16
LP, West—1598.7	0.25	0.05	–	0.35	0.74
ZP, East—2898.3	0.21	0.31	0.21	0.17	–
LP, South—3893.0	0.22	0.14	0.15	0.06	0.14
LP, East—5796.5	0.1	0.15	0.1	0.34	0.20
ZP, Center—17,881.9	0.4	0.56	0.19	0.68	0.08
LP, North—22,972.4	0.33	0.14	0.64	0.06	0.21
LP, Center—22,986.2	0.18	0.7	0.09	0.1	0.1
ZP, North—22,990.8	0.32	0.22	0.6	0.7	0.2
ZP, South—129,792.3	0.09	0.65	0.5	0.33	0.1

– The species are not found in this area

Analyzing the data, it should be noted that the Pb content in the aboveground parts was lower than one. But for some species, the shoot/root ratio was greater than one: *A. repens*—1.5 (ZP, West), *A. alba*—1.6 (ZP, "Center"), *B. inermis*—1.36 (ZP, "Center") and 2.9 (ZP, South) (Table 6.7). But the fact that it is not a natural phenomenon suggests that such data do not exist in other areas and it is likely the result of atmospheric pollution, rather than metal translocation from roots to aboveground parts.

Thus, there were found no plants in full compliance with the definition hyperaccumulator of heavy metals. But according to the literature, content of Zn and Pb in nonaccumulating plants in the contaminated soil must be less than 1000 mg/kg for Zn and less than 100 mg/kg for Pb [40]. In this case, Pb and Zn contents for the studied species were much greater than for nonaccumulating plants on the contaminated soil. Pb concentration values for *D. glomerata*, *B. inermis*, and *A. alba* were much higher than 100 mg/kg (the threshold concentration for Pb nonaccumulators) and the concentration of Zn was greater than 1000 mg/kg (the threshold concentration of Zn for nonaccumulators) for all species in the highly contaminated soil. Although these species are not concentrated in the aboveground organs, they accumulate metals in significant amounts in the roots. Thus, the study of wild grass species of flora in Kazakhstan, widely distributed in the contaminated area around metallurgical plants, showed that the investigated species accumulated heavy metals in amounts close to the generally accepted threshold concentration for plants-hyperaccumulators, but they are not fully consistent with this status.

The mean Pb concentration in plant parts of *A. alba*, *B. inermis*, and *D. glomerata* was much higher than 100 mg/kg and the mean Zn concentration for all species on highly contaminated soils was >1000 mg/kg. To estimate the effect of heavy metals on growth parameters and metal-accumulating ability in a comparative aspect, these species were chosen for further studies, including the screening of

Table 6.8 Root and shoot biomass of wild grass species, g/м^2

Variants	*Agropyron repens*	*Dactylis glomerata*	*Phleum pratense*	*Setaria viridis*
Shoots[a]				
Zn—1000 mg/kg	305.0 ± 13.1	345.0 ± 15.8	0	150.0 ± 5.5
Pb—1000 mg/kg	395.0 ± 15.5	350.0 ± 13.1	800.0 ± 41.2	280.0 ± 1.0
Cu—100 mg/kg	425.0 ± 17.2	340.0 ± 16.0	655.0 ± 30.2	115.0 ± 4.0
Cd—100 mg/kg	440.0 ± 20.2	320.0 ± 11.0	570.0 ± 21.1	245.0 ± 9.8
Roots[b]				
Zn—1000 mg/kg	605.0 ± 19.2	16.0 ± 0.55	0	40.0 ± 1.2
Pb—1000 mg/kg	1060.0 ± 4.2	39.0 ± 1.3	50.0 ± 2.2	90.0 ± 3.0
Cu—100 mg/kg	840.0 ± 4.3	40.0 ± 1.9	45.0 ± 1.9	25.0 ± 0.7
Cd—100 mg/kg	1050.0 ± 38.0	30.0 ± 1.4	27.0 ± 0.8	45.0 ± 1.8

[a] The differences across species and variants are not significant: $P > 0.05$ ($P = 0.22$, $P = 0.14$ for shoots and roots, respectively)

[b] The differences across species are significant $P < 0.05$ ($P = 1.46\text{E-}06$), across variants are not significant—$P > 0.05$ ($P = 0.26$)

these species for their ability to accumulate heavy metals in hydroponic conditions and their applicability for removal of trace metals from soils spiked with metals. The ability of wild grass species to accumulate trace metals in field conditions was studied by sowing their seed on artificially contaminated soils.

6.2.3 Experiments on Artificially Contaminated Soils

6.2.3.1 Effect of Heavy Metals on Plant Biomass

The shoot and root biomass of these wild grass species was compared to assess the abundance of trace metals in the soils. The root biomass of plant species from 1 m^2 of each heavy metal treatment increased in the following order (Table 6.8): Zn—*A. repens* > *S. viridis* > *D. glomerata* > *P. pretense* ($p<0.05$) Pb—*A. repens* > *S. viridis* > *P. pratense* > *D. glomerata* ($p<0.01$); Cu—*A. repens* > *P. pratense* > *D. glomerata* > *S. viridis* ($p<0.05$); Cd—*A. repens* > *S. viridis* > *D. glomerata* ≥ *P. pratense*; in this treatment, the differences between species were significant ($p<0.01$), with the exception of *D. glomerata* vs. *S. viridis* ($p>0.05$).

Shoot biomass of plant species from 1 m^2 for each treatment increased in the following order: Zn—*D. glomerata* ≥ *A. repens* > *S. viridis* > *P. pratense*; the differences between species were significant ($p<0.01$) with the exception of *D. glomerata* vs. *A. repens* ($p>0.05$); Pb—*P. pretense* > *A. repens* ≥ *D. glomerata* > *S. viridis* ($p<0.05$); the difference between *A. repens* and *D. glomerata* was not significant ($P>0.05$); Cu—*P. pratense* > *A. repens* ≥ *D. glomerata* > *S. viridis*; $P<0.01$ between all species with the exception of *A. repens* vs. *D. glomerata* ($P>0.05$); Cd—*P. pratense* > *A. repens* > *D. glomerata* > *S. viridis* ($P<0.05$ between all species). The mean shoot biomass of *P. pratense* was highest among all species in the presence of Cu, Cd, and Pb, and the shoot and root biomass of *S. viridis* was the lowest among all grasses. In the presence of Zn *D. glomerata* had the highest biomass, *P. pratense* the lowest. *A. repens* accumulated the highest root biomass in all treatments compared with other species.

Thus, *D. glomerata* and *A. repens* were tolerant to all four metals. The shoot biomass of these species was relatively high (Table 6.8). *S. viridis* was tolerant to Pb and sensitive to Zn, Cu, and Cd. *P. pratense* was relatively sensitive to all metals. *A. repens*. A comparison of shoot biomass in all treatments showed that, among metals, Zn was distinguishable by a stronger negative effect on biomass (305 g/m^2). In the presence of other metals (Pb, Cu, Cd), this species produced an approximately equal biomass (395, 425, 440 g/m^2, respectively, $p>0.05$). The greatest root biomass was observed in the treatments with Pb and Cd and the lowest in the treatment with Zn.

P. pretense. Zn fully inhibited the growth of *P. pratense*. In the presence of Pb, the mean shoot biomass was highest, and in the presence of Cu and Cd, the biomass was almost equal. The mean shoot biomass in different treatments decreased in the following order (g/m^2): Pb (800.0) > Cu (655.0) ≥ Cd (570.0); root biomass decreased in the following order (g/m^2): Pb (50.0) ≥ Cu (45.0) > Cd (27.0) ($p<0.01$) with the exception of Pb vs. Cu ($p>0.05$).

Table 6.9 Shoot/root ratio of trace metals content

Species	*Agropyron repens*	*Dactylis glomerata*	*Phleum pratense*	*Setaria viridis*
Zn	0.87	0.39	0	0.48
Pb	0.28	0.1	0.03	0.08
Cu	0.53	0.16	0.17	0.12
Cd	0.45	0.22	0.07	0.01

The differences across species and metals are significant—$P<0.05$ ($P=0.046$, $P=0.005$ for shoots and roots, respectively)

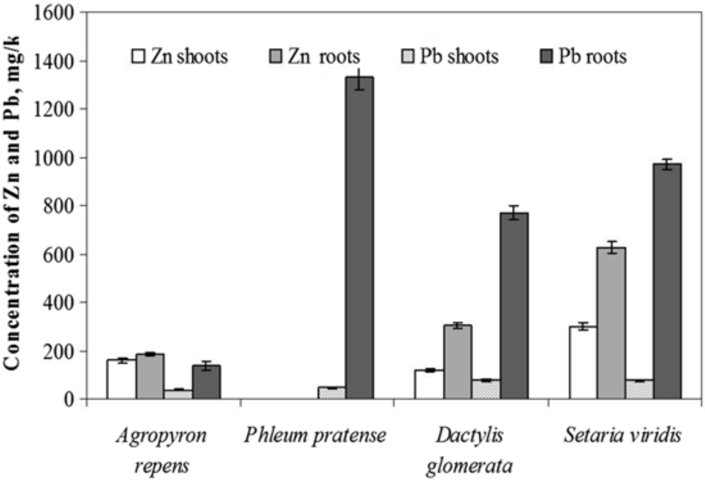


Fig. 6.4 Zinc and lead content in the plant parts of wild grass species. Differences between species according to two-way ANOVA test are significant at $P<0.05$ ($P=1.98E\text{-}32$; $7.63E\text{-}24$ for shoots and roots, respectively). LSD for roots—1067 and for shoots—210 at $P=0.95$. Values represent mean ± Standard Error (SE)

D. glomerata. In all treatments the shoot biomass was approximately equal (g/m^2): Pb (350.0) ≥ Zn (345.0) ≥ Cu (340.0) ≥ Cd (320.0) ($p>0.05$). The root biomass had the highest means in the presence of Pb and Cu. The mean root biomass decreased in the following order (g/m^2): Cu (40.0) ≥ Pb (39.0) > Cd (30.0) > Zn (16.0) ($p<0.01$), with the exception of Cu vs. Pb ($P>0.05$).

S. viridis. The shoot biomass decreased in the following order (g/m^2): Pb (280.0) > Cd (245.0) > Zn (150.0) > Cu (115.0) ($p<0.01$); root biomass (g/m^2): Pb (90.0) > Cd (45.0) ≥ Zn(40.0) > Cu (25.0) ($p<0.001$) with the exception of Zn vs. Cd ($P>0.05$).

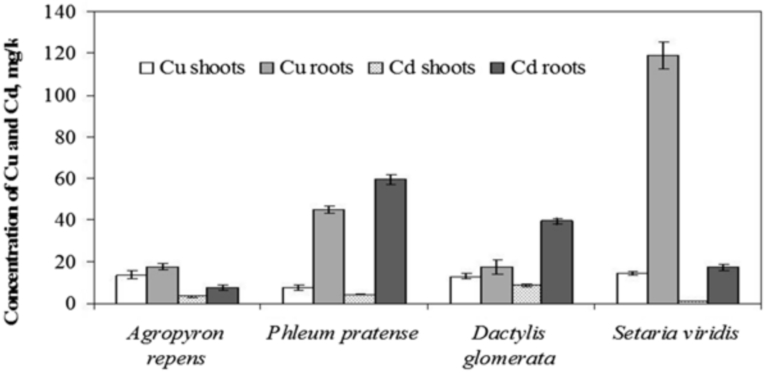


Fig. 6.5 Copper and cadmium content in plant parts of wild grass species. Differences between species according to two-way ANOVA test are significant at $P<0.01$ ($P=6.6\text{E-}35$; 2.09E-37 for shoots and roots, respectively). LSD for roots—105 and for shoots—9 at $P=0.95$. Values represent mean ± Standard Error (SE)

6.2.3.2 Content of Heavy Metals in Plant Parts of Wild Grass Species

The content of heavy metals in plant parts was determined. Different plant parts accumulated high concentrations of metals. The shoot/root ratio was <1 for all species (Table 6.9). *S. viridis* accumulated the highest amount of Zn in shoots and roots, and Pb in shoots (Fig. 6.4). Cu was most accumulated in the roots. Cd was least accumulated by this species than by other species (Fig. 6.5). The shoot/root ratio was <1 for all metals. The lowest shoot/root ratio was for Pb. The concentration of metals in the shoots decreased in the following order (mg/kg): Zn (299.1) > Pb (76.9) > Cu (14.3) > Cd (0.2); in the roots: Pb (971.2) > Zn (627.4) > Cu (119.1) > Cd (17.3).

P. pratense was the most sensitive grass to the presence of Zn in the soil. This plant did not germinate in Zn-contaminated soil. *P. pratense* accumulated Pb in the roots >1000 ppm, i.e., 1330 mg/kg. Cd accumulated by the roots of *P. pratense* was the highest among all the grass species assessed (59.4 mg/kg) (Fig. 6.5). The shoots of *P. pretense* accumulated the least Cu compared with the other heavy metals (Fig. 6.5). The shoot/root ratio for all metals was <1 and was the lowest among all species. The concentration of metals in the shoots decreased in the following order (mg/kg): Pb (45.3) > Cu (7.5) > Cd (4.2); in the roots: Pb (1330.0) > Cd (59.4) > Cu (44.9).

D. glomerata accumulated a considerable amount of Pb in the roots. In the shoots, the concentration of Zn was lowest among all species (Fig. 6.5). The concentration of metals in the shoots of *D. glomerata* decreased in the following order (mg/kg): Zn (118.4) > Pb (79.3) > Cu (13.0) > Cd (8.7); in the roots: Pb (770.4) > Zn (304.1) > Cd (39.4) > Cu (17.5) (Figs. 6.4 and 6.5). *A. repens* accumulated the least amount of Pb in the shoots and Cd in the roots. The shoot/root ratio was <1. The

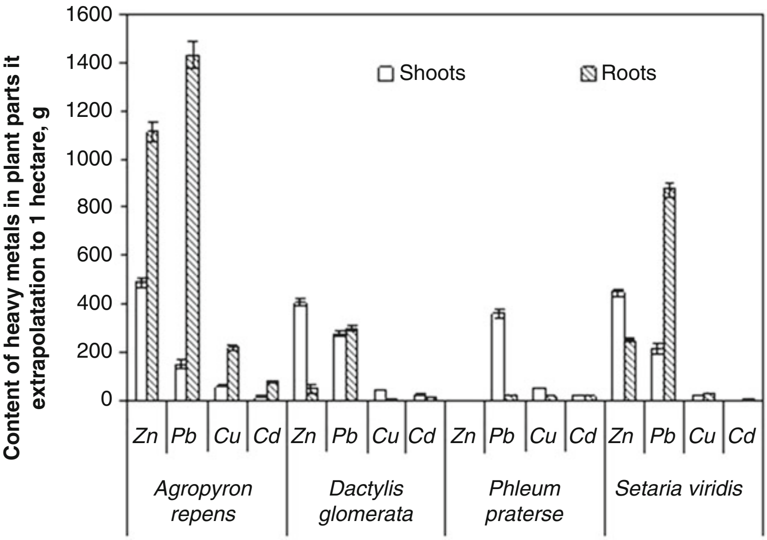


Fig. 6.6 Content of trace metals in plant biomass in extrapolation to 1 ha. Differences between species and metals are according to two-way ANOVA test significant at $P<0.05$ ($P=8.63\text{E-}16$; 5.14E-19 for shoots and roots, respectively). LSD for Pb—1314 and for Zn—1608 at $P=0.95$. Values represent mean ± Standard Error (SE)

concentration of metals in the shoots of *A. repens* decreased in the following order (mg/kg): Zn (159.7) > Pb (38.2) > Cu (13.7) > Cd (3.4); in the roots: Zn (184.1) > Pb(135.2) > Cu (25.8) > Cd (7.5) (Fig. 6.4). Thus, the highest Zn concentration was observed in the roots and shoots of *S. viridis*. Pb was greatly accumulated in the roots of *P. pratense*, *S. viridis,* and *D. glomerata*. The shoot/root ratio was <1 for all species (Table 6.9).

The removal efficiency of trace metals by roots and shoots of plants was calculated using the means of plant part biomass and the concentration of trace metals in plant parts according to equation: g/ha = Concentrations of metals (g/kg) × yield (kg/m^2) × 10,000; preliminarily, the mean of metal concentrations in mg/kg was converted into g/kg, yield in g/m^2 into kg/m^2, and the coefficient 10,000 is necessary to convert 1 m^2 into 1 ha. The removal of Zn by shoots extrapolated into 1 ha increased in the following order (g/ha): *A. repens* (487.1) > *S.viridis* (448.7) > *D. glomerata* (408.5); by the roots: *A. repens* (1113.8) > *S. viridis* (251.4) > *D. glomerata* (48.7) (Fig. 6.6).

Thus, the shoots and roots of *A. repens* removed the most Zn, whereas the shoots and roots of *D. glomerata* the least. The roots of *A. repens* removed the most Zn and the roots of *D. glomerata* the least. The removal of Pb decreased in the following order (g/ha): by shoots: *P. pratense* (362.4) > *S. viridis* (215.3) > *D. glomerata* (277.6) > *A. repens* (150.1); by roots: *A. repens* (1433.0) > *S. viridis* (874.1) > *P. pratense* (665.0) > *D. glomerata* (300.5) (Fig. 6.6). *Ph. pratense* was removed the most amounts of Pb by the shoots, *A. repens*—the lowest one. *A. repens* removed the most amounts of Pb by the roots, *D. glomerata*—the lowest ones. As for removal rates of Cu by the shoots, the species were disposed in the following order (g/ha): *A. repens* (58.2) > *P. pratense* (49.1) > *D. glomerata* (44.2) > *S. viridis* (16.5); by the roots—*A. repens* (216.7) > *S. viridis* (29.8) > *P. pratense* (20.2) > *D. glomerata* (7.0) (Fig. 6.6). The shoots of *A. repens* had the most removal rate of Cu, and the shoots of *S. viridis*—the lowest one. *A. repens* had the highest removal rate by roots and *D. glomerata*—the lowest one.

The removal of Cd by the shoots extrapolated to 1 ha decreased in the following order (g/ha): *D. glomerata* (27.8) > *P. pratense* (23.9) > *A. repens* (15.0) > *S. viridis* (0.5); roots—*A. repens* (78.8) > *P. pratense* (16.0) > *D. glomerata* (11.8) > *S. viridis* (7.8) (Fig. 6.6). Thus, the most removal rate of cadmium was observed in the shoots of *D. glomerata* and in the roots of *A. repens*. In general, all analyzed species were tolerant to trace metals. They accumulated varied amounts of trace metals mainly in the roots. All these species can be used for phytoremediation of contaminated soils, particularly for phytostabilization, due their ability to accumulate trace metals in the roots. The following species such as *D. glomerata*, *B. inermis*, *A. repens*, *A. alba,* and *P. pratense* accumulated Zn and Pb in great amounts mainly in the roots from heavily contaminated soils. Therefore, these species can be the candidates for using them in *phytostabilization*.

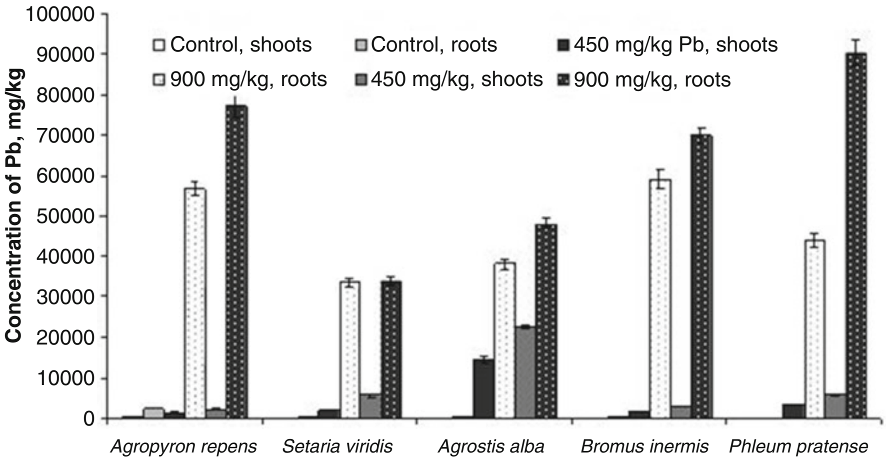


Fig. 6.7 Lead content in plant parts. Differences between treatments according to two-way ANOVA test are significant at $P < 0.05$, ($P = 5.33\text{E-}12$; 5.15E-19 for shoots and roots, respectively). LSD for roots—14,425 and for shoots—5000 at $P = 0.95$. Values represent mean ± Standard Error (SE)

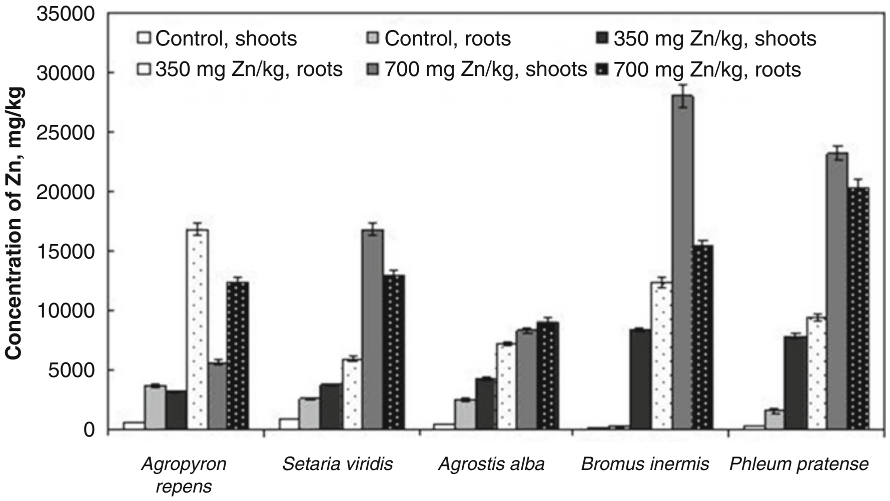


Fig. 6.8 Zinc content in plant parts. Differences between treatments according to two-way ANOVA test are significant at $P<0.05$ ($P=1.261E\text{-}37$; 2.03E-34 for shoots and roots, respectively). LSD for roots—4477 and for shoots—6469 at $P=0.95$. Values represent mean ± Standard Error (SE)

6.2.4 Hydroponic Experiments

The metal accumulation ability of grass species in hydroponic conditions at extremely high concentrations of zinc and lead (450, 900 mg/L of Pb and 350, 700 mg/L of Zn) was studied. The analysis of trace metals in plant parts has shown the highest level of lead concentration in the roots (Fig. 6.7). The highest level of lead at 900 mg/L was accumulated in the roots of the species: *P. pratense* (90,281.9 mg/kg), *A. repens* (77,137.9 mg/kg) and *B. inermis* (69,991.8 mg/kg).

The lowest level of lead was found in the roots of *S. viridis* (33,974.6 mg/kg). The shoot/root ratio for all species at the concentration 900 mg Pb/L was <1 and decreased in the following order: *A. alba* (0.47)>*S. viridis* (0.17)>*P. pratense* (0.06)>*B. inermis* (0.04)>*A. repens* (0.027). At concentration 900 mg Pb/L, the content of lead in shoots decreased in the following order: *A. alba*>*P. pretense*>*S. viridis*>*B. inermis*>*A. repens*; in roots—*P. pretense*>*A. repens*>*B. inermis*>*A. alba*. The highest level of Pb was detected in the shoots of *A. alba* (22,670.0 mg/kg) and the lowest in the shoots of *A. repens* (2091.3 mg/kg). The study of Zn content in plant parts has shown that all species except *A. repens* accumulated Zn mainly in the shoots (700 mg Zn/L). Shoot/root ratio of Zn for all species except of *A. alba* and *A. repens* was >1. *A. alba* accumulated approximately equal amount of Zn in both roots and shoots, whereas *A. repens* accumulated mainly in the roots (Fig. 6.8).

The shoot/root ratio decreased in the following order: *B. inermis* (1.8)>*S. viridis* (1.3)>*P. pratense* (1.14)>*A. alba* (0.92)>*A. repens* (0.46). The highest level of Zn was detected at 700 mg Zn/L concentration in shoots and roots of *B. inermis* and *P. pratense*, the lowest in *A. repens* and *A. alba*. Concentration of Zn in shoots was decreased in the following order: *B. inermis*>*P. pratense*>*S. viridis*>*A. alba*>*A. repens*; in roots: *P. pratense*>*B. inermis*>*S. viridis*≥*A. repens*>*A. alba*. *P. pratense* and *B. inermis* were distinguishable by high accumulation of Zn in the shoots and roots. Thus, in hydroponic conditions, Pb at high concentrations was accumulated mainly by the roots for all species and the shoot/root ratio was <1. At a high concentration of Zn, it was accumulated mainly by the shoots except those of *A. repens*, whereas *A. alba* accumulated Zn in approximately equal amounts in both roots and shoots. *P. pretense* and *B. inermis* were distinguishable from the other species by accumulating Zn in their shoots to a high level. These species also accumulated a large amount of Pb in the roots.

As mentioned above, there are several types of phytoremediation of soils contaminated with heavy metals. Plants-hyperaccumulators, which mainly accumulate heavy metals in the aboveground parts, are mainly used for phytoextraction of heavy metals from the soil. *Phytotabilization* is a type of phytoremediation and is defined as a technology, which aims to immobilize heavy metals in the root zone and prevent the distribution of metals in the soil profile. *For phytostabilization,* it is very important for the property of plants to reduce the transport of heavy metals to aboveground parts, to avoid the promotion of metals through the food chain [186, 187]. Herbaceous plants are usually resistant to trace amounts of metals and play an important role in phytoremediation [171, 172, 188]. They have well-developed adventitious roots, the unique morphology of the root system [171], the high biological productivity [171], and therefore, have an additional advantage for use in phytostabilization. The tested plant species *D. glomerata*, *B. inermis*, *A. repens*, *Ag. Alba,* and *Ph. pratense* accumulated Zn and Pb in high concentrations mainly in the roots in highly contaminated soils. *A. repens* is known as a plant that can be used for stabilization of Pb in soil [189]. These species are the most suitable candidates for their use for phytostabilization of metals on the contaminated area.

6.2.5 Effect of Humic Acids on Bioavailability of Cadmium and Lead

Humic acids are the widespread nature of physiologically active compounds; they are in the soil organic matter and determine its fertility. The structure is defined by the presence of humic acids slightly condensed and substituted aromatic rings and carboxylic, alcoholic and phenolic groups [190–192], and therefore, they have an important role in the transport, bioavailability, and solubility of heavy metals. *Agropyron repense* L. plants were grown in the soils on the pots (1 m^2 for each variant) where the following concentrations of metals in the form of $Pb(NO_3)_2$ and $CdSO_4$ were added: 250 mg Cd/kg and 1000 mg Pb/kg. After 7 days humic acids were added. As a source of humic acid (HA) "Potassium humate" was used (LTD "Kairat and Co"), which contain 8 g/L of humic acids. According to the instruction,

Table 6.10 Cadmium concentration in plant parts of *Agropyron repens* L

Variants	Shoots, мг/кг	% to control	Roots, mg/kg	% to control
Control (-Cd; -HA)	1.2±0.02	100	3.2±0.06	100
HA1 (2.5 g/m^2)	1.4±0.04	117	1.6±0.03	50
HA2 (5.0 g/m^2)	0.9±0.007	75	1.38±0.02	43
Cd (250 mg/kg)	6.6±0.17	550	200.7±4.81	6272
Cd+HA1	5.8±0.13	483	325.6±9.11	10,175
Cd+HA2	14.0±0.29	1167	421.0±7.578	13,156

Table 6.11 Lead concentration in plant parts of *Agropyron repens* L.

Variants	Shoots, мг/кг	% to control	Roots, mg/kg	% to control
Control (-Pb; -HA)	10.8±0.28	100	43.2±0.99	100
HA1 (2.5 g/m^2)	8.2±0.18	76	21.2±0.55	49
HA2 (5.0 g/m^2)	8.5±0.17	79	30.2±0.69	70
Pb (1000 mg/kg)	12.2±0.34	113	2518.5±52.8	5830
Pb+HA1	200.0±4.8	1852	4900.5±132.3	11,344
Pb+HA2	252.6±6.82	2339	1942.8±40.79	4497

the drug of HA was dissolved for obtaining necessary quantities of HA. The following quantities of HA were added to the soil: 12.5 g/m^2 (HA1) and 25 g/m^2 (HA2). Then the seeds of *Agropyron repense* L. were planted in the soil.

There were nine variants: control (without metals and humic acids), HA1 (12.5 g/m^2), HA2 (5.0 g/m^2), Cd (250.0 mg/kg), Cd+HA1 (250.0 mg/kg Cd+12.5 g/m^2 HA), Cd+HA2 (250.0 mg/kg Cd+5.0 g/m^2 HA), Pb (1000.0 mg/kg), Pb+HA1 (1000.0 mg/kg + 12.5 g/m^2 HA), Pb+HA2 (1000.0 mg/kg +5.0 g/m^2 HA). After 1 month, plants were collected for analysis. The content of cadmium in aboveground organs in variant with HA2 slightly reduced as compared to control variant. It was possibly due to chelation with humic acids trace amounts of heavy metals in soil or increased green biomass in the presence of HA (in this case, the concentration of metals is reduced by dilution and quantity of metals per unit weight is decreased). Concentration of Cd in the shoots and, especially, in the roots in variant Cd+HA2 increased significantly as compared to variants with Cd, but without HA (Table 6.10).

In variant (Cd+HA1), cadmium content in roots was 1.6 times more than in variant without HA Cd (250 mg/kg), while in variant (Cd+HA2) concentration of Cd in roots was more than two times. Concentration of Cd in the shoots in variant (Cd+HA1) was significantly lower than in the variant without HA (Cd (250 mg/kg)), and at higher concentration of HA (Cd+HA2) the cadmium content was more than two times as compared to variant without HA (Cd (250 mg/kg)). This indicates that a certain concentration of humic acids may increase the uptake of cadmium by roots and translocate it to the shoots. The similar results were obtained by previous studies. Humic acids increased the cadmium concentration in the shoots of *Nicotiana tabacum* from 30.9 to 39.9 mg/kg [134]. Humic acids decrease pH value, which promote higher heavy metals availability. Another reason of high bioavailability of heavy metals in the presence of humic acids is that plants may take up cadmium complexes with humic acid fragments, which result from microbiological degradation. The addition of humic acids reduces the lead content in the organs of plants grown without added metal (HA1 and HA2) as compared to control plants (Table 6.11).

In the presence of lead in the soil without humic acid (Pb (1000 mg/kg)), the content of this metal increased sharply in the roots and in the aerial organs was slightly higher than the control (13 %). The addition of humic acids in an amount of 2.5 g/m^2 (Pb + HA1) greatly increased the lead content in the aerial parts (16.4 times) and roots (1.9 times). Interestingly, the increasing amounts of HA in the soil (Pb + HA2) reduced the concentration of lead in the roots in 1.3 times as compared to variant Pb (1000 mg/kg), and in the shoots lead content in this variant was increased in 20.7 times as compared to variant without HA (Pb (1000 mg/kg)). Possibly that the Pb concentration in roots decreased in the roots is reduced by its translocation to aerial parts. According to other authors, the addition of humic acids to soil increased the Zn, Cu, Pb, and Cd content of tobacco plants from sludge-polluted soil. Similar results were obtained by previous studies [135, 193]. It has been found that Cd are mainly associated with low molecular weight fractions of humic acids (<1000 D), whereas Pb binds to high molecular weight fraction of humic acids (10,000 D). Their complexes with low molecular weight compounds have higher value of stability constant, more easily transported across cellular membranes than the complexes with high molecular substances that can cause a greater bioavailability of cadmium in the presence of humic acids [133].

6.3 Conclusion

Phytoremediation — the use of plants for the extraction of organic and inorganic contaminants from soil and waste water — attracted the attention of many researchers in recent years. Compared with the physical and chemical methods of purification of polluted soil from heavy metals, the method of cleanining by plants is less expensive, more efficient, and safe. The screening of grass species widely spread around metallurgic plants of East Kazakhstan in hydroponic and field conditions has shown that almost all studied grass species accumulate trace metals mainly in the roots in great amounts. The following wild grass species such as *D. glomerata*, *B. inermis*, *A. repens*, *A. alba,* and *P. pratense* can be used for phytostabilization. The experiments with addition of humic acids for phytoextraction of metals showed the possibility of applying of different chelators of trace metals, like EDTA, humic acids, etc., to enhance the removal efficiency of metals by plants from soils [194–196].

References

1. Panin MS, Melnik MS (2008) Tyazhelie metallic v ovoshnih kulturah v semipalatinskoi oblasti. In: Abstract of the international scientific-practical conference “Heavy metals and radionuclides in the environment”. The State Pegagogical University, Semey, 15–18 October 2008
2. Karpova EA (2012) Soderzhanie tyazhelih metallov v selskhozyaistvennih kulturah: vlianie airotechnogennoi nagruzki vblizi megapolisa. Paper presented at 6th international scientific conference “Heavy metals, radionuclides in the environment”, The State Pedagogic University, Semey, 4–7 February 2012
3. Kanibolotskaya UM (2013) Analyz soderzhania tyazhelih metallov v rasteniah (*Artemisia austriaca Jacq, Agropyron pectinatum (Bieb.) Beauv., Potentilla bifurca L.)* i pochve v Pavlodarskoi oblasti. HAA-n Shinzhleh yhaan satguul 10(1):113–118
4. Manara A (2012) Plant responses to heavy metal toxicity. In: Furini A (ed) Plants and heavy metals, Springer Briefs in Biometals. Springer, New York. doi:10.1007/978-94-007-4441-7_2
5. Morsy AA, Salama KH, Kamel HA, Mansour MMF (2012) Effect of heavy metals on plasma membrane lipids and antioxidant enzymes of *Zygophyllum* species. Eurasia J Biosci 6:1–10
6. Le Gall H, Philippe F, Domon J-M, Gillet F et al (2015) Cell wall metabolism in response to abiotic stress. Plants 4(1):112–166. doi:10.3390/plants4010112
7. Cheng S (2003) Effects of heavy metals on plants and resistance mechanisms. Environ Sci Pollut Res 10(4):256–264
8. Haribabu TE, Sudha PN (2011) Effect of heavy metals copper and cadmium exposure on the antioxidant properties of the plant Cleome gynandra. IJPAES 1(2):80–87
9. Aldoobie NF, Beltagi MS (2013) Physiological, biochemical and molecular responses of common bean (*Phaseolus vulgaris* L.) plants to heavy metals stress. Afr J Biotechnol 12(29):4614–4622
10. Sharma SS, Dietz K-J (2006) The significance of amino acids and amino acid-derived molecules in plant responses and adaptation to heavy metal stress. J Exp Bot 57(4):711–726
11. Hossain Z, Komatsu S (2013) Contribution of proteomic studies towards understanding plant heavy metal stress response. Front Plant Sci 3:12. doi:10.3389/fpls.2012.00310
12. Prasad MNV, Stralka K (2002) Physiology and biochemistry of metal toxicity and tolerance in plants. Kluwer Academic, Dordrecht, 432p
13. Emamverdian A, Ding Y, Mokhberdoran F et al (2015) Heavy metal stress and some mechanisms of plant defense. Scientific World J. Article ID 756120, pp 1–18. http://dx.doi.org/10.1155/2015/756120
14. Shkolnik N, Alekseeva-Popova IV (1983) Rastenia v extremalnih usloviah mineralnogo pitania. Nauka, Leningrad, p 176
15. Chaney RL, Brown SL, Li Y-M et al (1995) Potential use of hyperaccumulator plant species to decontaminate metal polluted soils. Mining Environ Manage 3(3):9–11
16. Raskin I, Smith RD, Salt DE (1997) Phytoremediation of metals: using plants to remove pollutants from the environment. Curr Opin Biotech 8:221–226
17. Öztürk M, Ashraf M, Aksoy A, Ahmad MSA (eds) (2015) Phytoremediation for green energy. Springer (eBook). http://www.springer.com/us/book/9789400778863191
18. Chaney RL, Malik M, Li YM et al (1997) Phytoremediation of soil metals. Curr Opin Biotech 8:279–284
19. Prasad MN (2003) Prakticheskoe ispolzovanie rastenii dlya vosstanovlenia ecosistem, zagyaznennihtiazhelimi metallami. Fisiologia rastenii 50(5):764–780
20. Terry N, Zayed AM (1994) Selenium volatilization in plants. In: Frankenberger JW, Benson S (eds) Selenium in the environment. Marcel Dekker, New York, pp 343–369
21. Raskin I (1996) Plant genetic engineering way help with environmental cleanup. Proc Nat Acad Sci USA 93:3164–3166
22. Oruc HH (2010) Fungicides and their effects on animals. In: Carisse O (ed) Fungicides, http://www.intechopen.com/books/fungicides/fungicides-and-their-effects-on-animal

23. Bull S (2011) HPA compendium of chemical hazards inorganic mercury/elemental mercury. Health Protection Agency. CRCE HQ, HPA 33p
24. Mendez MO, Maier RM (2008) Phytostabilization of mine tailings in arid and semiarid environments—an emerging remediation technology. Environ Health Perspect 116:278–283
25. Cotter-Howells JD, Champness PE, Charnock JM (1999) Mineralogy of Pb-P grains in the roots of *Agrostis capillaris* L. by ATEM and EXAFS. Min Mag 63(6):777–789
26. Huang JW, Cunningham SD (1996) Lead phytoextraction: species variation in lead uptake and translocation. New Phytol 134:75–84
27. Ensley BD (2000) Rationale for use of phytoremediation. Using plants to clean up the environment. In: Raskin I, Ensley BD (eds) Phytoremediation of toxic metals. Wiley, New York, pp 3–11
28. Chaney RL, Ryan JA (1994) Risk based standards for arsenic, lead and cadmium in urban soils. New Phytol 103:1305–1309
29. Ernst W (1975) Physiology of metal resistance in plants. In: Proceedings of international conference on heavy metals in the environment. 27–31 October, Toronto, Canada. J Environ Qual 11:121–136. doi:10.2134/jeq1978.00472425000700040037x
30. Dhote S, Dixit S (2009) Water quality improvement through macrophytes. Environ Monit Assess 152:149–153
31. Favas PJC, Pratas J, Varun M et al (2014) Phytoremediation of soils contaminated with metals and metalloids at mining areas: potential of native flora. Environ Risk Assess Soil Contamin. pp 485–517. http://dx.doi.org/10.5772/5746913,27,28
32. Li Y-M, Chaney RL, Brewer E et al (2003) Development of a technology for commercial phytoextraction of nicke: economic and technical considerations. Plant Soil 249:107–115
33. Broadly MR, White PJ, Hammond JP et al (2007) Zinc in plants. New Phytol 173:677–702
34. Palmergen MG, Clemens S, Williams LE (2008) Zinc biofortification of cereals: problems and solutions. Trends Plant Sci 13:464–473
35. Vazquez MD, Poschenreider C, Barcelo J et al (1994) Compartmentation of zinc in roots and leaves of the zinc hyperaccumulator *Thlaspi caerulescens*. Bot Acta 107:243–250
36. Chaney RL, Li Y-M, Scott JA (1999) Improving metal hyperaccumulator wild plants to develop commercial phytoextraction systems: approaches and progress. In: Terry N, Banuelos GS (eds) Phytoremediation of contaminated soil and water. CRC Press, Boca Raton, 97p
37. Lombi E, Zhao FJ, Dunham SJ (2000) Cadmium accumulation in populations of *Thlaspi caerulescens* and *Thlaspi goesingense*. New Phytol 145(1):11–20. doi:10.1046/j.1469-8137.2000.00560.x
38. Kramer U, Cotter-Howels JD, Charnock JM et al (1996) Free histidine as a metal chelator in plants that accumulate nickel. Nature 379:635–638
39. Chaney RL, Li YM, Scott JA (1998) Improving metal hyperaccumulator wild plants to develop commercial phytoextraction systems: approaches and Progress. Wiley, New York, 37p
40. Brooks RR (1998) Plants that hyper accumulate heavy metals. CAB International, Wallingford
41. Bert V, Bonnin I, Saumiyou-Laprade P (2002) Do *Arabidopsis halleri* from nonmetallicolous populations accumulate zinc and cadmium more effectively than those from metallicolous populations? New Phytol 155:47–57
42. Hamon RE, Holm PE, Lorenz SE et al (1999) Metal uptake by plants from sludge-amended soils: caution is required in the plateu interpretation. Plant Soil 216:53–64
43. McGrath SP (1998) Phytoextraction for soil remediation. In: Brooks RR (ed) Plants that hyperaccumulate heavy metals. CAB International, Wallingford, pp 261–287
44. Lasat MM, Fuhrman M, Ebbs SD (2003) Phytoextraction of radio cesium-contaminated soil: evaluation of cesium-137 bioaccumulation in the shoots of three plant species. J Environ Qual 27:165–169
45. Baker AJ, McGrath SP, Reeves RD (2000) Metal hyperaccumulator plants: a review of the ecology and physiology of a biochemical resource for phytoremediation of metal polluted soils. In: Terry N (ed) Phytoremediation of contaminated soil and water. Lewis Publishers, Boca-Raton, pp 85–108

46. Takahashi M, Nakanishi H, Kawasaki N et al (2001) Enhanced tolerance of rice to low iron availability in alkaline soils using barley nicotianamine aminotransferase genes. Nat Biotechnol 19(5):417–418
47. Inoue H, Higuchi K, Takanishi M et al (2003) Three rice nicotinamine synthase genes, OsNAS1, OsNAS2, OsNAS3 are expressed in cells involved in long distance transport of iron and differentially regulated by iron. Plant J 36:366–381
48. Negishi T, Nakanishi H, Yazaki J (2002) cDNA microarray analysis of gene expression during Fe-deficiency stress in barley suggests that polar transport of vesicles is implicated in phytosiderophore secretion in Fe-deficient barley roots. Plant J 30(1):83–94
49. Takagi S (1976) Naturally occurring iron-chelating compounds in oat- and rice-root washings. I. Activity measurement and preliminary characterization. Soil Sci Pl Nutr 22:423–433
50. Marshner H, Romheld V, Kissel M (1986) Different strategies in higher plants in mobilization and uptake of iron. J Plant Nutr 9:695–713
51. Takahashi M, Yamaguchi H, Nakanishi H et al (1999) Cloning two genes for nicotianamine aminotransferase, a critical enzyme in iron acquisition (Strategy II) in graminaceous plants. Plant Physiol 121:947–956
52. Sugiura Y, Tanaka H, Mino Y et al (1981) Structure, properties, and transport mechanism of iron (III) complex of mugineic acid, a possible phytosiderophore. J Am Chem Soc 103:6979–6982
53. Takagi S, Nomoto K, Takemoto S (1984) Physiological aspect of mugineic acid, a possible phytosiderophore of graminaceous plants. J Plant Nutr 7:469–477
54. Nishiyama R, Kato M, Nagata S et al (2012) Identification of Zn–nicotianamine and Fe–20-deoxymugineic acid in the phloem sap from rice plants (*Oryza sativa* L.). Plant Cell Physiol 53(2):381–390
55. Klatte M, Schuler M, Wirtz M (2009) The analysis of *Arabidopsis* nicotianamine synthase mutants reveals functions for nicotianamine in seed iron loading and iron deficiency responses. Plant Physiol 150:257–271
56. Mari S, Gendre D, Pianelli K et al (2006) Root-to-shoot long-distance circulation of nicotianamine and nicotianamine–nickel chelates in the metal hyperaccumulator *Thlaspi caerulescens*. J Exp Bot 57(15):4111–4122
57. Nomoto K, Sigiura Y, Takagi S (1987) Mugineic acids, studies on phytosiderophores. In: Winkellmann G, Van der Helm D, Neilands JB (eds) Iron transport in microbes, plants and animals. VCH Publishers, New York, pp 401–425
58. Ma JF, Nomoto K (1996) Effective regulation of iron acquisition in graminaceous plant. The role of mugineic acids as phytosiderophores. Physiol Plan 97:609–617
59. Treeby M, Marchner H, Romheld V (1989) Mobilization of iron and other micronutrient cations from a calcareous soil by plant-borne, microbial, and synthetic metal chelators. Plant Soil 114:217–226
60. Neilands JB (1986) Siderophores in relation to plant growth and disease. Ann Rev Plant Physiol 37:187–208
61. Suzuki M, Takahashi M, Tsukamoto T (2006) Biosynthesis and secretion of mugineic acid family phytosiderophores in zinc-deficient barley. Plant J 48:85–89
62. Tolay I, Erenoglu B, Romheld V et al (2001) Phytosiderophore release in Aegilops tauschii and Triticum species under zinc and iron deficiencies. J Exp Bot 52(358):1093–1099
63. Shenker M, Fan TW, Crowley DE (2001) Phytosiderophores influence on cadmium mobilization and uptake by wheat and barley plants. J Environ Qual 30(6):2091–2098
64. Demidchik VV, Sokolik AI, Urin VM (2001) Postuplenie medi v rastenia i raspredelenie v kletkah, tkaniah i organah. Uspehi Sovr Biol 121(2):190–197
65. Toshihiro Y, Hirotaka H, Yoshiyuki M et al (2006) Cadmium inducible fe deficiency responses observed from macro and molecular views in tobacco plants. Plant Cell 25(4):365–373
66. Alekseeva UE, Lepkovich IP (2003) Kadmii I zink v rasteniah lugovih fitocenosov. Agrohimia 9:66–69

67. Hill KA, Lion LW, Ahner BA (2002) Reduced Cd accumulation in Zea mays: a protective role for phytosiderophores? Environ Sci Technol 36(24):5363–5368
68. Gries D, Brunn S, Crowley DE (1995) Phytosiderophore release in relation to micronutrient metal deficiencies in barley. Plant Soil 172:299–308
69. Chlegel R, Kynast R, Schwarzzacher T et al (1993) Mapping of genes for copper efficiency in rye and the relationship between copper and iron efficiency. Plant Soil 154(1):61–65
70. Yehuda Z, Shenker M, Romheld V (1996) The role of ligand exchange in the uptake of iron from microbial siderophores by gramineous plants. Plant Physiol 112:1273–1280
71. Weber M, Harada E, Vess C et al (2004) Comparative microarray analysis of Arabidopsis thaliana and Arabidopsis halleri roots identifies nicotianamine synthase, a ZIP transporter and other genes as potential metal hyperaccumulation factors. Plant J 37:269–281
72. Pianelli K, Mari S, Marque SL et al (2005) Nicotianamine over-accumulation confers resistance to nickel in *Arabidopsis thaliana*. Transgen Res 14:739–748
73. Salt DE, Rauser WE (1995) Mg-ATPase dependent transport of phytochelatins across the tonoplast of oat roots. Plant Physiol 107:1293–1301
74. Ahner BA, Morel FMM (1995) Phytochelatin production in marine algae. Limnol Oceanograph 40(4):649–665
75. Cobbett CS (2000) Phytochelatins and their roles in heavy metal detoxification. Plant Physiol 123:825–832
76. Oven M, Raith K, Neubert HH (2001) Homo-phytochelatins are synthesized in response to cadmium in Azuki beans. Plant Physiol 126:1275–1280
77. Rauser WE (1995) Phytochelatins and related peptides. Plant Physiol 109:1141–1149
78. Ruttkay-Nedecky B, Nejd L, Gumulec J et al (2013) The role of metallothionein in oxidative stress. Int J Mol Sci 14:6044–6066. doi:10.3390/ijms14036044, www.mdpi.com/journal/ijms
79. Breen JG, Nelson E, Miller RK (1995) Cellular adaptation to chronic cadmium exposure: intracellular localization of metallothionein protein in human trophoblast cells. Teratology 51(4):266–272
80. Van Hoof NA, Hassinen VH, Hakvoork HWJ (2001) Enhanced copper tolerance in *Silene vulgaris* (Moench) Gracke populations from copper mines is associated with increased transcript levels of 2b-type metallothioneine gene. Plant Physiol 126:1519–1526
81. Grennan AK (2011) Metallothioneins, a diverse protein family. Plant Physiol 155:1750–1761
82. Dahmani-Muller H, Van-Oort F, Gelie B et al (2000) Strategies of heavy metal uptake by three plant species growing near a metal smelter. Environ Pollut 109:231–238
83. Neumann D, Zur Nieden U (1995) How does *Armeria maritima* tolerate high heavy metal concentrations? Plant Physiol 146(5–6):704–717
84. Schat H, Llugany M, Vooijs R et al (2002) The role of phytochelatins in constitutive and adaptive heavy metal tolerances in hyperaccumulator and non-hyperaccumulator metallophytes. J Exp Bot 53:1–12
85. Okuyama M, Kobayashi Y, Inouhe M (1999) Effect of some heavy metal ions on copper-induced metallothionein synthesis in yeast *Saccharomyces cerevisiae*. Biometals 12(4):307–314
86. Garsia-Hernandes M, Murphy A, Taiz L (1998) Metallothioneins 1 and 2 have distinct, but overlapping expression patterns in *Arabidopsis*. Plant Physiol 118:387–389
87. Jack E, Hakvoort HVJ, Reumer A et al (2007) Real time PCR-analysis of metallothioneine-2B expression in metallicolous and non metallicolous populations in *Silene vulgaris* (Moench) Garcke. Environ Exp Bot 59:84–91
88. Schmager MEV, Oven M, Grill E (2000) Detoxification of arsenic by phytochelatins in plants. Plant Physiol 122:739–802
89. Grill E, Luffler S, Winnacker E-L (1989) Phytochelatins, the heavy-metal-binding peptides of plants, are synthesized from glutathione by a specific g-glutamylcysteine dipeptidyl transpeptidase (phytochelatin synthetase). Proc Nat Acad Sci USA 86:6838–6842
90. Clemens S, Palmgren MG, Kramer U (2002) A long way ahead: understanding and engineering plant metal accumulation. Trends Plant Sci 7:309–315

91. Batista BL, Nigar M, Mestro A et al (2014) Identification and quantification of phytochelatins in roots of rice to long-term exposure: evidence of individual role on arsenic accumulation and translocation. J Exp Bot 65(6):1467–1479. doi:10.1093/jxb/eru018 First published online: 5 March 2014 http://jxb.oxfordjournals.org/open
92. Wu Z, Zhang C, Yan J et al (2013) Separation and quantification of cysteine, glutathione and phytochelatins in rice (*Oryza sativa* L.) upon cadmium exposure using reverse phase ultra performance liquid chromatography (RP-UPLC) with fluorescence detection. Anal Methods 5:6147–6152
93. Tsuji N, Hirayanagi N, Iwabe O et al (2003) Regulation of phytochelatin synthesis by zinc and cadmium in marine green alga, Dunaliella tertiolecta. Phytochemistry 62(3):453–459
94. Kvesitadze GI, Hatisashvili GA, Sadunishvili TA et al (2002) Meatbolism antropogennih toksikantov v visshih rasteniah. Nauka, Moskva, 197p
95. Xiang C, Oliver DJ (1998) Glutathione metabolic genes coordinately respond to heavy metals and jasmonic acid in *Arabidopsis*. Plant Cell 10:1539–1550
96. Lee J, Reeves RD, Brooks RR et al (1977) Isolation and identification of citrato-complex of nickel from nickel-accumulating plants. Biochemistry 16:1503–1505
97. Lebedeva AF, Savanina IV, Barskyi EL (1998) Ustoichivost cyanobakterii I microvodoroslei k deistviu tiazhelih metallov. Rol metallosvyasivaushih belkov. Vest. MGU 2:42–49
98. Inouhe M, Ito R, Ito S (2000) Azuki bean cells are hypersensitive to cadmium and do not synthesize phytochelatins. Plant Physiol 123:1029–1036
99. Gupta SC, Goldsborough PB (1991) Phytochelatin accumulation and cadmium tolerance in selected tomato cell lines. Plant Physiol 97:306–312
100. Burdin KS, Polyakova UE (1987) Metallothioneini, ih strjenie I funkcii. Usp Sovr Biol 103(3):390–400
101. Dominguez JR, Guttierez-Alcala G, Romero LC (2001) The cytosole O-acetylserine (thiol) lyase gene is regulated by heavy metals and can function in cadmium tolerance. J Biol Chem 276:9297–9302
102. Delhaize E, Jackson PJ, Lujan LD (1989) Poly (g-glutamylcysteinyl) glycine synthesis in *Datura innoxia* and binding with cadmium. Plant Physiol 89(2):700–706
103. Gachot B, Tauc M, Wanstoc F (1994) Zinc transport and metallothionein induction in primary cultures of rabbit kidney proximal cells. Biochim Biophys Acta 191(2):291–298
104. Harmens H, Den Hartog PR, Verkleij JA (1993) Increased zinc tolerance in *Silene vulgaris* (Moench) Garcke is not due to increased production of phytochelatins. New Phytol 103:1305–1309
105. Howden R, Andersen CR, Gobbett CS (1995) A cadmium-sensitive, glutathione-deficient mutant of *Arabidopsis thaliana*. Plant Phys 107:1067–1073
106. Lombi E, Zhao FJ, McGrath SP et al (2001) Physiological evidence foe high-affinity transporter highly expressed in *Thlaspi caerulescens* ecotype. New Phytol 149:53–60
107. Zhu YL, Pilon-Smits EAH, Jouanin L et al (1999) Overexpression of glutathione synthetase in Indian mustard enhances cadmium accumulation and tolerance. Plant Physiol 119:73–80
108. Foyer CH, Haliwell B (1976) The presence of glutathione and glutathione reductase in chloroplasts: a proposed role in ascorbic acid metabolism. Planta 133:21–25
109. Marrs K (1996) The functions and regulation of glutathione S-transferases in plants. Ann Rev Plant Physiol Plant Mol Biol 47:127–158
110. Zenk MH (1996) Heavy metal detoxification in higher plants: a review. Gene 179:21–30
111. Chen J, Zhou J, Goldsbrough PB (1997) Characterization of phytochelatin synthase from tomato. Physiol Plant 101:165–172
112. Steffens JC (1990) The heavy metal-binding peptides of plants. Ann Rev Plant Physiol Plant Mol Biol 41:553–575
113. Foyer CH, Souriau N, Perret S et al (1995) Over-expression of glutathione reductase but not glutathione synthetase leads to increases in antioxidant capacity and resistance to photoinhibition in poplar trees. Plant Physiol 109:1047–1057

114. Schneider S, Bergmann L (1995) Regulation of glutathione synthesis in suspension cultures of parsley and tobacco. Bot Acta 108:34–40
115. Rauser WE, Schupp R, Rennenberg H (1991) Cysteine, γ-glutamylcysteine and glutathione levels in maize seedlings. Distribution and translocation in normal and cadmium-exposed plants. Plant Physiol 97:128–138
116. Goldbrough PB (1998) Metal tolerance in plants: the role of phytochelatins and metallothioneins. In: Terry N, Banuelos GS (eds) Phytoremediation of trace elements. CRC Press, Boca Raton, pp 221–233
117. Cheung WY (1984) Calmodulin: its potential role in cell proliferation and heavy metal toxicity. Fed Proc 43:2995–2999
118. Marchiol L, Leita L, Martin M et al (1996) Physiological responses of two soybean cultivars to cadmium. J Environ Qual 25:562–566
119. Petit CM, van de Geijn SC (1978) In vivo measurements of cadmium (115 mM Cd) transport and accumulation in steams of intact tomato plants (*Lycopersicon esculentum* Mill). I. Long distance transport and local. Planta 138:137–143
120. Kos B, Grčman H, Leštan D (2003) Phytoextraction of lead, zinc and cadmium from soil by selected plants. Plant Soil Environ 49:548–553
121. Dede G, Ozdemir S, Dede HO (2012) Effect of soil amendments on phytoextraction potential of Brassica juncea growing on sewage sludge. Int J Environ Sci Technol 9:559–564
122. Hong PKA, Li C, Banerji SK, Regmi T (1999) Extraction, recovery and biostability of EDTA for remediation of heavy metal-contaminated soil. J Soil Contam 8:81–103
123. Sun B, Zhao FJ, Lombi E et al (2001) Leaching of heavy metals from contaminated soils using EDTA. Environ Pollut 113:111–120
124. Jiang XJ, Luo YM, Zhao QG (2003) Soil Cd availability to Indian mustard and environmental risk following EDTA addition to Cd-contaminated soil. Chemosphere 50:813–818
125. Farid M, Ali S, Shakoor MB, Bharwana SA et al (2013) EDTA assisted phytoremediation of cadmium, lead and zinc. Int J Agron Plant Prod 4(11):2833–2846
126. Barona A, Aranguiz I, Elias A (2001) Metal associations in soils before and after EDTA extractive decontamination: implications for the effectiveness of further clean-up procedures. Environ Pol 113:79–85
127. McGrath SP, Zhao FJ, Lombi E (2002) Phytoremediation of metals, metalloids and radionuclides. Adv Agron 75:1–56
128. Vassil A, Kapulnik Y, Raskin I, Salt DE (1998) The role of EDTA in lead transport and accumulation by Indian mustard. Plant Physiol 117:447–453
129. Blaylock MJ, Salt DE, Dushenkov S et al (1997) Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents. Environ Sci Technol 31:860–865
130. Kayser AK, Wenger A, Keller W (2000) Enhancement of phytoextraction of Zn, Cd and Cu from calcareous soil: the use of NTA and sulfur amendments. Environ Sci Technol 34:1178–1183
131. Robinson BH, Mills TV, Petit D et al (2000) Natural and induced cadmium accumulation in poplar and willow: implications for phytoremediation. Plant Soil 227:301–306
132. Platonov VV, Lebedeva GF, Proskuryakova VA et al (2005) Himicheskaya modifikasia guminovih preparatov s celiu povishenia ich biologicheskoi aktivnosti. ZHPH 78(8):1384
133. Grzybowsky W (2000) Comparison between stability constants of cadmium and lead complexes with humic substances of different molecular weight isolated from Baltic Sea water. Oceanologia 42(4):473–482
134. Evangelouye MWH, Daghan H, Schaeffer A (2004) The influence of humic acids on the phytoextraction of cadmium from soil. Chemosphere 57:207–213
135. Topcuoğlu B (2013) Effects of humic acids on the phytoextraction efficiency of sludge applied soil. IJCEBS 1(1) http://www.isaet.org/images/extraimages/IJCEBS%200101106.pdf

136. Seyyedi M, Moghaddam PR, Shahriari R et al (2013) Allelopathic potential of sunflower and caster bean on germination properties of dodder (*Cuscuta compestris*). Afr J Agric Res 8(7):601–607
137. Kupidłowska E, Gniazdowska A, Stpien J et al (2006) Impact of sunflower (*Helianthus annuus* L.) extracts upon reserve mobilization and energy metabolism in germinating mustard (*Sinapis alba* L.) seeds. J Chem Ecol 32:2569–2583
138. Lesage E, Meers E, Vervaeke P et al (2005) Enhanced phytoextraction: II. Effect of EDTA and citric acid on heavy metal uptake by Helianthus annuus from a calcareous soil. Int J Phytoremediation 7(2):143–152
139. Gulz PA, Gupta SK, Schulin R P (2003) Enhanced phytoextraction of arsenic from contaminated soil using sunflower. In: Abstracts of the 7th international conference on the biogeochemistry of trace elements (7th ICOBTE), Vol I–II, pp 148–149. Uppsala, Sweden, 15–19 June 2003
140. Boonyapookana B, Parkpian P, Techapinyawat S et al (2005) Phytoaccumulation of lead by sunflower (*Helianthus annuus*), tobacco (*Nicotiana tabacum*), and vetiver (*Vetiveria zizanioides*). J Environ Sci Health, Part A, Tox Hazard Subst Environ Eng 40(1):117–137
141. Begonia GB (1997) Comparative lead uptake and responses of some plants grown on lead contaminated soils. J Mississippi Acad Sci 42:101–106
142. Lee I, Baek K, Kim H et al (2007) Phytoremediation of soil co-contaminated with heavy metals and TNT using four plant species. J Environ Sci Health A Tox Hazard Subst Environ Eng 42(13):2039–2045
143. Solhi M, Hajabbasi MA, Shareatmadari H (2005) Heavy metals extraction potential of sunflower (*Helianthus annuus*) and canola (*Brassica napus*). Caspian J Env Sci 3(1):35–42
144. Raskin I, Nanda Kumar PBA, Dushenkov V, Salt DE (1994) Bioconcentration of heavy metals by plants. Curr Op Biol 5:285–290
145. Romeiro S, Lagôa AMM, Furlani PR (2006) Lead uptake and tolerance of *Ricinus communis* L. Braz. J Plant Physiol 18(4):18–25
146. Zhi-xin N, Sun LN, Sun TH et al (2007) Evaluation of phytoextracting cadmium and lead by sunflower, ricinus, alfalfa and mustard in hydroponic culture. J Environ Sci (China) 19(8):961–975
147. Vwioko DE, Anoliefo G, Fashemi SD (2006) Castor oil grown in soil contaminated with spent lubricating oil. J App Sci Environ Manag 10(3):127–134
148. Marchiol L, Assoaris S, Sacco P et al (2004) Phytoextraction of heavy metals by canola (*Brassica napus*) and radish (*Raphanus sativus*) grown on multicontaminated soil. Environ Pollut 132(1):21–27
149. Kvesitadze GI, Hatisashvili GA, Sadunishvili TA et al (2005) Meatbolism antropogennih toksikantov v visshih rasteniah. Nauka, Moskva, 197p
150. Pilon-Smits E, Pilon M (2002) Phytoremediation of metals using transgenic plants. Crit Rev Plant Sci 21(5):439–456
151. Krystofova O, Zitka O, Krizkova S et al (2012) Accumulation of cadmium by transgenic tobacco plants (*Nicotiana tabacum* L.) carrying yeast metallothionein gene revealed by electrochemistry. Int J Electrochem Sci 7:886–907
152. Bizily SP, Rugh CL, Meagher RB (2000) Phytodetoxification of hazardous organomercurials by genetically engineered plants. Nat Biotechnol 18:213–217
153. Rugh CL, Bizily SP, Meagher RB (2000) Phytoreduction of environmental mercury pollution. In: Raskin I, Ensley BD (eds) Phytoremediation of toxic metals—using plants to clean up the environment. Wiley, New York, pp 151–171
154. Raskin I, Ensley BD (2000) Phytoremediation of toxic metals: using plants to clean up the environment. Wiley, New York, p 303
155. Bizily SP, Kim T, Kandasamy MK (2003) Subcellular targeting of methylmercury lyase enhances. Its specific activity for organic mercury detoxification in plants. Plant Physiol 131:463–467

156. Brewer EP, Saunders JA, Angle JS et al (1999) Somatic hybridization between the zinc accumulator Thlaspi caerulescens and Brassica napus. Theor Appl Genet 99:761–771
157. Koprivova A, Kopriva S, Jager D et al (2002) Evaluation of transgenic poplars over expressing enzymes of glutathione synthesis for phytoremediation of cadmium. Plant Biol 4:664–670
158. Ow DW (1996) Heavy metal tolerance genes-prospective tools for bioremediation. Res Conserv Recycling 18:135–149
159. Blaylock MJ, Huang JW (2000) Phytoextraction of metals. In: Raskin I, Ensley BD (eds) Phytoremediation of toxic metals: using plants to clean up the environment. Wiley, New York, pp 53–70
160. Hetland MD, Gallagher JR, Daly DJ et al (2001) Processing of plants used to phytoremediate lead-contaminated sites. In: Leeson A, Foote EA, Banks MK, Magar VS (eds) Proceedings of the 6th international in situ and on-site bioremediation symposium. Phytoremediation, wetlands, and sediments, San Diego, Battelle Press, Columbus, Richland, 4–7 June 2001, pp 129–136
161. Kumar PB, Dushenkov AN, Motto VH et al (1995) Phytoextraction: the use of plants to remove heavy metals from soils. Environ Sci Technol 29:1232–1238
162. Nicks L, Chambers MF (1994) Nickel farm. Discover, p 19
163. Iyer PVR, Rao TR, Grover PD (2002) Biomass thermochemical characterization, 3rd edn. Indian Institute of Technology, Delhi, India, p 38
164. Bridgwater AV, Meier D, Radlein D (1999) An overview of fast pyrolysis of biomass. Org Geochem 30:1479–1493
165. Lasat MM (2001) American association for the advancement of science environmentals science and engineering fellow. The use of plants for the removal of toxic metals from contaminated soils. Environmental Protection Agency, New York, 2001, 33p
166. Shariphanova AS, Belgubayeva AE (2009) Soderzhanie tiazhelih metallov v hvoe b steble eli sibirskoi (*Picea obovata* Ledeb) v usloviah markakolskogo gosudarstvennogo prirodnogo zapovednika. Kazakhstannin industrialik-innovacialik damuindagi gilimnig roli, Ust-Kamenogorsk: VKGU, pp 163–166
167. Tasekeev M (2004) Bioremediacia txichnih promyshlennih othodov. Promyshlennost Kazakhstana 5(26):59–63
168. Panin MS (1998) Influence of anthropogenic activity and human agrochemical activity on migration of heavy metals in system “soil-plant”. In: International conference on the state and rational use of soils in Kazakhstan, 12–15 September 1998, Almaty, Kazakhstan, pp 76–79 (Abstract)
169. Prasad MNV (2006) Stabilization, remediation and integrated management of metal-contaminated ecosystems by grasses (Poaceae). In: Prasad MNV, Sajwan KS, Ravi Naidu (eds) Trace elements in the environment: biogeochemistry, biotechnology and bioremediation. CRC Press, Boca Raton, pp 405–424
170. Vernay P, Gauthier-Moussard C, Hitmi A (2007) Interaction of bioaccumulation of trace metal chromium with water relation, mineral nutrition and photosynthesis in developed leaves of Lolium perenne L. Chemosphere 68:1563–1575
171. Li T, Yang X, Lu L et al (2009) Effects of zinc and cadmium interactions on root morphology and metal translocation in a hyperaccumulating species under hydroponic conditions. J Hazard Mater 169:734–741
172. Zhang X, Xia H, Li Z et al (2010) Potential of four for age grasses in remediation of Cd and Zn contaminated soils. Biores Technol 101(6):2063–2066
173. Boisson S, Le Stradic S, Collignon J (2015) Potential of copper-tolerant grasses to implement phytostabilisation strategies on polluted soils in South D.R. Congo. Environ Sci Poll Res. First online: 8 October 2015, pp 1–13. http://www.link.springer.com/article/10.1007%2Fs11356-015-5442-2

174. Comino E, Fiorucci A, Menegatti S, Marocco C (2009) Preliminary test of arsenic and mercury uptake by Poa annua. Ecol Eng 35:343–350
175. Mateos-Naranjo E, Redondo-Gómez S et al (2008) Growth and photosynthetic responses to copper stress of an invasive cord grass, Spartina densiflora. Marine Environ Res 66:459–465
176. Deram A, Languereau-Leman F, Howsam M et al (2008) Seasonal patterns of cadmium accumulation in *Arrhenatherum elatius* (Poaceae): influence of mycorrhizal and endophytic fungal colonization. Soil Biol Biochem 40:845–848
177. Abhilash PC, Powell JR, Singh HB et al (2012) Plant-microbe interactions: novel applications for exploitation in multipurpose remediation technologies. Trends Biotechnol 30(8): 416–420. doi:10.1016/j.tibtech.2012.04.004
178. Frérot H, Lefèbvre C, Gruber W et al (2006) Specific interactions between local metallicolous plants improve the phytostabilization of mine soils. Plant Soil 282(1):53–65
179. Hartley W, Dickinson NM, Riby P et al (2009) Arsenic mobility in brownfield soils amended with greenwaste compost or biochar and planted with Miscanthus. Environ Pollut 157:2654–2662
180. Dunn CE (1992) Bigeochemical exploration for deposits of the noble metals. In: Brooks RR (ed) Noble metals and biological systems: their role in medicine, mineral exploration and the environment. CRS Press, Boca Raton, pp 47–89
181. LMN/SOP-06 (2006). Digestion of hard samples for heavy metal determination in the flame, 10p
182. Ovcharov KE (1979) Physiologicheskie osnovi vshozhesti semian. Nauka, Moskva, 278p
183. Hoagland DR, Broyer TC (1940) General nature of process of salt accumulation by roots with description of experimental methods. Am J Bot 3:431–444
184. LMN/SOP-08 (FLAA) (2001) Work process of Analyst 300 Perkin Elmer (in the flame), 14p
185. Atabayeva S, Sarsenbayev B, Prasad MNV et al (2010) Accumulation of trace metals in grasses of Kazakhstan: relevance to phytostabilization of mine waste and metal-smelting areas. AAJPSB Special issue: Kazakhstan Plant Science and Biotechnology, pp 91–97
186. Wenzel W, Adriano D, Salt D et al (2000) Phytoremediation: a plant-microbe-based remediation system. In: Bioremediationof contaminated soils. Agronomy Monograph no 37, 508p
187. Ghosh M, Singh SP (2005) A review on phytoremediation of heavy metals and utilization of its byproducts. App Ecol Environ Res 3(1):1–18
188. Wang Y, Shi J, Lin Q et al (2007) Trace metal availability and impact on activity of soil microorganisms along a Cu/Zn contamination gradient. J Environ Sci 19:848–853
189. Glass DI (1999) US International markets for phytoremediation 1999–2000. D.J. Glass Associates, Needham, 266p
190. Hofrichter M, Steinbuchel A (2001) Biopolymers, vol. 1, Lignin, humic substances and coal. Wiley Europe-VCH, Weinheim
191. Stadnikov GL (1973) Proishozhdenie uglei i nefti, 3rd edn. Nauka, Leningrad
192. Lagier T, Feuillade G, Matejka G (2000) Interactions between copper and organic macromolecules: determination of conditional complexation constants. Agronomie 20:537–546
193. Li Z, Shuman LM (1996) Heavy metal movement in metal-contaminated soil profiles. Soil Sci 161:656–666
194. Koopmans GF, Römkens PFA, Fokkema MJ et al (2008) Feasibility of phytoremediation to remediate cadmium and zinc contaminated soils. Environ Pollut 156:905–914
195. Saifullah E, Meers M, Qadir P et al (2009) EDTA assisted Pb-phytoextraction. Chem 10:1279–1291
196. Vamerali T, Bandiera M, Mosca G (2010) Field crops for phytoremediation of metal-contaminated land. Environ Chem Lett 8:1–17

Chapter 7
Bio-based Methods for Wastewater Treatment: Green Sorbents

Alaa El Din Mahmoud and Manal Fawzy

7.1 Introduction

The global environment is under great stress due to urbanization and industrialization as well as population pressure on the limited natural resources [1]. The availability of water resources is becoming increasingly scarce; the consumption and exploitation of water resources, along with an exponential increase in population have caused water pollution [2–4]. About 80 % of the world's population lives in areas with high water security threats, the most severe category encompassing 3.4 billion people, almost all in developing countries [5]. One of the Millennium Development Goals (MDGs) is to halve, by 2015, the proportion of people that did not have access sustainable sanitation. In 2002, 1.1 billion people did not have access to a reliable water supply and 2.6 billion people lacked access to adequate sanitation [6]. Moreover, the needs of water for agriculture, industry, and domestic have steadily increased. These withdrawals are projected to continue increasing, placing further pressure on aquatic ecosystems [5]. Regarding Fig. 7.1, United States Environmental Protection Agency (USEPA) stated that heavy metals are the most contaminants in industrial effluents [7].

Most of the pollutants and heavy metals discharged in industrial effluents ultimately find their way to aquatic ecosystems. Metals are omnipresent constituents in the biosphere, vital to our industry, infrastructure, and daily life. Since the industrial revolution, metals have increasingly been redistributed in the environment, with accumulation in terrestrial and aquatic habitats being associated with adverse effects on the biota and human health [8]. Heavy metals are elements having atomic weights between 63.5 and 200.6, and specific gravity greater than 5.0 [9]. Heavy metals are

---

A. El Din Mahmoud (✉) • M. Fawzy
Environmental Sciences Department, Faculty of Science, Alexandria University,
Alexandria 21511, Egypt
e-mail: alaa-mahmoud@alexu.edu.eg

A.A. Ansari et al. (eds.), *Phytoremediation*, DOI 10.1007/978-3-319-40148-5_7

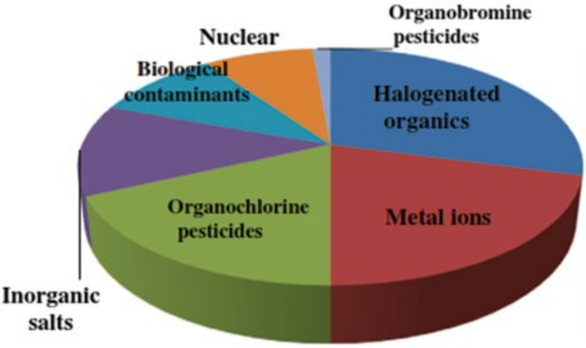


Fig. 7.1 Distribution of contaminants regulated by United States Environmental Protection Agency (USEPA) (Reproduced from [7])

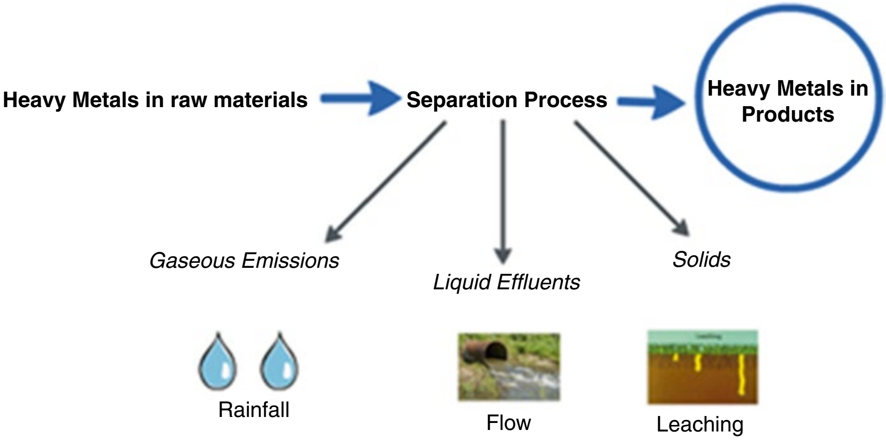


Fig. 7.2 Heavy metals sources in water bodies

the main group of inorganic contaminants, and a considerable large area of land is contaminated with them due to use of sludge, pesticides, fertilizers, and emissions from municipal waste incinerators, car exhausts, and smelting industries [10]. For example, heavy metals can be existed into water during rainfall, and they are leached from solids into surface streams and rivers. Moreover, effluents from industries are often discharged directly into rivers or other receiving surface water bodies [11] as shown in Fig. 7.2.

Toxic heavy metals of particular concern in treatment of industrial wastewaters include: mercury, lead, cadmium, zinc, copper, nickel, and chromium [9, 12] and the metals that defined as priory pollutants are: Pb, Cr, Hg, Se, Zn, As, Cd, Au, Ag, Cu, and Ni [13]. Various methods exist for the removal of heavy metal ions from wastewater which include chemical precipitation, ion exchange, reverse osmosis, solvent extraction, and adsorption. The traditional adsorbent material was Activated Carbon (AC). However, the use of (AC) is restricted due to high cost and its adsorption capacity gets lower after regeneration process in comparison with the virgin-activated carbon [14].

Among all the treatment processes mentioned, phytoremediation is one new cleanup concept that involves the use of plants to clean contaminated water [15]. It includes two uptake processes: an initial fast, reversible, metal-binding process (biosorption); and a slow, irreversible, ion sequestration step, bioaccumulation [16]. As a specific term, biosorption is used to depict a method that utilizes materials of biological origin biosorbents formulated from nonliving biomass for the removal of target substances from aqueous solutions. Biosorption "traditionally" covers sequestration of heavy metals as well as rare earth elements and radionuclides or metalloids, but the research and applications extended to the removal of organics, namely dyes [17]. The "bio" prefix refers to the involvement of biological entity, which is living organisms, dead cells and tissues, cellular components or products. The ultimate goal of these efforts is to provide an economical and eco-friendly technology, efficiently working also at metal levels below 10 mg L^{-1}. These are the features that living as well as dead biomass could be challenged for [18].

The natural capacity of microorganisms, fungi, algae, and plants to take up heavy metal ions and radio nuclides and, in some cases, to promote their conversion to less toxic forms has sparked the interest of (micro) biologists, biotechnologists, and environmental engineers for several decades. Consequently, various concepts for "bio-removal" of metals from waste streams and bioremediations of contaminated environment are being proposed, some of which were brought to pilot or industrial scale [19–23]. There are generally three routes to follow considering "bio-removal" of metallic species from solutions. The first two rely on properties of living cells and involve active metal uptake-bioaccumulation (i.e., plasma membrane mediated transport of metal ion into cellular compartment) and eventual chemical conversion of mobile metal to insoluble forms. The later may occur in the cytoplasm, at the cell surface or in the solution by precipitation of metal ion with metabolites, via redox reactions or by their combination [24]. The effectiveness of the process will depend on the (bio) chemistry of particular metal and on metabolic activity of eligible organism, which is in turn affected by the presence of metal ions. To this point, the use of metallotolerant species or physical separations of the production of metal-precipitating metabolite from metal precipitation in contaminated solution produce viable methods for treatment of industrial effluents [25]. Several of them are to various extents dependent on or involve the metabolism-independent metal uptake event at the cell wall by polysaccharides, associated molecules, and functional groups. This metal sequestration capacity is commonly known as biosorption, which itself represents the third potent way of "bio-removal" of metals from solution [18].

The majority of biosorption studies tested one-factor-at-a-time on the biosorption process. For example, equilibrium and kinetics models [4]. However, few studies examined three or four parameters and their interaction on the biosorption process using the factorial experimental design [16, 26–29]. The choice of such experimental design has two main objectives:

- Minimize bias.
- Minimize the variability of observations, with the aim of obtaining powerful statistical tests and precise estimates.

The aim of this chapter is to provide an overview of wastewater treatment methodologies with special references to phytoremediation. Biosorption mechanism and key factors controlling it are also elucidated.

7.2 Conventional Treatment Methods for Wastewater

There is growing consideration to abandon the conventional water treatment methods because of their high cost and environmental impact. Table 7.1 gives a summary of the pros and cons of some of the used technologies for the wastewater treatment. Each method mentioned in Table 7.1 has its own limitations in industrial applications, for instance, low selectivity, complex to operate, high capital, and energy costs. In addition, it is also inefficient in treating waste streams that contain low concentrations of contaminants and may fail when handling wastes of complex chemistry [30].

Table 7.1 Pros and Cons of conventional treatment methods for wastewater

Conventional treatment methods	Pros	Cons	References
Chemical precipitation	• Low capital cost, simple operation • Effectively treat inorganic effluent with a metal concentration of higher than 1000 mg/L	• The demand of a large amount of chemicals • Generates sludges • Generally, it cannot be used to handle low concentration of metal wastewater, which is below 100 mg/L	[31–33]
Ion exchange	• Effective to treat inorganic effluent with a wide metal concentration of less than 10 mg/L to higher than 100 mg/L	• Require pretreatment	[9, 31, 32]
	• No sludge generation	• Suitable ion-exchange resins are not available for all heavy metals	
	• Metal recovery	• Expensive • Ion-exchange resins must be regenerated by chemical reagents when they are exhausted and the regeneration can cause serious secondary pollution	

(continued)

Table 7.1 (continued)

Conventional treatment methods	Pros	Cons	References
Coagulation–flocculation	• Shorter time to settle out suspended solids	• Sludge production • Extra operational cost for sludge disposal • Generally, coagulation flocculation can't treat the heavy metal wastewater completely. Therefore, coagulation flocculation must be followed by other treatment techniques • Large consumption of chemicals	[9, 34]
Reverse Osmosis	• Purifies water by removing salts such as calcium, magnesium, sodium ion, chloride ion, copper ion, as well as bacteria	• Low recovery • Brine disposal • High maintenance	[35]
Ultrafiltration (UF)	• Potentially applicable in many areas such as high water flux, high water purity, membrane fouling, and high mechanical, chemical, and thermal stability	• Since the pore sizes of UF membranes are larger than dissolved metal ions in the form of hydrated ions, these ions would pass easily through UF membranes	[9, 35]
Microfiltration	• Separates larger size particles such as suspended solids, fixed solids, and microorganisms • The membranes exhibit high porosity and have distinct pores	• Permeates micrometer-sized particles present in water	[36]

7.3 Biosorption Vs. Bioaccumulation

Biosorption is a process with some unique characteristics. It can effectively sequester dissolved metals from very dilute complex solutions with high efficiency. This makes biosorption an ideal candidate for the treatment of high volume low concentration complex wastewaters. It is a physical–chemical process, simply defined as the removal of substances from solution by biological material. This is a property of both living and dead organisms, and has been heralded as a promising biotechnology because of its simplicity, analogous operation to conventional ion-exchange technology, apparent efficiency, and the availability of biomass wastes [37, 38]. On the other hand, bioaccumulation is metabolically active and is performed by living cells [39]. It is the accumulation of contaminant via all routes available to the organism [40]. Biosorption and bioaccumulation differ in that in the first process pollutants are bound to the surface of cell wall; passive and based mainly on the "affinity" between the sorbent and sorbate and in the second, they become also accumulated inside the cell; it is based on active metabolic transport [41]. The comparison between biosorption and bioaccumulation process is illustrated in Table 7.2.

Table 7.2 Comparison between biosorption and bioaccumulation process

Features	Biosorption	Bioaccumulation	References
Process	• Passive process • Adsorption; metals are bound with cellular surface	• Active process • Absorption; metals are bound with cellular surface and interior	[39]
Biomass	Growth independent; not alive (Single-stage)	Alive (Double-stage)	[39]
Rate of uptake	Usually rapid. Most biosorption mechanisms are rapid.	Usually slower than biosorption. Since intracellular accumulation is time-consuming	[41]
Metal affinity	High under favorable conditions	Toxicity will affect metal uptake by living cells, but in some instances high metal accumulation depends on the toxicity of the pollutant	[41]
Storage	Easy to store and use	External metabolic energy is needed for maintenance of the culture	[41]
Versatility	• Metal uptake may be affected by anions or other molecules • Extent of metal uptake usually pH dependent • The binding sites can accommodate a variety of ions	• Requires an energy source; dependent on plasma membrane ATPase activity • Not very flexible. Prone to be affected by metal/salt conditions	[41]
Selectivity	Poor as Variety of ligands involved. However, selectivity can be improved by modification/ processing of biomass	Better than biosorption, but less than some chemical technologies	[41]

(continued)

Table 7.2 (continued)

Features	Biosorption	Bioaccumulation	References
pH	The solution pH strongly influences the uptake capacity of biomass However, the process can be operated under a wide range of pH conditions	In addition to uptake, the living cells themselves are strongly affected under extreme pH conditions	[41]
Regeneration and Reuse	High possibility of biosorbent regeneration, with possible reuse over a number of cycles	Since most toxicants are intracellularly accumulated, the chances are very limited	[31]
Cost	Usually low	• Usually high • The process involves living cells and cell maintenance is cost prone	[41]

Previous studies reported that dead biomass or agricultural waste accumulates heavy metal ions greater extent than living cells. As the changes that occur in the cell structure after the cells are dry-killed, affect adsorption in a positive manner [42]. However, [43] studied biosorption of Pb^{2+}, Cd^{2+}, Cu^{2+}, and Ni^{2+} by nonviable and viable granular sludge biomass. The first uptake process is biosorption or passive uptake. It involves the binding of metal ions to the cell surface and the second uptake process is intracellular uptake, active uptake or bio-accumulation. It was found that the efficiency of nonviable cells in biosorbing metal ions may be less than that of the living cells but the use of nonviable biomass offers the following advantages over viable cells:

- Metal removal is not subject to toxicity limitations of living biomass
- No requirements for growth media and nutrients
- Biosorbed metal ions can be easily desorbed and biomass can be reused
- Biomass can be stored for a long period of time
- Biosorption that tends to be rapid

Therefore, nonviable biomass was used to overcome the disadvantages of using viable biomass. Also, the major advantages of biosorption are low cost, high efficiency, minimization of chemical or biological sludge, regeneration of biosorbents, and possible metal recovery. The successful of such biosorption process depends on using suitable biosorbents. Thus, characteristics of a suitable biosorbent for its successful application to industrial scale can be as the following [44]:

- Operation over wide range of pH, temperature, and other physicochemical parameters
- No secondary pollutants released
- Good stability under acidic/alkaline environments
- Good uptake capacity towards different ions

- Cost-effective
- No pretreatment necessity
- Sequential removal of metal ions
- Easy desorption and reuse ability
- No requirement of chemical modification or immobilization
- Easy adaptability to different system designs

7.4 Factors Affecting Biosorption Process

7.4.1 Contact Time

The contact time between adsorbent and adsorbate has a significant role to reach equilibrium in biosorption experiments. Liu et al. [45], Singanan and Peters [46] and Mahmoud et al. [47] concluded that the rate of adsorption is higher at the first hour of the biosorption process due to availability of a large number of active sites on the biosorbents' surface, then it becomes slower in the range of hour to three hours as these sites are exhausted by the rate at which the adsorbate is transported from the exterior to the interior sites of the biosorbents. With this account, the adsorption capacity or removal percentage did not vary significantly as a general for most heavy metals after 3 h.

7.4.2 Agitation Rate

Agitation rate is also an important factor in biosorption process because mass transfer resistance can minimize the uptake of metal ions from the aqueous solutions. The boundary layer resistance could be affected by the rate of agitation [48]. When increasing the agitation rate, the diffusion rate of a solute from the bulk liquid to the liquid boundary layer surrounding particles becomes higher due to the enhanced turbulence and the decrease in the thickness of the liquid boundary layer. Wong et al. [49] found that the uptake of Pb^{2+} and Cu^{2+} increased with increasing agitation rate and the maximum uptake was at 250 rpm.

7.4.3 Metal Ion Concentration

The removal percentages of most heavy metals were decreased with increasing the concentration of these metals. This is because the biomass surface area available for metal biosorption at low metal ion concentration was higher. Moreover, the ability of active sites to fully absorb the metal ions at lower concentrations is meant that the ratio of active adsorption sites to the initial metal ions is larger, resulting in higher removal efficiency. After that, with increasing metal ion concentration, the functional groups on biomass surface could be saturated, and there were a few available active sites on the biomass surface so the metal ions are competed for the available binding sites [50–52]. This is in line with previous studies done by Amarasinghe and Williams [53], Abdel-Aty et al. [54] and Mahmoud et al. [47].

7.4.4 pH of the Aqueous Solution

It is one of the important factors that significantly influence metal sorption [4, 55]. At lower pH values, the H_3O^+ ions compete with the metal ions for the exchange sites in the sorbent [56]. Arief et al. [57] explained this finding by the fact that when the concentration of H^+ ions was high, Cd^{2+} ions must compete with H^+ ions in order to attach to the surface functional groups of the agricultural wastes. Also, they found that when the pH value rise, fewer H^+ ions exist, and consequently, Cd^{2+} ions have a better chance to bind at free binding sites. As pH increased, the negative charge density on the adsorbent surface increases due to deprotonation of the metal-binding sites [58]. Similar comments were made by Anirudhan and Sreekumari [59]. They concluded that the increase in metal removal with an increase in pH can be explained on the basis of a decrease in competition between proton and the metal cations for the same functional groups and by the decrease in positive charge of the adsorbent which results in a lower electrostatic repulsion between the metal cations and the surface.

7.4.5 Biomass Dose

It strongly influences the biosorption process because the number of binding sites available for adsorption on the biosorbents is determined by biomass dose in the aqueous solutions [60]. Low biosorbent dose yielded lower percentage removal efficiencies because all biosorbents had a limited number of active sites, which would have become saturated above a certain metal concentration [61]. An increase in the biomass dose generally increased the amount of solute biosorbed, due to the increased surface area of the biosorbent, which in turn increases the number of binding sites [62].

7.4.6 Types of Biomass

A considerable number of bacteria, fungi, algae and yeasts, and different wastes and by-products of the agriculture and food industry have been investigated for their biosorbent metal properties [38]. Indeed, the choice of the biosorbent should consider both efficiency and economy. The efficiency of the process will depend on the biomass chemical composition which varies significantly for different species within the same genus or order [63].

7.4.6.1 Bacterial Surface Display of Metal-Binding Sites

Bacterial surface display has been proved a viable approach for a wide range of medical, industrial and environmental applications. Metal binding by biomolecules of structural components or excreted polymers of bacteria is generally fortuitous and relative efficiencies depend on attributes of the metal ion as well as on reactivity of provided ligands [18]. Shi et al. [64] investigated the efficiency of *Pannonibacter phragmitetus* on the reduction of Cr (VI) from aqueous solution. The maximum rate of Cr removal was found to be 562.8 mg L^{-1} h^{-1}. Miranda et al. [65] have been isolated two species of cyanobacteria, *Oscillatoria laetevirens* and *Oscillatoria trichoides* from a polluted environment and studied for their Cr (VI) removal efficiency from aqueous solutions, the highest removal through biosorption for living biomass was achieved between pH5 and 5.9 and for dead biomass at pH2. Of the two species, living cells of *O. trichoides* were most effective for which removal was 38.7 mg g^{-1} and reached 51.6 % of the total Cr (VI) at 30 mg L^{-1} at pH5–5.9.

Biosorption of hexavalent chromium using biofilm of *Escherichia coli* ASU 7 supported on granulated activated carbon (GAC), lyophilized cells of *Escherichia coli* ASU 7 and granulated activated carbon has been investigated by Gabr et al. [66]. The maximum adsorption removal ($q_{\max}$) of hexavalent chromium calculated from Langmuir equation for biosorption by biofilm, GAC, and bacteria are 97.7, 90.7, and 64.36 mg/g, respectively. The results demonstrate that biofilm supported on GAC, which prepared by impregnation method could be used as promising biosorbent for the removal of Cr (VI) ions from aqueous solutions.

7.4.6.2 Fungal Biosorption and Biosorbents

The common filamentous fungi can sorb heavy metals from aqueous solutions. Fungal biosorption largely depends on parameters such as pH, metal ion and biomass concentration, physical or chemical pretreatment of biomass, presence of various ligands in solution, and to a limited extent on temperature. The cell wall fraction of biomass plays an important role in the sorption of heavy metals [18]. Trivedi and Patel [67] studied the biosorption efficiency of tropical white-rot basidiomycete on chromium (VI) removal from aqueous solutions. It was found that the pretreatment of fungal biomass with acid resulted in 100 % metal adsorption compared to only 26.64 % adsorption without any pretreatment. Aksu and Balibek [68] studied the biosorption of chromium (VI) from saline solutions on dried *Rhizopus arrhizus* the results showed that the maximum chromium (VI) sorption capacity was for 78.0 mg/g of sorbent.

Srinivasan and Viraraghavan [69] have used two fungal biomasses of *Mucor rouxii* and *Absidia coerulea* along with chitosan and walnut shell media for the removal of oil from water. Moreover, it was found that Nonviable *M.rouxii* biomass is more effective than *A. coerulea* biomass in removing oil from water. The adsorption capacities for standard mineral oil, vegetable oil, and cutting oil were 77.2, 92.5, and 84 mg/g of biomass, respectively. However, these capacities using *M. rouxii* biomass were less than those obtained with chitosan and walnut shell media.

7.4.6.3 Plants and Agricultural Wastes as Biosorbents

Biosorption onto plants and raw agricultural waste is a low-cost treatment technique for the removal of contaminants, including heavy metals, from water and wastewater. Aoyama et al. [70] studied the biosorption of Cr (VI) from wastewater on using Japanese cedar Cryptomeria japonica bark. Igwe and Abia [71] investigated the biosorption efficiency of some agricultural wastes as maize husk on the removal of Cd(II), Pb(II), and Zn(II) ions from aqueous solutions. It was found that the modification of the biosorbent by EDTA enhanced the biosorption capacity. Jain et al. [72] studied the biosorbent efficiency of sunflower *Helianthus annuus* waste for Cr (VI) removal from wastewater under different experimental conditions and biosorbent treatments, either in boiling water or in formaldehyde. Obtained efficiencies were 81.7 and 76.5 % for boiled and formaldehyde treated biosorbent, respectively (4.0 g/L) biosorbent dose.

Zein et al. [73] investigated the biosorption efficiency of mangosteen shell, Garcinia mangostana shell for the removal of Pb(II), Cd(II), and Co(II). The sorption capacity of Pb(II), Cd(II), and Co(II) reached 3.56 mg/g, 3.15 mg/g, and 0.34 mg/g, respectively. García-Rosales and Colín-Cruz [74] investigated the efficiency of Maize (Zea mays) stalk sponge as a biosorbent for lead in aqueous solutions, it was found that Zea mays biosorbent is effective in reducing Pb(II) concentrations in industrial wastewater. Ibrahima et al. [75] suggested the use of an abundantly available agricultural waste modified soda lignin from oil palm empty fruit bunches, for the removal of lead (II) ions from aqueous solution. Tan et al. [76] has the biosorption efficiency of dried *Azolla filiculoides* on the removal of Basic Organic (BO) as a target pollutant from aqueous solution. The obtained results showed that the removal ratio of BO from wastewater containing 100 mg/L BO reached 79.3 %. Thus, this high biosorption capacity indicates the high efficiency of *Azolla filiculoides* biomass for the removal of BO from industrial wastewater.

Carro et al. [77] studied the biosorption capacity of *Pteridium aquilinum* for the removal of mercury from aqueous solution. It was found this sorption process takes place via neutral species and almost 100 % of mercury was sorbed at pH values above 5. Ashraf et al. [78] have investigated the biosorption capacity of *Mangifera indica* on the removal of Pb(II), Cu(II), Zn(II), and Ni(II) ions from aqueous solution. The percent removal of these metal ions reached 82.76 % for lead, 76.60 % for copper, 63.35 % for zinc, and 59.35 % for nickel. Lü et al. [79] tested the biosorption efficiency of lawny grass for the removal of Cd (II) from aqueous solution the biosorption capacity of Cd(II) reaches its maximum at 145(mg/g), thus revealing the efficient performance of lawny grass on metal ions removal.

The biosorption of Cd (II) from aqueous solution using *Ananas comosus* (AC) peel, *Parkia speciosa* (PS) pods and *Psidium guajava* (PG) peel was evaluated by Foo et al. [80]. It was found that the adsorption capacities reached 18.21 mg/g (AC peel), 25.64 mg/g (PS pods), and 39.68 mg/g (PG peel). Jeon [81] studied the biosorption efficiency of Rice hulls on the removal of copper ions from aqueous solution. It was found that the maximum removal capacity of copper ions was 11.83 mg/g.

Thus, rice hulls could be sufficiently used as a promising biosorbent in the copper ion removal process. Ding et al. [82] studied the biosorption efficiency of tea waste on the removal and the recovery of U (VI) from the aqueous solution. The removal and recovery percentages were up to 86 % and 80 %, respectively. Moreover, it was found that the biosorption of U(VI) by tea waste is a physical multilayer adsorption.

The capability of durian shell waste biomass as a novel and potential biosorbent for Cr (VI) removal from aqueous solution has been investigated by Kurniawan et al. [83] the maximum biosorption capacity of durian shell was 117 mg/g. Shukla and Vankar [84] studied the adsorption efficiency of Araucaria leaves on the removal of Cr (VI) ions from aqueous solution. The biosorption efficiency observed was maximum 100 %. Khoramzadeh et al. [85] have investigated the biosorption efficiency of Sugarcane Bagasse for Mercury removal from aqueous solutions. Moreover, Mahmoud et al (Mahmoud, A.E.D. and M. Fawzy, Statistical Methodology for Cadmium (Cd(II)) Removal from Wastewater by Different Plant Biomasses. Journal of Bioremediation & Biodegradation, 2015) studied the behavior of two different plant biomasses; rice straw (*Oryza sativa*) and dragon tree leaves (Dracaena draca) on the cadmium biosorption.

7.5 Biosorption Isotherms

Biosorption isotherm is a graphical representation expressing the relation between the mass of sorbed metal at constant temperature per unit mass of biosorbent q_e (mg/g) solid phase concentration of the sorbate and liquid phase metal concentration at equilibrium (Ce). It illustrates the metal distribution between the liquid and solid phases at various equilibrium concentrations, thus providing information concerning the biosorption mechanism and revealing how efficiently a given biosorbent interacts with the sorbate. Also, it is used as indication to estimate the economic feasibility of biosorbent for specific commercial applications [86, 87]. Biosorption isotherms can be generated based on theoretical principles. Three biosorption isotherm models have been tested in this study, namely, Langmuir, Freundlich, and Temkin, in order to describe the equilibrium characteristics of adsorption.

7.5.1 Langmuir Isotherm

Langmuir isotherm is an analytical equation basically developed for gas phase adsorption onto the homogeneous glass and metal surfaces [18, 88]. The assumptions of the Langmuir isotherm are:

(a) Adsorption energy is constant on all sites (all sorption sites are uniform)
(b) Adsorbed atoms or molecules are adsorbed at definite, localized sites
(c) Each site can accommodate only one molecule or atom (only one sorbate)
(d) There is no interaction between sorbed species
(e) One sorbate molecule reacts with only one active site

7.5.2 *Freundlich Isotherm*

Freundlich isotherm is applicable to both monolayer and multilayer adsorption, and it is based on made two assumptions which are first, heterogeneous surface energies that is, exponential variation in site energies second, surface adsorption is not the rate-limiting step [89, 90].

7.5.3 *Temkin Isotherm*

Temkin isotherm assumes that the heat of sorption or adsorption decreases linearly with the surface coverage, that is the energy of adsorption decreases as the degree of completion of the sorptional centers of biosorbent increases. This is mainly due to the adsorbent–adsorbate interactions [91, 92].

7.6 Biosorption Kinetics

Biosorption kinetic studies are essential to select the optimum conditions for full-scale batch process. The kinetic parameters, which are helpful for the prediction of biosorption rate, provide significant information for designing and modeling the biosorption process. Biosorption kinetics was analyzed based on the pseudo first-order and the pseudo second-order kinetic models to find the optimum biosorption kinetic constants [88, 93].

7.6.1 *Pseudo First-Order Equation*

The adsorption models were first described by the Lagergren pseudo first-order model [90, 93] that illustrates the sorption rate based on the sorption capacity. It is the ideal model of sorption, 1:1; (i.e.,) one sorbate molecule occupies one activated site.

7.6.2 *Pseudo Second-Order Equation*

The sorption kinetics was described by the pseudo second-order model [94]. It has been applied for the analysis of the kinetics of chemisorption processes. However, it relies on the assumption that the rate of occupation of sorption sites is proportional to the squares of the unoccupied sites. In other words, it assumes that an adsorbate molecule is adsorbed on two sorption sites; thus, two molecules to one active site [95]. Aoyama and Kishino [70] investigated the biosorption of Cr (VI) from aqueous solution on japanese cedar (*Cryptomeria japonica*) bark. The obtained results at different temperatures obeyed Langmuir adsorption isotherm.

Aksu and Balibek [68] investigated the biosorption efficiency of dried *Rhizopus arrhizus* and in salt-containing medium on the removal of chromium (VI) from aqueous solution. Experimental data fitted Langmuir–Freundlich sorption model. Sorption capacity of dried *R. arrhizus* used in this study is q_e 114.9 mg/g. Pseudo first-order, pseudo second-order, and saturation type kinetic models described the biosorption kinetics accurately at all chromium (VI) concentrations in the absence and in the presence of changing concentrations of salt all theoretical data obtained from kinetic models were in good agreement with the experimental results.

Elangovan et al. [96] investigated the removal of Cr(VI) and Cr(III) from aqueous phase using different aquatic weeds. The adsorption process for all biosorbents was a second-order process. The maximum sorption capacity for Cr(III) achieved using reed mat was 7.18 mg/g. However, in case of Cr(VI), mangrove leaves were the best for its removal (8.87 mg/g), followed by water lily (8.44 mg/g). Thus, aquatic weeds seem to be a promising biosorbent for the removal of chromium ions from water environment.

Gokhale et al. [97] studied the biosorption of immobilized *Spirulina platensis* on the removal of chromium (VI) from aqueous solution. The percent adsorption was 99 % from an aqueous solution containing 100 mg/L chromium (VI). Experimental data fitted the Freundlich adsorption isotherm. Gupta and Rastogi [98] investigated the biosorption efficiency of raw and acid-treated *Oedogonium hatei* for the removal of hexavalent chromium from aqueous solutions. Experimental data fitted both Langmuir and Freundlich isotherm models. The biosorption capacities of the raw and acid-treated algae were 31 and 35.2 mg Cr (VI) per g of dry biosorbent, respectively. The pseudo first-order kinetic model adequately describes the kinetic data in comparison to second-order model, and the process involving rate-controlling step is much complex involving both boundary layer and intra-particle diffusion processes.

Gabr et al. [66] investigated the biosorption of Escherichia coli supported on granulated activated carbon for the removal of Cr(VI) from aqueous solution. The biosorption equilibrium data fitted well to both Langmuir and Freundlich. Ibrahim et al. [75] investigated the biosorption capacity of modified soda lignin from oil palm on the removal of lead (II) from aqueous solutions experimental data fitted Langmuir isotherm equation, confirming the monolayer adsorption of lead (II) ions with a biosorption capacity of 46.72 mg/g at 47 °C. The biosorption followed the pseudo second-order equation. Biosorption of arsenic from aqueous solution by algae (*Maugeotia genuflexa*) biomass fitted Freundlich and Dubinin–Radushkevich (D–R) isotherm models. From the Langmuir model, the maximum monolayer biosorption capacity of the biosorbent was found to be 57.48 mg/g at pH6. Kinetic results indicated that the pseudo second-order kinetic model was well fitted to the experimental data [99].

Chen et al. [100] used *Phanero chaetechrysosporium* as a biosorbent for Cr(VI), the maximum removal for Cr(VI) was 344.8 mg/g as determined from the Langmuir isotherm. It was found that pseudo first-order Lagergren models best fitted the data than pseudo second-order Lagergren model. Kurniawan et al. [83] investigated the biosorption capacity of durian shell for the removal of Cr(VI) from synthetic wastewater. Experimental data obeyed Langmuir and Freundlich isotherm models ($R^2 > 0.99$). Also, the pseudo first order prevails over the pseudo second-order model. However, the maximum biosorption capacity of durian shell was 117 mg/g.

7.7 Modeling of Biosorption

Assessment of a solid–liquid sorption system is usually based on two types of investigations: equilibrium models (describe the sorption capacity as a function of chemistry) and kinetics models (describe the sorption history). Recent publications are focused on designing factorial experiments to yield the most relevant response from the wastewater experiments. The process of biosorption involves a solid phase (sorbent) and a liquid phase (solvent) containing a dissolved species (sorbate; metal ions) to be sorbed [63, 101]. The quality of sorbent material is judged according to how much sorbate it can attract and retain in an immobilized form after conducting factorial experimental design. The amount of metal are occupied up by plant biomass was calculated as the difference between the initial and final concentrations of metal after adsorption in the aqueous solution [102, 47].

$$q = V\frac{C_i - C_f}{S}$$

where q = metal ion uptake capacity (mg g^{-1}), C_i = initial concentration of metal in solution, before the sorption analysis (mg L^{-1}), C_f = final concentration of metal in solution, after the sorption analysis (mg L^{-1}). S = dry weight of biosorbent (g) and V = aqueous solution volume (L).

Moreover, the removal efficiency was calculated according to the following equation:

$$\text{Metal removal efficiency}\,\% = \frac{C_i - C_f}{C_i} \times 100$$

where C_i is initial metal concentration in aqueous solution; C_f is final metal concentration in aqueous solutions.

7.7.1 Equilibrium and Kinetics Models

In the equilibrium modeling, empirical models such as Langmuir and Freundlich models are used. The Langmuir model makes assumptions such as monolayer adsorption and constant adsorption energy while the Freundlich model deals with heterogeneous adsorption [103].

Langmuir model is expressed by the following equation [31]:

$$q_e = \frac{q_{\max} b C_e}{1 + b C_e}$$

where $q_{\max}$ is the maximum specific uptake corresponding to sites saturation (mg/g), b is the biomass metal-binding affinity (mg/L), and C_e is the metal residual concentration in solution (mg/L).

The feasibility of Langmuir isotherm can be expressed by a dimensionless constant separation factor [32].

$$\mathrm{SF} = \frac{1}{1 + b C_i}$$

where SF is the dimensionless equilibrium parameter or separation factor, b is Langmuir constant and C_i the initial metal ion concentration. The value of $\mathrm{SF} > 1$ indicates that biosorption is unfavorable, linear ($\mathrm{SF} = 1$), irreversible ($\mathrm{SF} < 1$), *or SF* value between 0 and 1 represents favorable biosorption.

Another equilibrium model is the Freundlich model. It is expressed as [31]:

$$q_e = K_f C_e^{1/n}$$

where K_f is the Freundlich adsorption constant, C_e is the metal residual concentration in solution (mg/L), and $1/n$ is the measure of adsorption intensity.

Both previous equations for Langmuir and Freundlich models are used to describe the sorption of single component in aqueous solutions. Table 7.3 illustrates advantages and disadvantages of both equilibrium models.

In kinetic models, the mechanism of biosorption process is needed to be examined such as mass transfer and chemical reaction. Thus, the linear pseudo first-order equation is given as follows [31, 104]:

$$\log\left(q_{\mathrm{eq}} - qt\right) = \log \mathrm{q}_{\mathrm{eq}} - \frac{K_1}{2.303} t$$

Table 7.3 Advantages and disadvantages of equilibrium models

Equilibrium models	Pros	Cons
Langmuir model	• Has Henry law • Finite saturation limit • Valid over a wide range of concentration	• Based on monolayer assumption
Freundlich model	• Simple expression • Has a parameter for surface heterogeneity	• Does not have Henry law • No saturation limit, not structured • Not applicable over wide range of concentration

where q_{eq} and q_t are the amounts of metal ions adsorbed at equilibrium and time t (mg/g), respectively, K_1 is the rate constant of pseudo first-order adsorption process (min^{-1}).

The linear pseudo second-order equation is given as follows:

$$\frac{t}{q_t} = \frac{1}{K_2 q_{\mathrm{eq}}^2} + \frac{1}{q_{\mathrm{eq}}} t$$

where K_2 is the equilibrium rate constant of pseudo second-order biosorption (g/mg min).

Many researchers investigated these models. For instance, Hameed, et al. [105] studied the sorption of basic dye from aqueous solutions by banana stalk waste. Sorption models of methylene blue (MB) onto the banana stalk waste was determined at 30 °C with the initial concentrations of MB in the range of 50–500 mg/L. Equilibrium data were fitted to the Langmuir, Freundlich models. The equilibrium data were best represented by the Langmuir isotherm model, with maximum monolayer adsorption capacity of 243.90 mg/g. The sorption kinetic data were analyzed using pseudo first-order, pseudo second-order models. It was found that the pseudo second-order kinetic model was the best applicable model to describe the sorption kinetics.

Biosorption of Pb (II) ions from aqueous solutions in a batch system using *Candida albicans* was investigated by Baysal et al. [106]. The optimum conditions of biosorption were determined by inspecting the initial metal ion concentration, contact time, temperature, biosorbent dose, and pH. Biosorption equilibrium time was observed in 30 min. The Freundlich and Langmuir adsorption models were used for the mathematical description of biosorption equilibrium, and isotherm constants were also evaluated. The maximum biosorption capacity of Pb (II) on *C. albicans* was determined as 828.50 ± 1.05, 831.26 ± 1.30, and 833.33 ± 1.12mg g−1, respectively, at different temperatures (25, 35, and 45 °C). Gupta and Rastogi [98] studied the hexavalent chromium, Cr(VI), biosorption by raw and acid-treated *Oedogonium hatei* from aqueous solutions. Batch experiments were conducted to determine the biosorption properties of the biomass. Both Langmuir and Freundlich isotherm equations could fit the equilibrium data at biomass dose of 0.8 g/L, contact time of 110 min, pH and temperature 2.0 and 318 K. Under the optimal conditions, the biosorption capacities of the raw and acid-treated algae were 31 and 35.2 mg Cr(VI) per g of dry adsorbent, respectively.

Hamissa et al. [107] investigated the potential of *Agave americana* fibers for Pb(II) and Cd(II) removal from aqueous solutions. Batch experiments were conducted as a function of pH, initial metal concentration, and temperature. Metal sorption followed pseudo second-order kinetics with excellent correlation. The Langmuir model and pseudo second-order kinetics were successfully applied to describe the sorption models. The maximum sorption capacity of *Agave americana* fibers was 40.0 mg.g^{-1} for Pb(II) and 12.5 mg g^{-1} for Cd(II), respectively, at 20 °C, pH5.0, contact time of 30–60 min and 5 g/L biomass concentration. Sometimes, these empirical models do not reflect any mechanisms of sorbate uptake and hardly have a meaningful physical interpretation for biosorption. Some authors have pointed out that the results from the empirical models cannot be extrapolated, and no predictive conclusions can be drawn for systems operating under different conditions [4].

7.7.2 Factorial Experimental Design

When we talk about factorial experimental design, we need to highlight the following expressions and definitions.

- *Factor*: a controllable experimental variable thought to influence response (as such metal ion concentration, temperature, biomass dose, flow rate of wastewater…).
- *Levels*: specific value of the studied factors (high value "+1" and low value "−1").
- *Response*: the outcome or result (e.g., metal removal efficiency from aqueous samples).
- *Interaction factors*: factors may not be independent, therefore combinations of factors may be important. If you have interaction effects, you cannot find the optimum conditions using OFAT (One Factor at a Time).

Thus, factorial experimental design is employed to define the most important factors affecting the metal removal efficiency as well as how the effect of one factor varies with the level of the other factors [108, 109]. The effect of a factor is defined as the change in response produced by a change in level of the factor. This is frequently called a main effect as it refers to the primary factors of interest in the experiment [108]. In addition the total number of experiments to be carried out in the factorial experimental design is much lower than the univariate procedure. It can reduce time and overall research cost [50, 110].

The factorial experimental design is associated with the following questions:

A. What factors should be involved in the design?
B. How many levels of each factor should be involved?
C. Which experimental units should be selected?
D. How can a factorial design be assessed to fit a real model?

The simplest factorial design involves two factors at two levels. The one-factor-at-a-time (OFAT) design is shown in Fig. 7.3a. However, Fig. 7.3b shows the points for the factorial designs that are starting with low levels and ending with high levels [111].

For example, full 2^3 factorial design requires only 8 runs versus 16 for an OFAT experiment. Hence, the ratio of the number of observations needed in a one-at-a-time experiment to the number required in a full 2^k factorial experiment grows linearly according to the following equation [112] and Fig. 7.4.

$$\frac{k+1}{2}$$

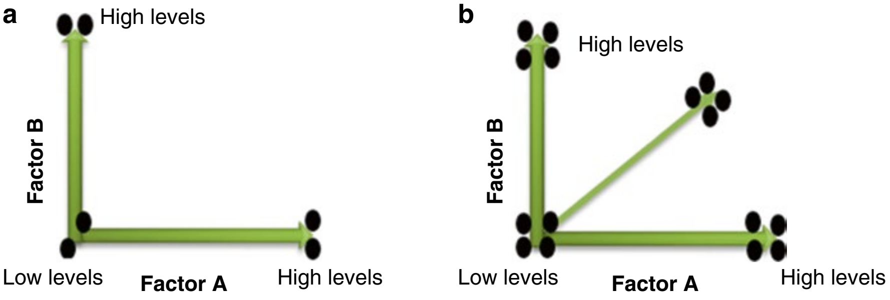


Fig. 7.3 (**a**) One-factor-at-a-time (OFAT), (**b**) two-level factorial design

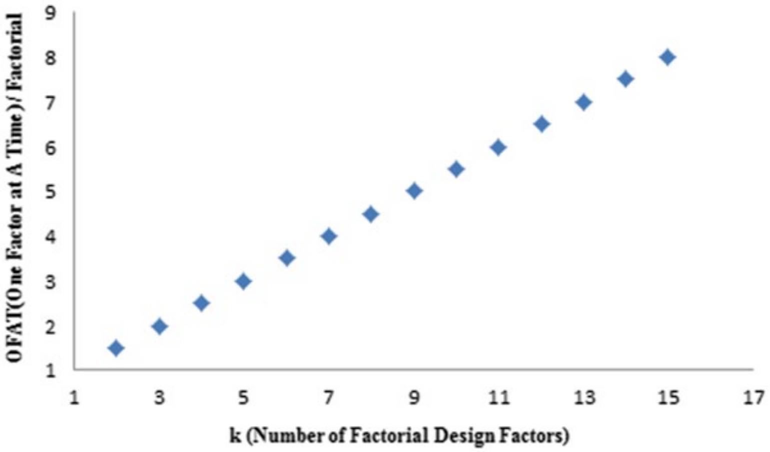


Fig. 7.4 Relative efficiency of one-at-a-time and factorial design

where *k* represents the number of factors in factorial design (Fig. 7.4).

Two levels of the three and four factors can be used, high level "+1" and low level "−1" (Tables 7.4 and 7.5). High level "+1" and low level "−1" can be changed to any numeric or text value according to the studied factors. In a full factorial experiment, responses are measured at all combinations of the factor levels. The combination of factor levels represents the conditions at which responses will be measured [113].

The codified mathematical model employed for the (2^3) factorial design is:

$$Y = A + a_1x_1 + a_2x_2 + a_3x_3 + a_4x_1x_2 + a_5x_1x_3 + a_6x_2x_3 + a_7x_1x_2x_3$$

While the codified mathematical model employed for the (2^4) factorial design is:

$$\begin{gathered} Y = A + a_1x_1 + a_2x_2 + a_3x_3 + a_4x_4 + a_5x_1x_2 + a_6x_1x_3 + a_7x_1x_4 + a_8x_2x_3 \\ + a_9x_2x_4 + a_{10}x_3x_4 + a_{11}x_1x_2x_3x_4 \end{gathered}$$

Table 7.4 Possible interactions of the factors levels (2^3) without replication

Interaction of factors levels (2^3)			
Number of experiments	Factor 1	Factor 2	Factor 3
1	−1	−1	−1
2	−1	−1	+1
3	−1	+1	−1
4	−1	+1	+1
5	+1	−1	−1
6	+1	−1	+1
7	+1	+1	−1
8	+1	+1	+1

where Y: Estimated value or estimation of the response, A: represents the global mean (constant), a: coefficients, x: experimental variables or factors that affect biosorption process.

Coefficients could be positive or negative value. The positive sign of the coefficient represents a synergistic effect, while a negative sign indicates an antagonistic effect for the studied factors [114]. As a result, agricultural wastes/by-products have been investigated as biosorbents for the optimum removal of heavy metals from aqueous solutions by a number of researchers. Based on the literature survey, optimization of heavy metals removal from wastewater has increased in the last 14 years as illustrated in Fig. 7.5.

Badr [26] studied the removal of Cd^{2+} and Pb^{2+} by *Eichhornia crassipes* from synthetic wastewater using factorial experimental design (2^3). Experiments were carried out at two markedly different levels of three factors: pH (2.0 and 6.0), temperature (T) (20 and 45 °C), and metal ion concentration (X) (10 and 1200 mg/L). Maximum removal was observed near pH = 6 for Cd^{2+} while that for Pb^{2+} was observed near pH = 2. The most significant effect for Cd^{2+} and Pb^{2+} biosorption by *Eichhornia crassipes* was ascribed to pH and the interaction effects of T. pH and X. pH that have a significant influence on the Cd^{2+} and Pb^{2+} removal efficiency. The best removal percentage of Cd^{2+} was 85.0 % when pH = 6, X = 10 mg/L, and T = 20 °C while for Pb^{2+}, it was 84.0 % when pH = 2, X = 1200 mg/L, and T = 20 °C.

The potential of *Phragmites australis* biomass for the removal of Cd^{2+} and Pb^{2+} from synthetic wastewater using factorial design (2^3) was studied by Fawzy [16]. Experiments were carried out at two markedly different levels of three factors: pH (2.0 and 6.0), temperature (T) (20 and 45 °C), and metal ion concentration (X) (10 and 1200 mg/L). The most significant effect for Cd^{2+} and pb^{2+} biosorption was ascribed to pH. The interaction effects of T pH and T X have a significant influence on the Cd^{2+} removal efficiency while, the main Cr(VI) into Cr(III) in acidic medium. These studies were conducted at initial concentration 100 mg/L and 32 ± 0.5 °C with constant amount of biomass (0.15 g) at the constant pH6 ± 0.1 (except for Hg and Cu at pH5.5 ± 0.1). Maximum removal percentage for Pb, Hg, Cd, Cu, Zn, Co, Mn, Ni, and Cr(VI) were 81 %, 49.65 %, 21.6 %, 16.2 %, 11.20 %, 12.75 %, 11.55 %, 7.65 %, and 78.15 %, respectively. Garg et al. [115] studied the removal of Cd^{2+} from

Table 7.5 Possible interactions of the factors levels (2^4) without replication

Interaction of factors levels (2^4)				
Number of experiments	Factor 1	Factor 2	Factor 3	Factor 4
1	+1	+1	−1	−1
2	−1	−1	−1	+1
3	+1	−1	−1	+1
4	−1	+1	+1	−1
5	−1	+1	−1	+1
6	−1	−1	+1	−1
7	−1	+1	+1	+1
8	−1	−1	−1	−1
9	−1	−1	+1	+1
10	−1	+1	−1	−1
11	+1	+1	−1	+1
12	+1	−1	+1	−1
13	+1	+1	+1	−1
14	+1	−1	−1	−1
15	+1	+1	+1	+1
16	+1	−1	+1	+1

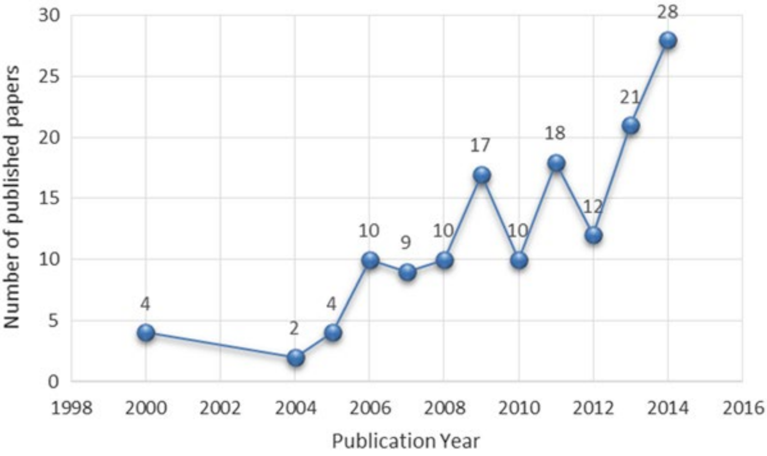


Fig. 7.5 Number of journal papers related to “optimization of heavy metals removal from wastewater” published between 2000 and 2014 (*Source*: Scopus)

aqueous solutions using sugarcane bagasse (SCB), maize corncob (MCC) and Jatropha oil cake (JOC). Batch experiments were carried out at various pH (2–7) and biosorbent dose (0.25–2 g) for a contact time of 1 h. The maximum adsorption of Cd^{2+} ions was observed at pH = 6 and dose= 2 g with percentage of 99.5 %, 99 % and 85 % for JOC, MCC, and SCB, respectively. The changes in FT-IR spectra confirmed the complexation of Cd^{2+} with functional groups present in the adsorbents.

Krishnani et al. [116] studied the sorption of eight metal ions Pb, Hg, Cd, Cu, Zn, Co, Mn and Ni by rice husk as a function of pH and metal concentrations and also for the reduction of Cr(VI) into Cr(III) in acidic medium. These studies were conducted at initial concentration 100 mg/l and 32 ± 0.5 °C with constant amount of biomass (0.15 g) at the constant pH 6 ± 0.1 (except for Hg and Cu at pH 5.5 ± 0.1). Maximum removal percentage for Pb, Hg, Cd, Cu, Zn, Co, Mn, Ni and Cr(VI) were 81 %, 49.65 %, 21.6 %, 16.2 %, 11.20 %, 12.75 %, 11.55 %, 7.65 % and 78.15 %, respectively.

Singh et al. [10] investigated the optimum environmental conditions for biosorption of Pb^{2+}, Cd^{2+}, and Cu^{2+} by *Trichoderma viride* using response surface methodology. The design variables of Box–Behnken for biosorption include: initial metal ions concentration (X = 20–100 mg/L), pH was selected (2–6), biomass dose (D = 0.25–0.125 g), and temperature (T = 20–40 °C). The removal of the metal ions increases with an increase in the biomass dose for all the metals ions containing 60 mg/L and pH = 4 of each of the investigated metals. The biosorption capacity of biosorbent increased with the increase in initial metal ions concentrations at the initial low temperature. At higher metal ions concentration uptake of the ions did not further increase with an increase of initial metal ions concentration resulting from the saturation of biosorbent surface. The removal decreases at higher metals ions due to the competing of the ions for the available binding site. The best removal percentage of Pb^{2+}, Cd^{2+}, and Cu^{2+} is 78.16, 76, and 63 % at X = 60, 20, and 60 mg/L; pH = 4, 4, and 6; D = 0.125, 0.125, and 0.075 g; and T = 40, 30, and 20 °C, respectively.

Varma et al. [113] studied the removal of cadmium from aqueous solutions using *Psidium guajava* leaves powder. The experiments were designed by 3–1 fractional factorial design. The factors in this study are metal concentration (50, 100, 150 mg/L), pH of aqueous metal solution (2, 4, and 6), and biomass dose (0.25, 0.5, and 0.75 g). Other variables such as speed of shaker adjusted at 160 rpm, volume of the aqueous solution 50 mL, and temperature 30 °C, and optimum agitation time 60 min were kept constant. The removal percentage of Cd^{2+} was 95.11 % at initial concentration = 90 mg/L, pH = 4, and biomass dose = 1 g. The increase in pH resulted in an increase in Cd uptake. Authors found that low sorption of Cd^{2+} in the $pH < 4$. This could be due to the competition with the H ions for metal-binding sites on the biomass cells, while the increase in pH favors metal sorption mainly because of negatively charged groups.

Park et al. [117] determined optimal condition for the Cr(VI) removal. Several factors such as pH, temperature, and contact time were studied. The removal efficiency of Cr (VI) increased with a decrease in pH or with an increase in temperature and contact time until equilibrium had been attained. Meanwhile, the total Cr removal efficiency increased with an increase of temperature and contact time till 60 h. Thus, 100 % of Cr (VI) removal and ~95 % of total Cr removal could be obtained at pH4 and 40 °C.

Seolatto et al. [3] studied the removal of lead, cadmium, and chromium by the Pequi Fruit Skin (*Caryocar brasiliense Camb.*) biomass and considered factors such as biomass dose (0.15, 0.75 g), pH (3, 5), and biomass size (0.2, 0.7 mm) that were studied using a factorial statistical design. The results showed that Pb^{2+} ions recorded the highest biosorption with an average of 16.78 mg/g and up to 80 % removal percentage. However, there was less removal of chromium and cadmium. The dose of biomass and pH were found to be the most important factors in the biosorption while grain size did not influence the biosorption process. Sulaymon et al. [118] studied biosorption of cadmium ions from simulated wastewater using rice husk with 100 mL of Cd^{2+} solution (concentration = 25 mg/L). The pH of the solutions was adjusted to the pH = 6 using 0.1 M NaOH or 0.1 M HNO_3. Highest removal efficiency (97 %) was obtained at 2.5 g of adsorbent, pH6, and contact time = 100 min.

Al-Qahtani [119] studied the biosorption of Cd^{2+} and Pb^{2+} on *Cyperus laevigatus* using the factorial design (2^3). The three factors were screened at two markedly different levels were pH (2.0 and 6.0), temperature (T) (20 and 45 °C), and metal ion concentration (X) (20 and 800 mg/L). The most significant effect for Cd^{2+} and Pb^{2+} biosorption was ascribed to (T). The best percentage of Cd^{2+} removal was 85 % when pH = 2, T = 45 °C, and X = 800 mg/L, but the best percentage of Pb^{2+} removal was 82 % when pH = 6, T = 45 °C, and X = 20 mg/L. Muhammad and Nwaedozie [120] studied the removal of lead and cadmium using marine algae-seaweed (*Ascophyllum nodosum*) as adsorbent for metal removal at two temperatures (23.5 °C and 37 °C) and four pH values (2, 5, 7, and 10). The maximum removal of lead was 93.41 % at pH2 and 53.13 % for cadmium at pH10. Temperature was found to have no significant effect on the adsorption process.

The biosorption efficiency of Cd^{2+} using rice straw was investigated by Nasr et al. [114]. Experiments studied the effect of three factors, biosorbent dose BD (0.1 and 0.5 g/L), pH (2 and 7), and initial Cd^{2+} concentration X (10 and 100 mg/L) at two levels "low" and "high." From 2^3 factorial design, the effects of BD, pH, and X achieved p value equals to 0.2248, 0.1881, and 0.1742, respectively, indicating that the influences are in the order $X > \text{pH} > \text{BD}$. Mahmoud et al. [47] investigated the removal of Cd^{2+} by dragon tree leaves using full factorial design. Results revealed that the optimum results (79.60 %) was attained at metal ion concentration = 10 ppm, pH = 7, and biomass dose = 0.5 g.

7.8 Quality Control/Quality Assurance

When biosorption experiments are conducted, errors can be occurred. These errors are due to cross-contamination from glassware or used chemicals and metal ions loss owing to sorption or volatilization [121, 122]. To reduce or prevent these errors, acidification of pH between 1 and 2 has been done to prevent metabolism by microorganisms and hydrolysis and precipitation. Also, cooling and freezing for reduction of bacterial activity and water samples should be stored in darkness and kept refrigerated (−4 °C) until the completion of analysis. Moreover, the shortest time between the sampling and the analysis increases the reliability of analytical results[123].

In this investigation, metal solutions should be stored in polyethylene bottles till their metal content was analyzed. Polyethylene or Teflon bottles are used in inorganic analysis to minimize loss of metal ions on their surfaces [124]. Also, metal solution samples were acidified down to pH ~ 2 (with HNO_3) till analyses. It has been recommended to acidify the sample down to pH ~ 2 to avoid adsorption on plastic bottle and prevents precipitation of metal hydroxides or adsorption of metal ions on the walls of the bottle. We will give some examples below.

7.8.1 Cleaning Glassware and Plastic Bottles

Glassware and plastic bottles must be washed with detergent overnight immersed in 10 % HNO_3 and rinsed with double distilled water (DDW) several times.

7.8.2 Blanks

Samples of metal solutions should be used without biosorbents to determine initial metal concentration.

7.8.3 Replicates

All experiments and measurements should be conducted in duplicates or triplicates.

7.9 Recommendations

Eco-friendly treatment processes for wastewater are major fundamentals for a developing and growing economy of developing countries. Therefore, it is crucial to implement such treatment processes. It is known that the environmental-based market for metal removal from industrial effluents is enormous [125]. So it is mandatory to apply economic analyses especially for the design of eco-friendly treatment plants [126]. As a result, when the adsorption process is considered, the kind of the used adsorbent is regarded as the most critical factor both on removal efficiency and total operational costs [126]. In general, the low-cost biosorbent should be [14]:

A. Efficient to remove many and different contaminants
B. Have high adsorption capacity and rate of adsorption
C. Have high selectivity for different concentrations.

References

1. Juwarkar A, Singh S, Mudhoo A (2010) A comprehensive overview of elements in bioremediation. Rev Environ Sci Bio/Technol 9:215–288. doi:10.1007/s11157-010-9215-6
2. Zimmo OR, Imseih N (2011) Overview of wastewater management practices in the Mediterranean region. In: Barceló D, Petrovic M (eds) Waste water treatment and reuse in the Mediterranean region. Springer, Berlin, pp 155–181
3. Seolatto AA, Silva Filho CJ, Mota DLF (2012) Evaluation of the efficiency of biosorption of lead, cadmium, and chromium by the biomass of Pequi Fruit Skin (Caryocar brasiliense Camb.). Chem Eng Trans 27:1974–9791
4. Gautam R, Chattopadhyaya M, Sharma S (2013) Biosorption of heavy metals: recent trends and challenges. In: Sharma SK, Sanghi R (eds) Wastewater reuse and management. Springer, The Netherlands, pp 305–322
5. Gaddis EB, Glennie PR, Huang Y, Rast W (2012). Water. GEO 5. United Nations Environment Programme, pp 97–132
6. WHO/UNICEF (2005) Water for life. In: Making it happen. World Health Organization, Geneva
7. Thatai S, Khurana P, Boken J, Prasad S, Kumar D (2014) Nanoparticles and core–shell nanocomposite based new generation water remediation materials and analytical techniques: a review. Microchem J 116:62–76
8. Sparks DL (2005) Toxic metals in the environment: the role of surfaces. Elements 1:193–197. doi:10.2113/gselements.1.4.193
9. Fenglian F, Wang Q (2011) Removal of heavy metal ions from wastewaters: a review. J Environ Manage 92:407–418
10. Singh R, Chadetrik R, Kumar R, Bishnoi K, Bhatia D, Kumar A, Bishnoi NR, Singh N (2010) Biosorption optimization of lead(II), cadmium(II) and copper(II) using response surface methodology and applicability in isotherms and thermodynamics modeling. J Hazard Mater 174:623–634. doi:http://dx.doi.org/10.1016/j.jhazmat.2009.09.097
11. Wang LK, Shammas NK (2009) Heavy metals in the environment. Taylor & Francis, Boca Raton
12. Mousavi HZ, Hosseynifar A, Jahed V, Dehghani SAM (2010) Removal of lead from aqueous solution using waste tire rubber ash as an adsorbent. Brazil J Chem Eng 27:79–87
13. Rezić I (2012) Cellulosic fibers—biosorptive materials and indicators of heavy metals pollution. Microchem J 107:63–69
14. Grassi M, Kaykioglu G, Belgiorno V, Lofrano G (2012) Removal of emerging contaminants from water and wastewater by adsorption process. In: Lofrano G (ed) Emerging compounds removal from wastewater. Springer, The Netherlands, pp 15–37
15. Singh D, Tiwari A, Gupta R (2012) Phytoremediation of lead from wastewater using aquatic plants. J Agric Technol 8:1–11
16. Fawzy M (2007) Biosorption of cadmium and lead by Phragmites australis L. biomass using factorial experimental design. Global J Biotechnol Biochem 2:10–20
17. Kaushik P, Malik A (2009) Fungal dye decolourization: recent advances and future potential. Environ Int 35:127–141
18. Kotrba P (2011) Microbial biosorption of metals—general introduction. Springer, New York
19. Bargar JR, Bernier-Latmani R, Giammar DE, Tebo BM (2008) Biogenic uraninite nanoparticles and their importance for uranium remediation. Elements 4:407–412
20. Macek T, Kotrba P, Svatos A, Novakova M, Demnerova K, Mackova M (2008) Novel roles for genetically modified plants in environmental protection. Trends Biotechnol 26:146–152
21. Muyzer G, Stams AJ (2008) The ecology and biotechnology of sulphate-reducing bacteria. Nat Rev Microbiol 6:441–454
22. Chaney RL, Angle JS, Broadhurst CL, Peters CA, Tappero RV, Sparks DL (2007) Improved understanding of hyperaccumulation yields commercial phytoextraction and phytomining technologies. J Environ Qual 36:1429–1443

23. Sheoran A, Sheoran V (2006) Heavy metal removal mechanism of acid mine drainage in wetlands: a critical review. Min Eng 19:105–116
24. Demirbas A (2008) Heavy metal adsorption onto agro-based waste materials: a review. J Hazard Mater 157:220–229. doi:http://dx.doi.org/10.1016/j.jhazmat.2008.01.024
25. Sud D, Mahajan G, Kaur M (2008) Agricultural waste material as potential adsorbent for sequestering heavy metal ions from aqueous solutions—a review. Bioresour Technol 99: 6017–6027
26. Badr NBE (2007) Statistical design of experiments as a tool for optimizing the biosorption of Pb^{2+} and Cd^{2+} on *Eichhornia crassipes* (Mart.) Solms. Global J Environ Res 2:33–42
27. Amini M, Younesi H, Bahramifar N, Lorestani AAZ, Ghorbani F, Daneshi A, Sharifzadeh M (2008) Application of response surface methodology for optimization of lead biosorption in an aqueous solution by *Aspergillus niger*. J Hazard Mater 154:694–702
28. Siyal A, Memon S, Khaskheli M (2012) Optimization and equilibrium studies of Pb (II) removal by Grewia Asiatica seed: a factorial design approach. Polish J Chem Technol 14:71–77
29. Calero M, Ronda A, Martín-Lara M, Pérez A, Blázquez G (2013) Chemical activation of olive tree pruning to remove lead (II) in batch system: factorial design for process optimization. Biomass Bioenergy 58:322–332
30. Xu Z, Dong J (2008) Synthesis, characterization, and application of magnetic nanocomposites for the removal of heavy metals from industrial effluents. In: Shah V (ed) Emerging environmental technologies. Springer, The Netherlands, pp 105–148
31. Sun J, Ji Y, Cai F, Li J (2012) Chapter five: heavy metal removal through biosorptive pathways. In: Sharma SK, Sanghi R (eds) Advances in water treatment and pollution prevention. Springer, The Netherlands, pp 95–145
32. Farooq U, Kozinski JA, Khan MA, Athar M (2010) Biosorption of heavy metal ions using wheat based biosorbents—a review of the recent literature. Bioresour Technol 101:5043–5053. doi:http://dx.doi.org/10.1016/j.biortech.2010.02.030
33. Zouboulis A, Lazaridis N, Karapantsios, TD, Matis K (2010) Heavy metals removal from industrial wastewaters by biosorption. Int J Environ Eng Sci, 1:57–78.
34. Ajila CM, Brar S, Verma M, Prasada Rao UJS (2012) Sustainable solutions for agro processing waste management: an overview. In: Malik A, Grohmann E (eds) Environmental protection strategies for sustainable development. Springer, The Netherlands, pp 65–109
35. Khan F, Wahab R, Rashid M, Khan A, Khatoon A, Musarrat J, Al-Khedhairy A (2014) The use of carbonaceous nanomembrane filter for organic waste removal. Appl Nanotechnol Water Res, pp 115–152
36. Khan F, Wahab R (2014) Nanomaterials with uniform composition in wastewater treatment and their applications. Appl Nanotechnol Water Res, pp 475–511
37. Bailey SE, Olin TJ, Bricka RM, Adrian DD (1999) A review of potentially low-cost sorbents for heavy metals. Water Res 33:2469–2479. doi:http://dx.doi.org/10.1016/S0043-1354(98)00475-8
38. Das N, Vimala R, Karthika P (2008) Biosorption of heavy metals: an overview. Ind J Biotechnol 7:159–169
39. Chojnacka K (2010) Biosorption and bioaccumulation—the prospects for practical applications. Environ Int 36:299–307. doi:http://dx.doi.org/10.1016/j.envint.2009.12.001
40. Bradl H (2005) Heavy metals in the environment: origin, interaction and remediation. Elsevier, Amsterdam
41. Gavrilescu M (2010) Biosorption in environmental remediation. In: Fulekar MH (ed) Bioremediation technology. Springer, The Netherlands, pp 35–99
42. Singh KK, Rastogi R, Hasan SH (2005) Removal of cadmium from wastewater using agricultural waste (rice polish). J Hazard Mater 121:51–58. doi:http://dx.doi.org/10.1016/j.jhazmat.2004.11.002
43. Hawari AH, Mulligan CN (2006) Biosorption of lead(II), cadmium(II), copper(II) and nickel(II) by anaerobic granular biomass. Bioresour Technol 97:692–700. doi:http://dx.doi.org/10.1016/j.biortech.2005.03.033

44. Vijayaraghavan K, Balasubramanian R (2015) Is biosorption suitable for decontamination of metal-bearing wastewaters? A critical review on the state-of-the-art of biosorption processes and future directions. J Environ Manage 160:283–296
45. Liu L-e, Liu J, Li H, Zhang H, Liu J, Zhang H (2012) Equilibrium, kinetic, and thermodynamic studies of lead (II) biosorption on sesame leaf. Bioresources 7:3555–3572
46. Singanan M, Peters E (2013) Removal of toxic heavy metals from synthetic wastewater using a novel biocarbon technology. J Environ Chem Eng doi:http://dx.doi.org/10.1016/j.jece.2013.07.030
47. Mahmoud AED, Fawzy M, Radwan A (2016) Optimization of cadmium (Cd^2) removal from aqueous solutions by novel biosorbent. Int J Phytoremed 18:619–625. doi:10.1080/15226514.2015.1086305
48. Singanan M, Peters E (2013) Removal of toxic heavy metals from synthetic wastewater using a novel biocarbon technology. J Environ Chem Eng 1:884–890. doi:http://dx.doi.org/10.1016/j.jece.2013.07.030
49. Wong K, Lee C, Low K, Haron M (2003) Removal of Cu and Pb by tartaric acid modified rice husk from aqueous solutions. Chemosphere 50:23–28
50. Lopez-Mesas M, Navarrete ER, Carrillo F, Palet C (2011) Bioseparation of Pb(II) and Cd(II) from aqueous solution using cork waste biomass. Modeling and optimization of the parameters of the biosorption step. Chem Eng J 174:9–17
51. Surchi KMS (2011) Agricultural wastes as low cost adsorbents for Pb removal: kinetics, equilibrium and thermodynamics. Int J Chem 3:103–112
52. Singh N, Gadi R (2012) Bioremediation of Ni(II) and Cu(II) from wastewater by the nonliving biomass of Brevundimonas vesicularis. J Environ Chem Ecotoxicol 4:137–142
53. Amarasinghe B, Williams R (2007) Tea waste as a low cost adsorbent for the removal of Cu and Pb from wastewater. Chem Eng J 132:299–309
54. Abdel-Aty AM, Ammar NS, Abdel Ghafar HH, Ali RK (2013) Biosorption of cadmium and lead from aqueous solution by fresh water alga Anabaena sphaerica biomass. J Adv Res 4:367–374. doi:http://dx.doi.org/10.1016/j.jare.2012.07.004
55. Verma KVR, Swaminathan T, Subrahmanyam PVR (1990) Heavy metal removal with lignin. Environ Sci Health Part A 25:243–265
56. Taty-Costodes VC, Fauduet H, Porte C, Delacroix A (2003) Removal of Cd(II) and Pb(II) ions, from aqueous solutions, by adsorption onto sawdust of Pinus sylvestris. J Hazard Mater 105:121–142. doi:http://dx.doi.org/10.1016/j.jhazmat.2003.07.009
57. Arief VO, Trilestari K, Sunarso J, Indraswati N, Ismadji S (2008) Recent progress on biosorption of heavy metals from liquids using low cost biosorbents: characterization, biosorption parameters and mechanism studies. Clean-Soil, Air, Water 36:937–962
58. Şahan T, Öztürk D (2014) Investigation of Pb(II) adsorption onto pumice samples: application of optimization method based on fractional factorial design and response surface methodology. Clean Technol Environ Policy 16:819–831. doi:10.1007/s10098-013-0673-8
59. Ahmaruzzaman M (2011) Industrial wastes as low-cost potential adsorbents for the treatment of wastewater laden with heavy metals. Adv Colloid Interface Sci 166:36–59. doi:http://dx.doi.org/10.1016/j.cis.2011.04.005
60. Chen G, Zeng G, Tang L, Du C, Jiang X, Huang G, Liu H, Shen G (2008) Cadmium removal from simulated wastewater to biomass byproduct of Lentinus edodes. Bioresour Technol 99:7034–7040. doi:http://dx.doi.org/10.1016/j.biortech.2008.01.020
61. Badr N, Al-Qahtani KM (2013) Treatment of wastewater containing arsenic using Rhazya stricta as a new adsorbent. Environ Monit Assess 185:9669–9681
62. Mudhoo A, Garg V, Wang S (2012) Heavy metals: toxicity and removal by biosorption. In: Lichtfouse E, Schwarzbauer J, Robert D (eds) Environmental chemistry for a sustainable world. Springer, The Netherlands, pp 379–442
63. Gadd GM (2009) Biosorption: critical review of scientific rationale, environmental importance and significance for pollution treatment. J Chem Technol Biotechnol 84:13–28
64. Shi Y, Chai L, Yang Z, Jing Q, Chen R, Chen Y (2012) Identification and hexavalent chromium reduction characteristics of Pannonibacter phragmitetus. Bioprocess Biosyst Eng 35:843–850

65. Miranda J, Krishnakumar G, Gonsalves R (2012) Cr6+ bioremediation efficiency of Oscillatoria laete-virens (Crouan & Crouan) Gomont and Oscillatoria trichoides Szafer: kinetics and equilibrium study. J Appl Phycol 24:1439–1454
66. Gabr RM, Gad-Elrab SM, Abskharon RN, Hassan SH, Shoreit AA (2009) Biosorption of hexavalent chromium using biofilm of E. coli supported on granulated activated carbon. World J Microbiol Biotechnol 25:1695–1703
67. Trivedi B, Patel K (2007) Biosorption of hexavalent chromium from aqueous solution by a tropical basidiomycete BDT-14 (DSM 15396). World J Microbiol Biotechnol 23:683–689
68. Aksu Z, Balibek E (2007) Chromium (VI) biosorption by dried Rhizopus arrhizus: effect of salt (NaCl) concentration on equilibrium and kinetic parameters. J Hazard Mater 145: 210–220
69. Srinivasan A, Viraraghavan T (2010) Oil removal from water using biomaterials. Bioresour Technol 101:6594–6600
70. Aoyama M, Kishino M, Jo TS (2005) Biosorption of Cr (VI) on Japanese cedar bark. Sep Sci Technol 39:1149–1162
71. Igwe JC, Abia AA (2007) Equilibrium sorption isotherm studies of Cd (II), Pb (II) and Zn (II) ions detoxification from waste water using unmodified and EDTA-modified maize husk. Electron J Biotechnol 10:536–548
72. Jain M, Garg VK, Kadirvelu K (2009) Chromium(VI) removal from aqueous system using Helianthus annuus (sunflower) stem waste. J Hazard Mater 162:365–372. doi:http://dx.doi.org/10.1016/j.jhazmat.2008.05.048
73. Zein R, Suhaili R, Earnestly F, Munaf E (2010) Removal of Pb (II), Cd (II) and Co (II) from aqueous solution using Garcinia mangostana L. fruit shell. J Hazard Mater 181:52–56
74. García-Rosales G, Colín-Cruz A (2010) Biosorption of lead by maize (Zea mays) stalk sponge. J Environ Manage 91:2079–2086
75. Ibrahim MM, Ngah WW, Norliyana M, Daud WW, Rafatullah M, Sulaiman O, Hashim R (2010) A novel agricultural waste adsorbent for the removal of lead (II) ions from aqueous solutions. J Hazard Mater 182:377–385
76. Tan C-y, Li G, Lu X-Q, Z-l C (2010) Biosorption of basic orange using dried A. filiculoides. Ecol Eng 36:1333–1340
77. Carro L, Anagnostopoulos V, Lodeiro P, Barriada JL, Herrero R, de Vicente MES (2010) A dynamic proof of mercury elimination from solution through a combined sorption–reduction process. Bioresour Technol 101:8969–8974
78. Ashraf MA, Maah M, Yusoff I (2010) Study of mango biomass (Mangifera indica L) as a cationic biosorbent. Int J Environ Sci Technol 7:581–590
79. Lü L, Lu D, Chen L, Luo F (2010) Removal of Cd (II) by modified lawny grass cellulose adsorbent. Desalination 259:120–130
80. Foo L, Tee C, Raimy N, Hassell D, Lee L (2012) Potential Malaysia agricultural waste materials for the biosorption of cadmium (II) from aqueous solution. Clean Technol Environ Policy 14:273–280
81. Jeon C (2011) Removal of copper ion using rice hulls. J Indus Eng Chem 17:517–520
82. Ding D-X, Liu X-T, Hu N, Li G-Y, Wang Y-D (2012) Removal and recovery of uranium from aqueous solution by tea waste. J Radioanalyt Nucl Chem 293:735–741
83. Kurniawan A, Sisnandy VOA, Trilestari K, Sunarso J, Indraswati N, Ismadji S (2011) Performance of durian shell waste as high capacity biosorbent for Cr (VI) removal from synthetic wastewater. Ecol Eng 37:940–947
84. Shukla D, Vankar P (2012) Efficient biosorption of chromium(VI) ion by dry Araucaria leaves. Environ Sci Pollut Res 19:2321–2328. doi:10.1007/s11356-012-0741-3
85. Khoramzadeh E, Nasernejad B, Halladj R (2013) Mercury biosorption from aqueous solutions by sugarcane bagasse. J Taiwan Inst Chem Eng 44:266–269
86. Lorenc-Grabowska E, Gryglewicz G (2007) Adsorption characteristics of Congo Red on coal-based mesoporous activated carbon. Dyes Pigments 74:34–40
87. Sarkar B, Xi Y, Megharaj M, Krishnamurti GS, Rajarathnam D, Naidu R (2010) Remediation of hexavalent chromium through adsorption by bentonite based Arquad® 2HT-75 organoclays. J Hazard Mater 183:87–97

88. Maurya NS, Mittal AK, Cornel P, Rother E (2006) Biosorption of dyes using dead macro fungi: effect of dye structure, ionic strength and pH. Bioresour Technol 97:512–521
89. Itodo A, Itodo H (2010) Sorbent capacities and intensities of thermochemically cracked shea nut shells for the removal of waste water dyestuff. Academia Arena 2:41–50
90. Ghaedi M, Hajati S, Karimi F, Barazesh B, Ghezelbash G (2013) Equilibrium, kinetic and isotherm of some metal ion biosorption. J Indus Eng Chem 19:987–992
91. Abdel Wahab O (2007) Kinetic and isotherm studies of copper (II) removal from wastewater using various adsorbents. Egyptian J Aquat Res 33(1):125–143
92. Rajoriya R, Prasad B, Mishra I, Wasewar K (2007) Adsorption of benzaldehyde on granular activated carbon: kinetics, equilibrium, and thermodynamic. Chem Biochem Eng Quart 21:219–226
93. Lagergren S (1898) Zur Theorie der sogenannten Absorption gelöster Stoffe. PA Norstedt & söner, Stockholm
94. Ho Y-S, McKay G (2000) The kinetics of sorption of divalent metal ions onto sphagnum moss peat. Water Res 34:735–742
95. Pillai SS, Mullassery MD, Fernandez NB, Girija N, Geetha P, Koshy M (2013) Biosorption of Cr (VI) from aqueous solution by chemically modified potato starch: equilibrium and kinetic studies. Ecotoxicol Environ Saf 92:199–205
96. Elangovan R, Philip L, Chandraraj K (2008) Biosorption of chromium species by aquatic weeds: kinetics and mechanism studies. J Hazard Mater 152:100–112
97. Gokhale S, Jyoti K, Lele S (2009) Modeling of chromium (VI) biosorption by immobilized Spirulina platensis in packed column. J Hazard Mater 170:735–743
98. Gupta V, Rastogi A (2009) Biosorption of hexavalent chromium by raw and acid-treated green alga Oedogonium hatei from aqueous solutions. J Hazard Mater 163:396–402
99. Sarı A, Uluozlü ÖD, Tüzen M (2011) Equilibrium, thermodynamic and kinetic investigations on biosorption of arsenic from aqueous solution by algae (Maugeotia genuflexa) biomass. Chem Eng J 167:155–161
100. Chen G-Q, Zhang W-J, Zeng G-M, Huang J-H, Wang L, Shen G-L (2011) Surface-modified Phanerochaete chrysosporium as a biosorbent for Cr (VI)-contaminated wastewater. J Hazard Mater 186:2138–2143
101. Sud D, Mahajan G, Kaur MP (2008) Agricultural waste material as potential adsorbent for sequestering heavy metal ions from aqueous solutions—a review. Bioresour Technol 99:6017–6027. doi:http://dx.doi.org/10.1016/j.biortech.2007.11.064
102. Abdel-Ghani N, Hegazy A, El-Chaghaby G (2009) Typha domingensis leaf powder for decontamination of aluminium, iron, zinc and lead: biosorption kinetics and equilibrium modeling. Int J Environ Sci Tech 6:243–248
103. Chen JP, Yang L, Lim S-F (2007) Emerging biosorption, adsorption, ion exchange, and membrane technologies. In: Wang L, Hung Y-T, Shammas N (eds) Advanced physicochemical treatment technologies. Humana Press, Totowa, pp 367–390
104. Özacar M, Şengil IA (2003) Adsorption of reactive dyes on calcined alunite from aqueous solutions. J Hazard Mater 98:211–224
105. Hameed B, Mahmoud D, Ahmad A (2008) Sorption equilibrium and kinetics of basic dye from aqueous solution using banana stalk waste. J Hazard Mater 158:499–506
106. Baysal Z, Cinar E, Bulut Y, Alkan H, Dogru M (2009) Equilibrium and thermodynamic studies on biosorption of Pb (II) onto Candida albicans biomass. J Hazard Mater 161:62–67
107. Hamissa AMB, Lodi A, Seffen M, Finocchio E, Botter R, Converti A (2010) Sorption of Cd(II) and Pb(II) from aqueous solutions onto Agave americana fibers. Chem Eng J 159:67–74. doi:http://dx.doi.org/10.1016/j.cej.2010.02.036
108. Montgomery DC (2001) Design and analysis of experiments. Wiley, New York
109. Abdel-Ghani NT, Hegazy AK, El-Chaghaby GA, Lima EC (2009) Factorial experimental design for biosorption of iron and zinc using Typha domingensis phytomass. Desalination 249:343–347
110. Saadat S, Karimi-Jashni A (2011) Optimization of Pb(II) adsorption onto modified walnut shells using factorial design and simplex methodologies. Chem Eng J 173:743–749. doi:http://dx.doi.org/10.1016/j.cej.2011.08.042

111. Anderson M, Whitcomb P (2007) Chapter 3: two-level factorial design. In: Anderson M, Whitcomb P (eds) DOE simplified: practical tools for effective experimentation, 2nd edn. CRC Press, Boca Raton, pp 1–30
112. Spall JC (2010) Factorial design for efficient experimentation. IEEE Control Syst 30(5): 38–53
113. Varma DSNR, Srinivas C, Nagamani C, PremSagar T, Rajsekhar M (2010) Studies on biosorption of Cadmium on Psidium guajava leaves powder using statistical experimental design. J Chem Pharmaceut Res 2:29–44
114. Nasr M, Mahmoud A, Fawzy M, Radwan A (2015) Artificial intelligence modeling of cadmium(II) biosorption using rice straw. Appl Water Sci 1–9. doi:10.1007/s13201-015-0295-x
115. Garg U, Kaur MP, Jawa GK, Sud D, Garg VK (2008) Removal of cadmium (II) from aqueous solutions by adsorption on agricultural waste biomass. J Hazard Mater 154:1149–1157. doi:http://dx.doi.org/10.1016/j.jhazmat.2007.11.040
116. Krishnani KK, Meng X, Christodoulatos C, Boddu VM (2008) Biosorption mechanism of nine different heavy metals onto biomatrix from rice husk. J Hazard Mater 153:1222–1234
117. Park D, Yun Y-S, Lee DS, Park JM (2011) Optimum condition for the removal of Cr (VI) or total Cr using dried leaves of Pinus densiflora. Desalination 271:309–314
118. Sulaymon AH, Mohammed AA, Al-Musawi TJ (2013) Competitive biosorption of lead, cadmium, copper, and arsenic ions using algae. Environ Sci Pollut Res 20(5):3011–3023
119. Al-Qahtani KM (2012) Biosorption of Cd and Pb on Cyperus laevigatus: application of factorial design analysis. Life Sci J 9:860–868
120. Muhammad M, Nwaedozie J (2012) Application of marine biomass for the removal of metals from industrial wastewater. Indian J Innovat Develop 1:36–44
121. EPA (2007) Test methods for evaluating solid wastes physical/chemical methods. pp. 1–28
122. Zhang C (2007) Fundamentals of environmental sampling and analysis. Wiley, New York
123. Marcovecchio JE, Botté SE, Freije RH (2007) Heavy metals, major metals, trace elements. In: Nollet ML (ed) Handbook of water analysis, 2nd edn. CRC Press, New York, pp 275–311
124. Mortimer M, Müller JF, Liess M (2007) Sampling methods in surface waters. In: Nollet ML (ed) Handbook of water analysis. CRC Press, New York, pp 1–45
125. Srivastava S, Goyal P (2010) Biosorption: application strategies. In: Novel biomaterials. Springer, Berlin, pp. 53–55
126. Ozdemir U, Ozbay I, Ozbay B, Veli S (2014) Application of economical models for dye removal from aqueous solutions: cash flow, cost–benefit, and alternative selection methods. Clean Technol Environ Policy 16:423–429

Chapter 8
Metal Hyperaccumulators: Mechanisms of Hyperaccumulation and Metal Tolerance

Abdul Razaque Memon

8.1 Introduction

Heavy metal contamination and toxicity is a major problem for both industrialized and the raw materials (especially metal ores) exporting countries. Heavy metals are important environmental pollutants and are toxic at very high concentrations. The major environmental problems which poses severe health risk are due to unregulated industrial activities, for example mining, smelting of metalliferous ores, combustion of fossil fuels, municipal waste, and several agricultural activities (unlimited use of fertilizers, pesticides, and herbicides) [1]. The metal-contaminated areas in the globe support unique plant species which can flourish in these high metal-containing soils [2]. All plants have the ability to absorb and accumulate trace amount of essential metals for their growth for example (Fe, Mn, Zn, Cu) from the soil and water but they have also the ability to accumulate non-essential heavy metals which include Cd, Cr, Pb, Ag, and Hg which have no known biological function [1]. However, excessive accumulation of these metals can be toxic to most plants. The ability of plants to both tolerate high levels of heavy metals, accumulate them to unusually high concentrations has evolved both independently and together in a number of plant species [3]. The basic hallmarks of hyperaccumulators plants are: an enhanced rate of heavy metal uptake in roots, highly efficient translocation of metals from root-to-shoot, and a greater ability to accumulate and sequester heavy metals in leaves. The hyperaccumulation phenomenon has growing attention in the past decade because of their potential use in phytoremediation [4].

Exploiting hyperaccumulating plant species and identifying metal accumulation genes is currently focal point for phytoremediation or phytomining [5]. Around 500 plant species are classified as heavy accumulator plants [6–8]. These plant species

---

A.R. Memon (✉)
Department of Molecular Biology and Genetics, Usak University,
1 Eylül Kamp., Usak 64200, Turkey
e-mail: abdulrezzak.memon@gmail.com; armemon@usak.edu.tr

A.A. Ansari et al. (eds.), *Phytoremediation*, DOI 10.1007/978-3-319-40148-5_8

have the ability to accumulate extremely high amounts of heavy metals in their leaves [9, 10]. Approximately 80 % of them accumulate Ni, and the second largest group (15 species) is hyperaccumulators of Zn and Cd [11]. Memon and co-workers, while working with 62 plant species in 39 genera and 27 families from the natural forest of central Japan, reported several multi-accumulator plant species concentrating several hundred folds Mn, Cu, Zn, Cd, Co, and Ni in their leaves [12–14]. *Ilex crenata*, *Acanthopanax sciadophylloides*, *Clethra barbinervis*, and *Pieris japonica* were found as bi- or multi-accumulator plants. *Arabidopsis hallari, Noccaea caerulescens* (*Thlaspi caerulescens*), *Brassica nigra*, and *Brassica juncea* have been widely studied to elucidate the mechanisms of heavy metal accumulation and tolerance in plants [15].

The use of molecular biology and genetic engineering techniques to engineer the plants with efficient metal uptake, transport, and high accumulation capacity in leaves or shoots will surely enhance the efficiency of phytoremediation of polluted areas. Several genes related to metal binding, for example metallothionein (MT) and phytochelatin (PC), and metal transporters (e.g., metal-transporting ATPases) have been cloned to develop transgenic plants which can detoxify and/or accumulate several toxic metals, for example, cadmium, lead mercury, arsenic, and selenium. After publishing Arabidopsis thaliana genome, further genome sequencing projects of *Arabidopsis lyreta*, *Arabidopsis hallari*, Nocea cerulusence, and other crop species in Brassicacceae like *Brassica rapa* and *B. juncea* using third-generation sequence technologies will open up new research avenues for the development of superaccumulator plants for phytoremediation.

In this chapter, I summarize the current state of knowledge concerning metal accumulation and detoxification mechanism in plants and the potential commercial application of this technology in phytoremediation.

8.2 Metal Accumulator Plants

Plants growing on contaminated and metalliferous soils can be divided into three basic groups: Metal excluders—plants that prevent large quantities of metals from entering the aboveground parts of the plants, but contain large amounts of metals within the roots. Metal indicators—accumulate metals in the aboveground parts of the plant, and the levels of metals in the tissues generally reflect the metal levels in the soil. Metal accumulators (also called hyperaccumulators)—concentrate levels of metals in their above-ground tissues that greatly exceed those present in soil or in nearby nonaccumulating species. Hyperaccumulators plants can be categorized according to the consistency of their metal accumulation behavior and are being divided in two distinct groups: obligate and facultative [3, 16]. The species which are endemic to type of metalliferous soil and always show high metal uptake at the level defined for hyperaccumulation are classified as obligate accumulators. On the other hand, facultative hyperaccumulators are species in which some individuals are hyperaccumulators and other individuals are not [17]. The facultative hyperaccumulator category includes particularly those species that occur on both metalliferous and nonmetalliferous (normal) soils showing hyperaccumulation only when they grow on metalliferous soils [16]. Hyperaccumulators contain more than 0.1 % of Ni, Co, Cu, Cr, and Pb or 1 % of Zn on a dry weight basis, irrespective of the soils' metal concentration [17]. Va der Ent et al. [16] suggested that the criteria which have been commonly used to delimit hyperaccumulation of some metals are unnecessarily conservative. They propose that the criteria for hyperaccumulation of Se and Tl be lowered to 100 µg/g dried leaf, the criteria for hyperaccumulation of Cu, Co, and Cr be lowered to 300 µg/g dried plant leaf, and the criterion for hyperaccumulation of Zn be lowered to 3000 µg/g dried plant leaf.

There are currently over 450 known hyperaccumulator plant species, with new ones regularly being discovered, so it is quite possible that there are still many unidentified hyperaccumulators [3]. On the other hand, it should be noted that there is no standardized procedure for naming, and declaring a plant to be a hyperaccumulator, and some of these hyperaccumulators could be deleted from the list if the trait is unconfirmed by repeat experimentation under controlled conditions [16]. Several plant species in *Brassicaceae* are reported to be most efficient in removing Zn, Cd, and Ni from the contaminated soils [2, 7]. Many *Brassica sp.* such as *B. juncea* L., *B. napus* L., and *B. rapa* L. showed moderately high Zn and Cd accumulation [18–20]. However, there are some specific plant species which have been identified as a good candidate for remediation of polluted soils, which include either high biomass plants such as willow [21, 22] or those that have low biomass but high hyperaccumulating characteristics such as *Noccaea* (*Thlaspi*) and some Arabidopsis species (e.g., *A. hallari*) [15].

Hyperaccumulators have been found in over 45 families, distributed all over the world in both tropical and temperate zones, proving that this trait has evolved independently more than once. The most interesting plant family for phytoextraction research is the *Brassicaceae* to which over 25 % of the thus discovered hyperaccumulators belong to, with *Noccacaea caerulescens* (previously *Thlaspi caeulescens*), *Arabidopsis halleri*, and *Alyssum* being the most studied ones. Ni accumulation is present in over 75 % of these taxa, while only five species have been found to accumulate Cd. Zn hyperaccumulation has also been observed less frequently with Zn hyperaccumulators including *A. halleri* and certain *Noccaea* species (e.g., *N. caerulescens*) [3, 23].

8.3 Factors Affecting Heavy Metal Uptake by Plants

There are various environmental factors that affect the uptake of metals, such as temperature, soil pH, soil cation exchange capacity, the type of plant, its size, its root system, the bioavailability of metals, soil moisture, etc. [24, 25]. The metal solubility and availability are strongly influenced by soil pH, which is a major factor in the bioavailability of metals in the soil. Many metal cations have been reported to be more soluble and available in soils with low pH (below 5.5) [26] and such findings have led to investigate the incorporation of acidifiers into metal-polluted soils to improve phytoextraction, but little research has been performed on these subjects, and it needs more attention.

Heavy metal cations often bind to soil particles because of soil cation exchange capacity (CEC), the CEC is a measure of the soils' ion exchange capacity. The higher the CEC of the soil means the greater the sorption and immobilization of the metals from the soil. One way of improving the heavy metal bioavailability is by addition of chelators to the soil, such as EDTA. However, the use of synthetic chelating agents could promote the leaching of contaminants into the soil. Chelators could be warranted for alkaline soils as the bioavailability of heavy metals decreases above pH 5.5.

8.4 Mechanisms of Metal Uptake, Translocation, and Tolerance

In order to cope with environmental stresses, plants have developed a wide range of strategies to cope with changes in environmental conditions and minimize the harmful effects of metal deficiency or toxicity. The possible responses include large alterations of gene expression, particularly of membrane transporters, responsible for uptake, translocation and sequestration of essential and nonessential mineral nutrients. The process of heavy metal phytoremediation can be roughly divided into four parts: (1) heavy metal uptake and transport to roots, (2) transfer from root to shoot, (3) heavy metal chelation, and (4) vacuolar compartmentalization.

8.4.1 Heavy Metal Uptake

In order to have effective phytoextraction capabilities for plants, the entrance of metal into root tissues must be rapid, and followed by immediate transport into the aboveground parts of the plant. Bioavailability of heavy metals to the plants is the primary determinant of phytoextraction effectiveness and it refers the available fraction of the total pollutant present in the soil and sediment. Even though the medium in which the plants are growing can have a high concentration of heavy metals, only a small fraction will be bioavailable. The bioavailability of heavy metals is limited because of their low solubility (Cr, Ag, Hg, or Sn) and strong binding to soil particles (Pb). This bioavailability of heavy metals in the soil can be altered by rhizospheric microbes and root exudates, such as siderophores, organic acids, and protons. The initial uptake is mobilized through the secreted molecules in the rhizosphere such as mugenic and aveic acids and specific membrane bound metal reductases and the proton extrusion from the roots [9, 27].

8.4.1.1 Transport to Root

After mobilization the metals are firstly bind to the cell wall, which is an ion exchanger of low selectivity. The uptake and transport through the plasma membrane is then mediated by secondary transporters such as channel proteins and/or H^+ coupled carrier proteins. A number of such membrane transporter gene families have been identified and characterized such as ZIP (ZRT, IRT-like protein), NRAMP (natural resistance-associated macrophage protein), YSL (yellow-stripe-like transporter), NAS (nicotinamine synthase), SAMS (S-adenosyl-methionine synthetase), FER (ferritin Fe (III) binding), CDF (cation diffusion facilitator), HMAs (heavy metal ATPases), and IREG (Iron regulated transporter) family [4, 15, 28].

There are 18 genes coding ZIPs in Arabidopsis, and they are predicted to be involved in the cellular uptake and mobilization of stored Zn [29]. Some ZIP genes have been found to be highly overexpressed in Zn/Cd accumulators such as *N. caerulescens* and *A. halleri* when compared to non-accumulating species [30, 31]. Differential expression of ZIP transporters has been observed between contrasting ecotypes (Ganges, a Cd hyperaccumulator and Prayon, a Cd non accumulator) of the Zn hyperaccumulator *N. caerulescens*. Four ZIP genes (TcIRT1, TcIRT2, TcZNT1, and TcZNT5) were found to be expressed only in the roots, indicating their specificity for metal uptake and transport in the roots. ZNT1 was found to be a mediator of high-affinity Zn transport capable of low-affinity Cd transport [32]. The differences in Cd accumulation between these two ecotypes were determined to be due to the differing expression levels of these proteins [33]. TcZNT1 expression was not influenced by Cd and the expression levels remained similar for both Ganges and Prayon ecotypes, but the TcZNT5 expression levels were lower in Ganges than in Prayon, and unaffected by Cd treatment. TcIRT1 showed increased expression in roots of both ecotypes when Cd was introduced, but the transcript levels of TcIRT2 were not detectable in either ecotype, regardless of the presence of Cd.

ABC family transporters (ATP-binding cassette) are one of the largest protein families in living organisms [34]. *A. thaliana* contains around 130 ABC transporters, with most still having undetermined functions [35]. Some Arabidopsis ABC transporters were found to take part in detoxification processes as well as plant growth and development [36–38]. At ATM3 is a ATP-binding cassette transporter in Arabidopsis thaliana which has been shown to be upregulated in roots of plants when exposed to Cd (II) and Pb(II) [39]. Arabidopsis plants with overexpressed AtATM3 showed enhanced Cd and Pb resistance when compared to wild-type, whereas those lacking AtATM3 were Cd-sensitive [40].

8.4.2 Transport from Roots to Shoots

The further movement of metals to aerial parts is controlled by root pressure and transpirational pull [41]. Several studies have shown the presence of free heavy metal ions in xylem sap, such as that Zn is present as free hydrated ZN^{2+} ions in *N.*

caerulescens [42] and that Cd occurs mainly in free ionic form in *A. halleri* [43]. These freely available metals for translocation from roots to shoots indicate that the sequestration of these metals in root vacuoles is limited and several studies have shown a much lower accumulation of Zn in root vacuoles and a faster translocation from roots to shoots in hyperaccumulator *N. caerulescens* compared to non-accumulator *N. arvense* [44]. A non-accumulating ecotype of *Sedum alfredii* Hance (NHE) was reported to retain 2.7 times more Zn in its root vacuoles compared to its hyperaccumulating ecotype (HE) [45].

8.4.2.1 Metal-Transporting ATPases

The efficient translocation of heavy metals from roots to shoots is heavily dependent on efficient xylem loading mechanisms by constitutive overexpression of the genes that code for transport systems from roots to shoots in accumulator plants [46]. Of high importance in this process are the P1B-type ATPases, also known as HMAs (Heavy Metal-Transporting ATPases). Their function is to transport heavy metals and a lot of studies have identified particular members of this family as being responsible for Cd and Zn loading into xylem from the surrounding vascular tissues. These superfamily of P-type ATPases transport transitional metal ions against their electrochemical gradient using the energy provided by ATP hydrolysis [47, 48]. On the basis of similarities in both sequence and function, these ATPases could be divided into multiple subfamilies and have been identified in both prokaryotes and eukaryotes [49]. These heavy metal-transporting P-type ATPases (HMAs) are generally located both in plasma membrane and vacuolar membrane. In Arabidopsis thaliana, there are eight HMA genes present and which constitutes a multigene family. The functional roles of these metal-transporting ATPases are diverse. HMA2, HMA3, and HMA4 are involved in zinc/cadmium/lead/cobalt transport, whereas HMA5, HMA6, HMA7, and AtHMA8 are involved in copper transport [50].

HMA1 transports copper but its role in zinc/cadmium/cobalt as well as calcium transport has also been reported [51]. The sub-compartmentalization of the metals in the cell is directly correlated with the different localization of metal transporters especially HMAs in cell. For example, HMA1 is located on the chloroplast envelope and is involved both in copper loading into the chloroplast and in zinc detoxification [52]. In *A. thaliana*, HMA2 and HMA4 play a critical role in the zinc and cadmium translocation from the roots to the shoot and are functionally redundant [53]. HMA3 in planta is located in the vacuolar membrane, with a high expression level in guard cells, hydathodes, vascular tissues, and the root apex and likely plays a role in the detoxification of Zn, Cd, cobalt, and Pb by participating in their vacuolar sequestration [54, 55]. HMA4 appears to be a major determinant of zinc and cadmium tolerance in metal accumulator plants in *Brassicaceae* [56].

8.4.2.2 Phylogenetic Analysis of Metal-Transporting ATPases

The HMA family makes an interesting target for the research of heavy metal transport. Because of the functional diversity of HMA2, HMA3, and HMA4 genes displayed in A. thaliana, in particular, given the functional diversity that the HMA2, HMA3, and HMA4 genes actually display in *A. thaliana*, it would be of great importance to determine the appropriate orthologs of the HMA genes in every plant species of interest. While developing such a strategy, the phylogenetic relationships were analyzed by linking the genes encoding Zn/Cd/Pb/Co P1B-ATPases from different plant species. The clear orthology relationships were defined among species belonging to a same botanical family, but these relationships were not clearly established with plants belonging to different botanical families [57]. Recently, it has been shown that rice HMAs display different functional properties from the Arabidopsis HMAs [58, 59].

In this context, we constructed a phylogenetic tree of genes tagged with "Heavy Metal Binding domain" using *Arabidopsis thaliana* and *Brassica rapa* databases (Muhovic and Memon unpublished data). The phylogenetic tree of this group includes all P1B ATPases as well as CCH, NAKR, and HIPP family genes (Fig. 8.1).

The phylogenetic tree gave us a clear picture of heavy metal-induced genes in Arabidopsis and Brassica. In green we marked the P-1B ATPases that were of special interest to us as they have been identified to play crucial roles in heavy metal accumulation, more specifically the xylem loading. Another large clade on the phylogenetic tree is the HIPP family group (Fig. 8.1). This is a HMA domain containing protein family of metallo-chaperone-like genes, characterized in *Arabidopsis thaliana*. They are linked to Cd detoxification, possibly by Cd binding, and the proteins of the group are commonly expressed in root tissues [60]. A third group was identified, consisting of copper chaperone CCH, ATX1, and NAKR gene family, some of which have been shown to regulate plant response to heavy metal homeostasis, dehydration, and abiotic stress [61–64]. The rest of the proteins in the phylogenetic tree are not fully characterized, as they are mostly annotated as "Heavy metal domain-associated" genes, or "heavy metal transport-associated" genes. The *A. thaliana* and *B. rapa* orthologs did not show any great divergence between them in a phylogenetic tree (Fig. 8.1). The genes listed in this study are a good target for future work and characterization, as we have seen that the P-1B ATPases and the HIPP genes have divergent functions related to metal detoxification. Further analysis is needed to construct more detailed networks of possible interactions and mechanisms involved in this vital process. One common annotation among them was "Copper transport," this can be explained by the fact that heavy metal transporters compete for any kind of metal ions available and so transport all heavy metals, due to their similar atomic structure [65].

The challenge of this research was to relate the above-mentioned knowledge about HMAs in *A. thaliana* and *B. rapa* to ortholog HMAs in other species in Brassicaceae. For example, Talke et al. [66] reported three copies of the sole HMA4 genes present in *A. halleri* while only one copy is present in *A. thaliana*. In this connection, a phylogenetic analysis was carried out by using the nucleotide sequences

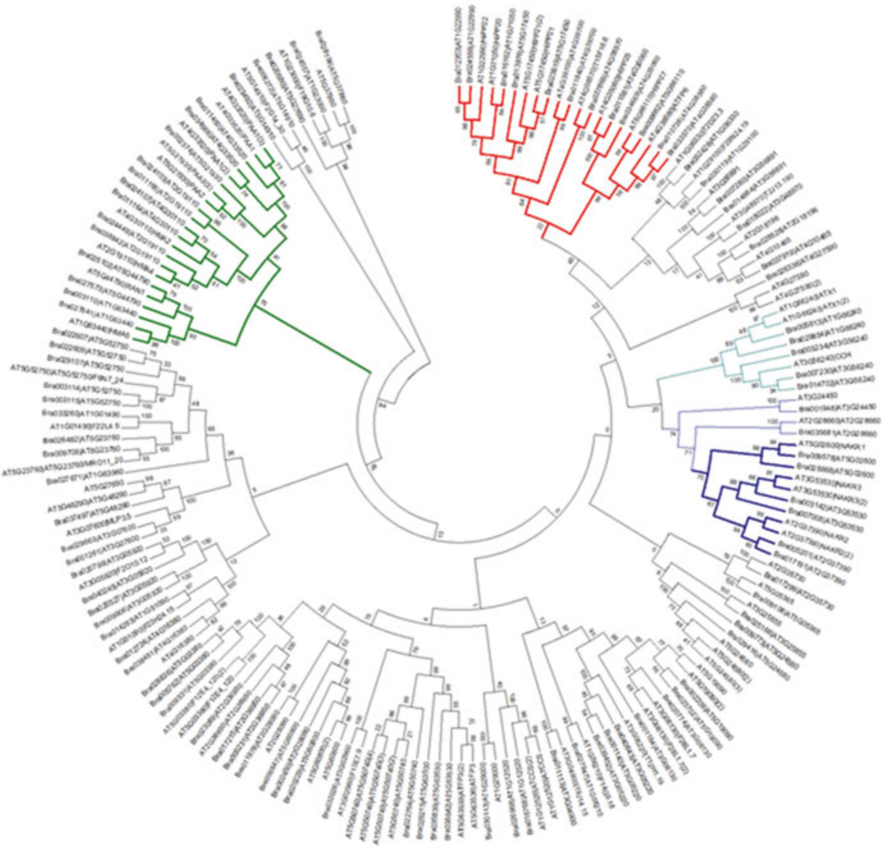


Fig. 8.1 A phylogenetic tree of all Heavy Metal Binding Domain genes in *Brassica rapa*, and *Arabidopsis thaliana*. Characterized gene families are marked with colors, *Green* for HMA P-1B family, *red* for HIPP family and NAKR genes are marked in *blue*, and ATX and CCH are marked in *light blue* (Muhovic and Memon unpublished data)

of the number of ortholog genes (HMAs) from different plant species (Fig. 8.2). Clearly, there was a great number of duplication events occurred independently after speciation. The phylogenetic tree built in Jal view software is shown in Fig. 8.2.

By observing phylogenetic trees, the results were in accordance with our expectations in a way that HMAs among Brassicaceae family are grouped based on their orthology relationship. Therefore, three paralogs of HMA4 in *Arabidopsis halleri* are close to HMA4 *Arabidopsis thaliana*. *A. halleri* HMA2 is close to *A. thaliana* HMA2 but its transcript level in *A. hallari* is reported to be lower than *A. thaliana* [66]. On the other hand, EpHMA2 and BrHMA2 transporters are closely related to BrHMA4-1 and BrHMA4-2 which are also involved in corresponding heavy metal transport and NcHMA4 is closely related to AlHMA1-2, BrHMA1-2, EpHMA1-2, EpHMA1-1, AlHMA1-1, AtHMA1, and BrHMA1-1 transporters (Fig. 8.2). Phylogenetic trees were constructed by using two different softwares, Jalview and ClustalW, in a way to confirm accuracy of data (Subasic and Memon unpublished data).

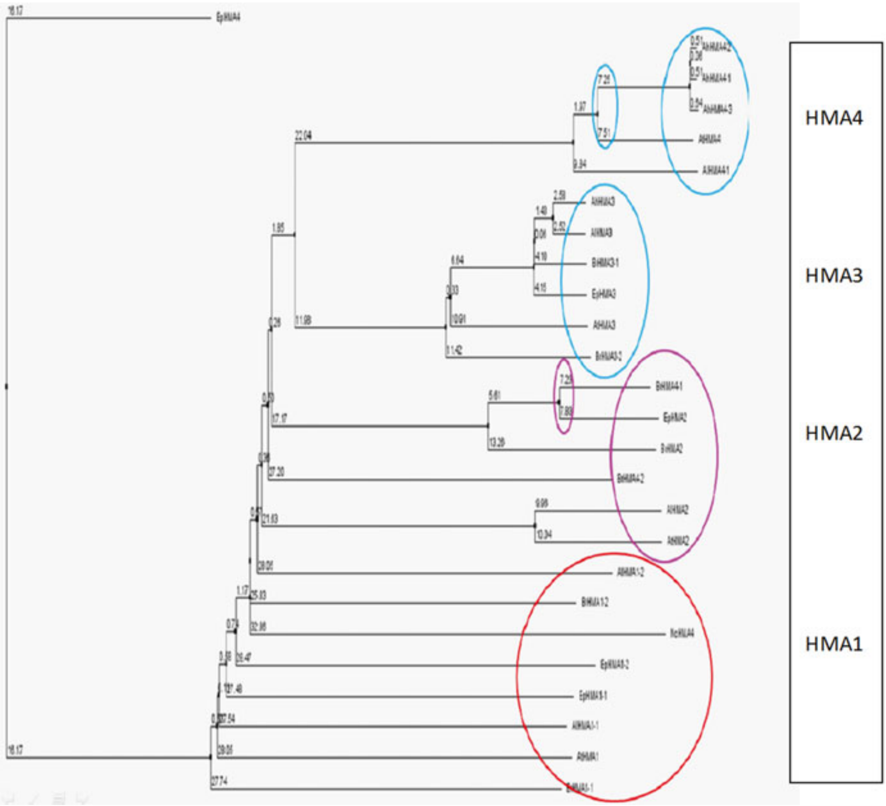


Fig. 8.2 Phylogenetic tree built by ClustalW by implementing Distance Matrix for orthologues genes in *Brassicaceae* family. Four clusters were observed: HMA4 (AhHMA4-1, AhHMA4-2, AhHMA4-3, AtHMA4 and AlHMA4-1), HMA3 (AhHMA3, AlHMA3, BrHMA3-1, EpHMA3, AtHMA3, BrHMA3-2), HMA2 (BrHMA4-1, EpHMA2, BrHMA2, BrHMA4-2, AlHMA2, AtHMA2), and HMA1 (AlHMA1-2, BrHMA1-2, NcHMA4, EpHMA1-2, EpHMA1-1, AlHMA1-1, AtHMA1, BrHMA1-1) (Subasic and Memon unpublished data)

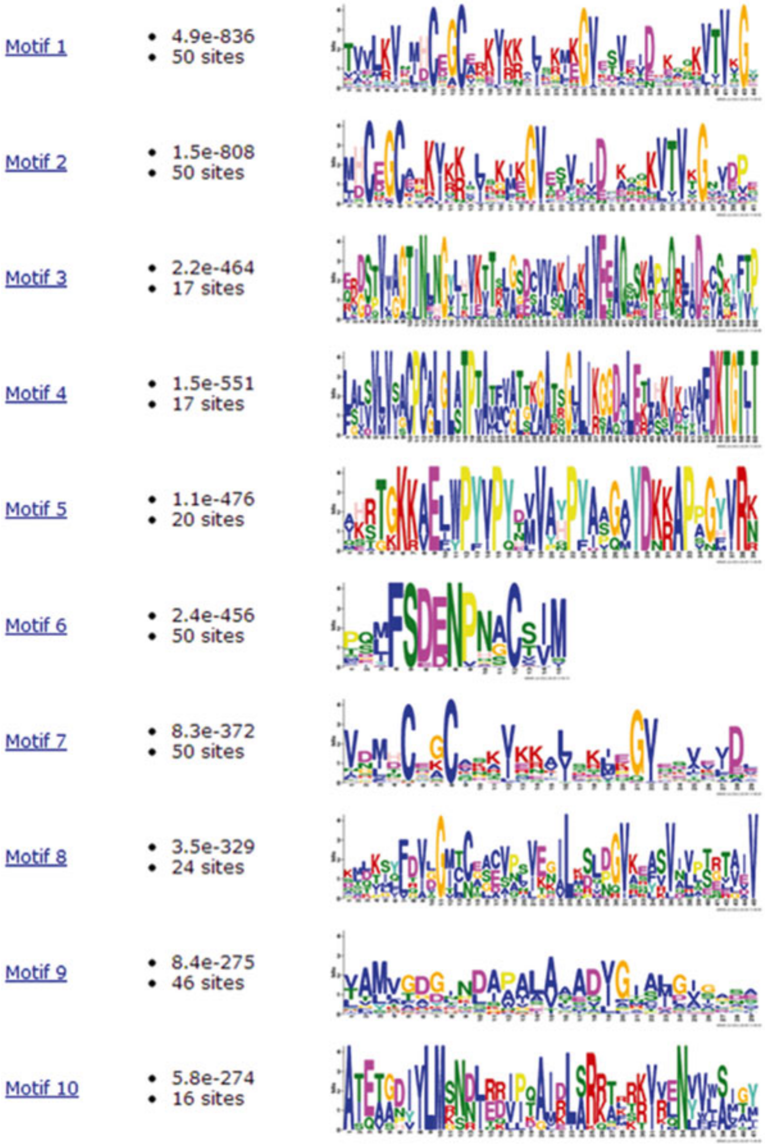
The Zn hyperaccumulation and full hypertolerance to Zn and Cd in *A. halleri* were considerably reduced when HMA4 expression was reduced through RNAi. Using RNAi to downregulate HMA4 expression, Hanikenne and Baurain [67] showed that full hypertolerance to Cd and Zn and Zn hyperaccumulation in *A. halleri* depend on the metal pump HMA4. Increased expression of HMA4 in *A. halleri* enhanced the Zn transport from the root symplasm into the xylem vessels essential factor for shoot Zn hyperaccumulation. High HMA4 expression in *A. halleri* is specified in *cis* elements of the promoter and amplified by gene copy number expansion. Increased expression of HMA4 in *A. halleri* enhanced the Zn flux from the root symplasm into the xylem necessary for shoot Zn hyperaccumulation, and works as a physiological master switch to upregulate Zn deficiency response gene expression in roots [67, 68]. In addition, Arabidopsis single mutant *hma4* accumulate more Zn in roots and less in shoots than the wild type [68]. On the other hand, the shoots of double mutant plants *hma2 hma4* show Zn deficiency symptoms and infertility, whereas root-to-shoot partitioning of other essential transition metals is unaffected. Application of the Zn exogenously rescued the Zn deficiency in the shoot [53]. Several reports suggest that the metal translocation rates in hyperaccumulator plants are not solely dependent on HMA4-mediated xylem loading, but there are other "upstream factors or mechanisms," responsible for the retention of metals in root cell vacuoles [69, 70]. These findings indicate that several regulatory mechanisms including the copy numbers of HMAs, their *cis* regulatory diversification, and other transport proteins (e.g., vacuolar transporters, etc.) play a central role in metal hyperaccumulation. A conserved heavy-metal-associated (HMA) domain has been found in a number of these heavy metal transport or detoxification proteins [PMID: 8091505] and contains two conserved cysteines that are probably involved in metal binding. Genes containing the heavy metal binding motif are of special interest to hyperaccumulation studies, as they make likely targets for discovering new proteins involved in hyperaccumulation and metal detoxification, and further studies are being carried out by our group in this connection.

8.4.2.3 Promoter Analysis of Metal-Transporting ATPases

Motif analyses in the promoter of HMAs from different species in Brassicaceae were performed by using the MEME (Multiple EM for Motif Elicitation) suite software toolkit [71, 72]. MEME is one of the most widely used tools for searching for novel "signals" in sets of biological sequences [71]. This application discovers the new transcription factor binding sites and protein domains. It works by searching for repeated, un-gapped sequence patterns that occur in the DNA or protein sequences. Zn^{2+}-ATPases, such as HMA2, HMA3, and HMA4 in Arabidopsis, have a CysCysXXGlu motif within a similar βαββαβ-fold HMA domain. In vitro studies show that the N-MBD domain of HMA2 binds Zn^{2+} and Cd^{2+} with high affinity and the conserved Cys and Glu residues function in coordination with both metals [60].

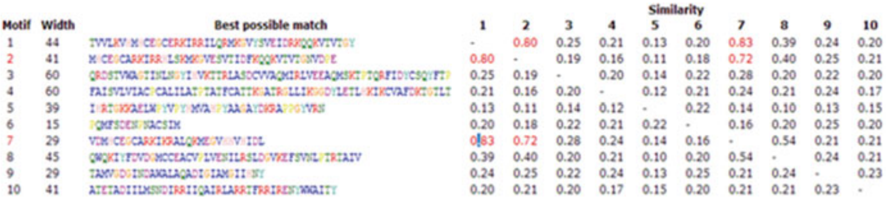
The MEME motif analysis suit gave us ten conserved motifs (Fig. 8.3). The first, second, and seventh have a high level of similarity (0.72–0.83), meaning that they are probably isoforms of the IPR006121 protein domain. Interesting differences were observed between the HMA4 gene versions among plant species. Interestingly, the HMA4 gene production *A. thaliana* contains an additional copy of motif 8, which is absent in all of the Brassica versions of this gene. On the other hand, HMA2 has two homologs in *Brassica rapa*, one of which lacks motif 4, which is expressed in the other copies of HMA2, as well as in PAA2 and HMA4 (data not shown). It remains to be seen what kind of effect this isoform has on the metal accumulation properties in *B. rapa* and other *Brassica* species (Muhovic and Memon unpublished data).

Further analysis of the conserved motifs showed that motif 8 is the same heavy metal binding motif as motifs 1, 2, and 7, but surprisingly it shows a lesser level of conservation of the two cysteines that are commonly thought to be involved in the metal binding property of this protein domain (see Fig. 8.4).



Motif 1	• 4.9e-836 • 50 sites
Motif 2	• 1.5e-808 • 50 sites
Motif 3	• 2.2e-464 • 17 sites
Motif 4	• 1.5e-551 • 17 sites
Motif 5	• 1.1e-476 • 20 sites
Motif 6	• 2.4e-456 • 50 sites
Motif 7	• 8.3e-372 • 50 sites
Motif 8	• 3.5e-329 • 24 sites
Motif 9	• 8.4e-275 • 46 sites
Motif 10	• 5.8e-274 • 16 sites

Fig. 8.3 The conserved motifs found in the P-1B ATPase family, this is the MEME suite output for sequences from the P-1B ATPase family. The larger letters indicate that the residue is more often expressed (more highly conserved) at that place. The first, second, and seventh motifs show two large blue C letters. These conserved cysteine residues indicate that these are probably one and the same motif and possibly a Heavy Metal Domain motif, which is found in all of the genes analyzed in the present work



			Similarity									
Motif	Width	Best possible match	1	2	3	4	5	6	7	8	9	10
1	44	[illegible]	-	0.80	0.25	0.21	0.13	0.20	0.83	0.39	0.24	0.20
2	41	[illegible]	0.80	-	0.19	0.16	0.11	0.18	0.72	0.40	0.25	0.21
3	60	[illegible]	0.25	0.19	-	0.20	0.14	0.22	0.28	0.20	0.22	0.20
4	60	[illegible]	0.21	0.16	0.20	-	0.12	0.21	0.24	0.21	0.24	0.17
5	39	[illegible]	0.13	0.11	0.14	0.12	-	0.22	0.14	0.10	0.13	0.15
6	15	[illegible]	0.20	0.18	0.22	0.21	0.22	-	0.16	0.20	0.25	0.20
7	29	[illegible]	0.83	0.72	0.28	0.24	0.14	0.16	-	0.54	0.21	0.21
8	45	[illegible]	0.39	0.40	0.20	0.21	0.10	0.20	0.54	-	0.24	0.21
9	29	[illegible]	0.24	0.25	0.22	0.24	0.13	0.25	0.21	0.24	-	0.23
10	41	[illegible]	0.20	0.21	0.20	0.17	0.15	0.20	0.21	0.21	0.23	-

Fig. 8.4 Distance calculation of all conserved motifs described in Fig. 8.3. There is a very high level of similarity between motifs 1, 2, and 7 (marked in *red*), indicating that they are actually just one and the same motif. It can also be seen that motif 8 has 0.39–0.54 similarities with the other HMA motifs (Muhovic and Memon unpublished data)

Motif 3 was found to be the E1–E2 ATPase superfamily domain, Motif 4 corresponded to the ZntA, cation transport ATPase involved in inorganic ion transport and metabolism. Motifs 5 and 6 were not found in the conserved domain database. Motif 9 was found to belong to the COG4087 superfamily of soluble P-type ATPases, and Motif 10 was found to be the ATPase 1B, copper or silver translocating P-type ATPase [73].

8.5 Heavy Metal Chelation

To avoid the buildup of toxic metal ions plants catalyze redox reactions and alter the chemistry of the metal ions, when possible. Good examples are the reduction of Cr^{6+} to Cr^{3+} in *Eichornia crassipes* [74], and As^{5+} to As^{3+} in *B. juncea* [75]. Certain heavy metals do not occur in different oxidation states, and thus have to be detoxified by chelation. Chelation refers to the process in which a molecule encircles and binds to a metal, thus rendering in unreactive. The major ligands responsible for chelation are phytochelatins (PCs), metallothionein, organic acids, and amino acids [4, 15, 76]. It is assumed that most hyperaccumulated metals are ligand bound, and while some aspects of ligand detoxification have been discovered, a complete picture of the different chelators involved at different stages of the transport and storage process of metals, in plants, is still unknown.

8.5.1 *Phytochelatins*

Phytochelatins are a family of thiol-rich peptides, which consist of repetitions of γ-Glu-Cys dipeptide followed by a C-terminal glycine with the basic structure $(\gamma\text{-glu-cys})_n$-Gly $[(PC)_n]$ where n is in the range of 2 to 11 [24]. PCs production is catalyzed via the constitutive PC synthetase (PCS) in the presence of metals or metalloids from glutathione (γ-glutamylcysteinylglycine, GSH) [76, 77].

Overexpression of AtPCS1 increased PC levels 14 times, and improved both metal resistance and accumulation in *N. tabacum*, but the root-to-shoot translocation of Cd^{2+} remained unaffected [78]. While a similar study performed on wheat *Triticuma estivum* (TaPCS1) by Gong et al. [79] resulted in reduced metal accumulation in root tissues. Constitutive overexpression of TaPCS in shrub tobacco *N. glaucum* substantially increased its tolerance to Pb^{2+} and Cd^{2+} and greatly improved accumulation of Cu^{2+}, Zn^{2+}, Pb^{2+}, and Cd^{2+} in shoots. The overexpressed gene conferred up to 36 times greater Pb^{2+} accumulation, and 9 times greater Cd^{2+} accumulation in the shoots of the transgenic NgTP1 under hydroponic conditions. In some cases phytochelatin synthase activity has been used as a marker of metal pollution [80, 81]. The results with tobacco BY2 cells have shown that PCS activity is markedly elevated with increasing concentration of cadmium (II) ions. The lowest concentration of the toxic metal ions increased around threefold PCS activity as compared to control samples and this activity was increased seven times when cells were treated with high amount of Cd^{2+}.

Efficient overexpression of AtPCS1 in *A. thaliana*, conferred greatly increased levels of transcript and doubled production of PCs in the transgenic lines, but paradoxically caused the plants to be hypersensitive to Cd and Zn. This can be explained by the occurrence of a non-physiological decrease in the GSH pool due to supraoptimal synthesis of GSH, which would interfere with metal homeostasis [82]. Similarly Cd^{2+}-sensitive phenotype was observed in another *A. thaliana* transformed AtPCS1 line under the control of a strong actin A2 promoter. However, the PC overproduction in this case conferred a markedly improved resistance to arsenate in media and transgene on arsenate exposure exhibited greatly elevated γ-glutamlycysteine and GSH levels [83]. Results from various studies suggest that PCs are in general not essential for hyperaccumulating phenotypes, but seem to play a likely role in arsenic detoxification [84, 85]. Arsenic is possibly chelated by GSH and PCs and form As^{III}tris-thiolate complexes through thiolate bonds [84].

8.5.2 Metallothioneins (MT)

MTs are low molecular mass, cystein-rich proteins (4–8 KDa) that were originally isolated from animals. Their primary structure generally contains conserved CC CXC and CXXC motifs which enhances the ability of MTs to mono and divalent cations especially Cu, Zn, and Cd [86]. The role of plant MTs is generally attributed to the homeostasis of essential heavy metals, especially for copper [81]. Over 50 MTs have been reported in various plants, and in Arabidopsis genome all four type of metallothionein genes have been identified [81]. The expression levels of metallothionein family members vary between plant populations; this has been associated with variations in Cu tolerance [87]. MTs of types 1, 2, and 3 are Cu regulated, and seem to act in Cu accumulation and transport of Cu into phloem [79, 80]. Cd hyperaccumulating populations of *N. caerulescens* have been reported to have overexpressed levels of MT family members, when compared to

Arabidopsis. Type 3 MT was particularly strongly expressed and further induced by Cu addition and several studies suggest that NcMT3 may be involved in Cu homeostasis [88–90]. Type 4 or Ec pMTs are distinct from that of Types 1–3 in terms of their cysteine topology and majority of the research work has shown its role in Zn accumulation [86].

Several biophysical studies have suggested its role in discrimination between Zn and Cd. MT overexpression can increase a plant's metal tolerance and a wide variety of MTs from different organisms have been overexpressed in plants; however, these transgenic plant experiments have been limited to lab assessments only [91]. Currently, the proposed functions for MTs in plants include metal detoxification, involvement in development and a role in protection from abiotic stress. Further research is needed to fully understand the role of MTs in phytoextraction and how do they fit in with other detoxification mechanisms [81, 92].

8.5.3 Histidine

Histidine (His) is involved in the chelation of heavy metals and is the most important amino acid that plays a significant role in metal ion homeostasis in plants [93]. It is capable of forming stable complexes with heavy metals such as Ni, Zn, and Cd, and is present at high concentrations in hyperaccumulator roots [94, 95]. A dose-dependent increase in His in xylem sap in the Ni hyperaccumulator *Alyssum lesbiacum* was observed, which was not found in the nonhyperaccumulator congeneric species, *A. montanum*. When His was exogenously supplied, it did not increase the Ni tolerance and/or Ni loading into the xylem in *A. lesbiacum* probably due to the larger quantity of His already present in *A. lesbiuacum.* On the other hand, Ni loading in xylem and tolerance was increased in the non-accumulator species *A. montanu* [96]. His overproducing transgenic lines of *A. thaliana* displayed elevated Ni tolerance, but did not exhibit increased accumulation of Ni in xylem sap or leaves. This suggests that there are additional factors required for His-dependant Ni xylem loading in *A. thaliana*. The mechanism of His-coupled Ni xylem loading is yet unknown [97]. It has been recently shown that most of the Ni in xylem sap of hyperaccumulator Alyssum plants is present as hydrated Ni^{2+} cation [98, 99].

8.5.4 Nicotianamine

Nicotianamine (NA) is produced in all plants from 3 *S*-adenosyl-methionine (SAM) by NA synthase (NAS) and forms strong complexes with most transition metal ions. There is evidence of the involvement of NA in metal hyperaccumulation, as higher transcript levels of several *NAS* genes were observed in both *A. halleri* and

Noccaea caerulescens [66, 100]. When compared to *A. thaliana*, *A. halleri*, an accumulator plant has been shown to have a higher NA content together with higher *AhNAS*2 transcript and protein levels in roots, and higher transcript levels of *AhNAS*3 in the shoots. A study performed with Thlaspi (Noccaea) provides evidence that nicotianamine is involved in Ni hyperaccumulation in Thlaspi, but not of Zinc, and that Ni and Fe compete for NA complexation [95]. Mari et al. [101] provided a direct evidence for NA–Ni complex formation in the xylem sap in the roots of Ni exposed plants and its translocation in the aerial part of a *N. caerulescens*. This transport of NA, Ni, and NA–Ni chelates was absent in the non-tolerant non-hyperaccumulator species *T. arvense*. TcNAS is constitutively expressed in the leaves and its expression was never observed in roots, even after high Ni concentration treatment of the plants. The exposure of the plant to Ni triggered the accumulation of NA in the roots which is most probably the result of its translocation from the leaves. Recently, Deinlein et al. [70] showed that increased NA in the roots enhanced the NA-Zn^{2+} translocation from roots to shoots in hyperaccumulator plant *A. halleri*. NAS2 expression was elevated in *A. halleri* roots when grown both in a hydroponic system and in near natural conditions on native soil. Suppression of Ah-NAS2 by RNA interference (RNAi) resulted in a significant reduction in Zn hyperaccumulation in *A. halleri* roots. This indicates that probably NA enhances symplastic mobility of Zn (II) and is therefore a key molecule for the translocation of Zn within plants [70].

8.5.5 YSL (Yellow Strip1-Like) Family Proteins

YSL (Yellow Strip1-Like) family proteins have been shown to be involved in heavy metal translocation, by mediation the loading in and out of xylem of nicotinamine-metal chelates [102]. Three members of this family (TcYSL3, TcYSL5. and YSL7), are constitutively overexpressed in roots and shoots of *N. caerulescens*, where they participate in vascular loading and translocation of nicotinamine-metal (most frequently nicotinamine-Ni) complexes [23].

8.5.6 MATE (Multidrug and Toxin Efflux) Family

Another kind of transport proteins involved in heavy metal translocation in hyperaccumulator plants is the MATE (Multidrug and Toxin Efflux) family [23]. FDR3 (Ferric reductase defective 3), a member of this family is constitutively expressed in roots of *N. caerulescens* and *A. halleri* and is localized at the root pericycle plasma membrane. It is involved in the xylem influx of citrate, which is necessary as a ligand for Fe transport, but the overexpression of FDR3 in hyperaccumulators suggests that it may play a role in the translocation of other metals, such as Zinc [23, 66, 103].

8.6 Metal Tolerance Proteins (MTPs)

MTPs (CDF transporter family) in plants are involved in transport of metal ions from the cytoplasm to the vacuole [104], and to the apoplast and endoplasmic reticulum [105]. The family of cation diffusion facilitators (CDFs) is phylogenetically ubiquitous carrier type transporters [106]. In prokaryotes they are involved in the efflux of Zn, but also Fe, Ni, Co, and Cd [107–109]. Their major role is to maintain metal homeostasis in the cell but some members appear to be part of the cell's metal resistance apparatus (e.g., FieF in *E. coli* and DmeF in *C. metallidurans*) [108]. CDF transporters have also been identified and characterized in eukaryotes [110] and like in prokaryotes they also transport divalent metal cations such as Zn, Cd, Co, Fe, Ni, and Mn [111] In plants, all known MTPs are localized to intracellular membranes, especially the tonoplast [34]; however, recent evidence supports their localization in the plasma membrane [112], pre-vacuolar compartment, trans-Golgi, and ER membranes [105].

There are 12 CDF members (MTPs) which have been identified in Arabidopsis genome and have been grouped according to the metal specificity (AtMTP1-4, 5, 12 in Zn-group, AtMTP8-11 in Mn-group, AtMTP6 in Fe/Zn-group, and AtMTP7 with no metal specificity annotation) [105]. Ten MTP genes have been also identified in the genome of an important crop species rice and MTP genes have also been cloned from number of other plant species, such as TcZTP1 from *N. caerulescens* [5], TgMTP1 from *T. goesingense* [113] and ShMTP1 from *Stylosantheshamata*, which are shown to have a role in hyperaccumulation or tolerance to Zn, Ni, and Mn, respectively. Analysis of the rice genome has identified ten MTP genes [110]. Eukaryotic CDF transporters are characterized by six transmembrane domains (TMDs) with cytoplasmic N and C termini, and a histidine-rich region between trans-membrane domains IV and V [106] that may act as a sensor of metal concentration [114]. The most conserved amphipathic TMDs I, II, V, and VI regions are likely to be involved in metal transport [115]. The CDF family can be divided into four phylogenetic groups [106], but groups I and III are the most interesting in plants since these are the ones involved in metal tolerance and accumulation [104].

To date, the only characterized proteins in plants are members of group 1, which includes the Zn vacuolar transporters AtMTP1 and AtMTP3 [116, 117]; and from group 9, which includes the trans-Golgi/pre-vacuolar compartment-localized AtMTP11 [105]. AtMTP1 is shown to be involved in Zn transport into the vacuole and may represent a Zn tolerance mechanism. AtMTP3, another tonoplast transporter is also reported to be involved in the Zn transport into the vacuole [104] but has distinct expression pattern than that of AtMTP [117]. Silencing of AtMTP3 or AtMTP1 and the disruption of AtMTP1 by T-DNA insertion [114, 116] led to Zn sensitivity, which suggested that the silenced or mutant plants cannot compensate the lack of one gene by expressing the other one, and therefore AtMTP3 and AtMTP1 have non-redundant functions in Zn tolerance [117]. Comparative transcriptomic analysis of roots and shoots indicated that AhMTP1 transcripts are constitutively higher in *A. halleri* than in *A. thaliana*, especially in leaves [118, 119] and is able to complement the Zn-sensitive phenotype of the zrc1cot1 mutant yeast [119].

A. halleri genome has five MTP1 paralogs, located at four loci, and with one copy not fixed in the population analyzed (AhMTP1-D) [120]. AhMTP1-A1 and AhMTP1-A2 are duplicated in tandem and thus linked, whereas AhMTP1-B, AhMTP1-C, and AhMTP1-D are segmentally duplicated [120]. AhMTP1-A1/AhMTP1-A2 and AhMTP1-B loci are the copies associated with high accumulation of MTP1 transcripts in shoots, upregulated upon high Zn concentration in roots, and are related with Zn hypertolerance [119, 120]. Interestingly, although all five copies were able to confer Zn tolerance to zrc1cot1 mutant yeast, AhMTP1-B was less competent than AhMTP1-C and AhMTP1-D [120]. These results indicate that although duplication of MTP1 loci could be the basis of Zn tolerance in *A. halleri*, the five MTP genes seem to be undergoing distinct evolutionary fates [120]. These studies suggest that high copy numbers and enhanced expression of MTP proteins are likely the main characteristics responsible for hypertolerant/hyperaccumulator phenotypes [119, 120]. It appears that genes from metal hyperaccumulator species have no special features to distinguish them from those of crop plants, and that the molecular and biotechnological approaches aiming at biofortified crops or for phytoremediation would be similar when using genes obtained from either group of plants. A few MTP proteins have been fully functionally characterized and the subcellular localization of these characterized proteins is still not clear. Therefore, further work is required before the roles of all family members are fully understood.

8.7 Other Metal Transporters

CaCA (Ca^{2+}/Cation Antiporter) superfamilies member MHX, is a vacuolar Mg^{2+} and Zn^{2+}/H^+ exchanger, it is present in leaves of *A. halleri* at much higher concentrations when compared to *A. thaliana*, and is proposed to play a role in Zn vacuolar storage. Since the transcript levels were to be similar in both species, a difference in the post-transcriptional regulation was postulated [121]. Other members of the CaCA family may play roles in metal detoxification. CAX (CationeXchanger) is a large family of membrane proteins, divided into true CAX (CAX1-CAX6) and CCX (Calcium cation exchanger) [25, 122]. All members of the “true” CAX appear to be involved in vacuolar sequestration, in particular of Cd. Experimental evidence exists that AtCAX2 and AtCAX4 are capable of transporting Cd^{2+} into the vacuoles and the overexpression of these two genes resulted in higher accumulation of Cd in root vacuoles [123]. The ABC (ATP-binding cassette) superfamily contains some transporters involved in vacuolar sequestration of various metals and xenobiotics. The members of MRP and PRD subfamilies are involved in the transport of chelated heavy metals or organic acids necessary for heavy metal transport. Strong evidence exists for a role in trace metal homeostasis, and they are expected to play a role in vacuolar sequestration [124].

8.8 Vacuolar Sequestration

The detoxification of heavy metal ions in aerial parts of hyperaccumulators relies mostly on ligand binding and removal from cytoplasm by transporting the metals into inactive compartments, vacuoles, and cell walls [125]. The main storage site for metals is the vacuole, especially for Cd and Zn ions and in some metal hyperaccumulators, the compartmentalization of metals in the vacuole is an important part of their tolerance mechanism. For example, most of the Ni in Ni hyperaccumulator *T. goesingense* was compartmentalized into leaf vacuole [126] and the high expression level of metal ion transporter TgMTP1 (Metal Tolerance Protein) is considered to be the main factor responsible for high metal accumulation in shoot vacuoles [113]. For example, efficient sequestration of Zn and Cd into the shoot vacuoles by MTP1 transporter seems to play a role in hypertolerance as well as in hyperaccumulation [111, 127, 128]. The MTP1 family was characterized at both the genomic and functional levels in *A. halleri* and displayed increase in gene copy number in *A. halleri* compared to *A. thaliana* or in *A. lyrata* [116, 117]. When ectopically overexpressed in *A. thaliana*, AtMTP1 confers enhanced zinc tolerance [129]. A single copy of MTP1 was present in *A. thaliana* and interestingly five paralogs were found to be present in *A. halleri*, AhMTP1-A1, -A2, -B, -C, and -D. These five paralogs shared only 91–93 % identity with their *A. thaliana* and *A. lyrata* orthologs [119, 120].

MTP3 is another vacuolar transporter and is induced by elevated levels of Zn and confers enhanced Zn tolerance in Arabidopsis roots [117]. AtMTP3 and AtMTP1 are functionally similar genes, but not redundant. AtMTP3 has a distinct expression patterns to that of AtMTP1 and shares 67.6 % sequence identity with the AtMTP1 [116, 117]. At protein level both have similar predicted secondary structure with six membrane domains, facing N-terminal and C-terminal ends toward cytoplasm and a histidine-rich region [111]. Both transporters are localized in the tonoplast membrane in yeast and plants but their spatial, temporal, and metal-responsive transcriptional regulation in the cell suggest that these transporters have different roles in plant Zn homeostasis [111, 125]. The vacuolar sequestration trait relies, at least partly, on constitutive overexpression of genes that encode proteins that transfer heavy metal across the tonoplast and are involved in excluding heavy metals from the cytoplasm.

8.9 Role of MicroRNAs in Metal Stress

MicroRNAs (miRNA) are endogenous non-coding small RNAs usually consisting of ~20–22 nucleotides for animals and ~20–24 nt for plants [130, 131]. They function as post-transcriptional regulators in eukaryotes and were reported to have crucial roles in the modulation of gene expression in plants [132]. miRNA genes are transcribed and excised into miRNAs and then recruited by RISC (RNA-induced silencing complex, including argonaute and other proteins) which combines with mRNA to inhibit or degrade the target mRNA [131]. The regulation of target gene expression via miRNAs is critical for numerous biological processes in both plants and animals [133–137].

Two major classes of small RNAs have been reported in plants and they are microRNAs (miRNAs) and small interfering RNAs (siRNAs). miRNAs are processed from single-stranded RNA precursors capable of forming imperfectly complementary hairpin structures by the RNase III enzyme DICER-LIKE1 (DCL1) or DCL4. They are known to base-pair their target mRNAs to repress their translation or induce their degradation in organisms [131, 136]. In plants only the mature miRNAs are conserved instead of miRNA precursors that are usually conserved in animals [131]. In plants miRNA-directed target cleavage can sometimes stimulate the production of dsRNA which is subsequently processed by a Dicer protein to produce siRNAs referred to as trans-acting siRNAs (ta-siRNAs) [138, 139].

MicroRNAs (miRNAs) are an integral component of the stress response network and regulate the expression of their target protein coding genes, many of which encode transcription factors that regulate the expression of other genes Liu et al. [140] have presented an online database for *Arabidopsis thaliana* miRNA function annotations which integrate various type of datasets, including miRNA-target interactions, transcription factor (TF) and their targets, expression profiles, genomic annotations and pathways, into a comprehensive database, and developed various statistical and mining tools, together with a user-friendly web interface. This database can serve as a comprehensive resource for exploring the functions of *Arabidopsis thaliana* miRNAs. Other databases like PASmiR database have also been developed [141] to provide a solid platform for collection, standardization, and searching of miRNA abiotic stress regulation data in plants. PASmiR database includes data from ~200 published studies, representing 1038 regulatory relationships between 682 miRNAs and 35 abiotic stresses in 33 plant species and is a comprehensive repository for miRNA regulatory mechanisms involved in plant response to abiotic stresses and is extremely useful for the plant stress physiology community.

A significant fraction of miRNAs are expressed when plants are subjected to a variety of abiotic and biotic stresses, such as drought [142, 143], salinity [144, 145], cold [146, 147], heat [148, 149], nutritional deficiency [150], oxidative stress [151], and heavy metal stress [152–155]. Recently, microRNAs (miRNAs) are emerged as important modulators of plant-adaptive response to heavy metal stress, and a detailed list of miRNAs involved in metal toxicity tolerance is given by [156]. A total of 19 Cd-responsive miRNAs were identified in rice and some of these miRNAs were targeted to the genes which encoded transcription factors, and proteins associated with metabolic processes or stress responses [152]. Zhou et al. [154] identified 84 conserved and non-conserved miRNAs (belonging to 37 miRNA families) from Cd-treated and non-treated *B. napus*, including 19 miRNA members that were not identified before. Most of the identified miRNAs were found to be differentially expressed in roots/shoots or regulated by Cd exposure. Using Solexa sequencing technology, Xu et al. have constructed two small RNA libraries from Cd-treated and Cd-free roots of radish seedlings [157] and several key responsive proteins or enzymes for heavy metal uptake and translocation were identified as target transcripts for a few conserved miRNAs. For example, rsa-miR156 targeted a transcript encoding a glutathione *S*-transferase 5 (GST5), whereas rsa-miR393 targeted phytochelatin synthase1 (PCS1). These data suggest that rsa-miR156 and rsa-miR393 could be involved in Cd detoxification and mediation via directing regulation of the GST5 and PCS1 genes in radish, respectively. miR395 is another conserved small RNA which regulates sulfate assimilation and distribution in many plant species including *Arabidopsis thaliana*, *B. napus*, and rice (Jone-Rhoades and Bartel 2004); [158, 159]. Recent data indicate that it is also involved in Cd detoxification in *B. napus*, possibly by overaccumulating the sulfate in their shoots from the soil [154]. Moreover, iron transporter-like protein and ABC transporter protein were shown to be targeted by rsa-miR159 and rsa-miR166, respectively [160]. These findings indicate that rsa-miR159 and rsa-miR166 might play an important role in Cd uptake and translocation in plants through regulating their corresponding targets.

Using the miRNA microarray approach, a total of nine miRNA families (miR156, miR162, miR168, miR166, miR171, miR396, miR390, miR1432, and miR444) were shown to be downregulated under Cd stress, whereas only miR528 was significantly upregulated in rice [152]. In *M. truncatula*, miR171, miR319, miR393, and miR529 were upregulated, whereas miR166 and miR398 were downregulated under Cd stress using a qRT-PCR-based assay [154]. miR398 is shown to regulate copper homeostasis in Arabidopsis via downregulating the expression of Cu, Zn-superoxide dismutase (CSD), a scavenger of superoxide radicals [152]. The downregulation of miR398 expression is important for the induction of CSD1 and CSD2 mRNA levels under high Cu stress [151]. Transcription factors are generally the targets for plant metal-responsive miRNAs. For example, TCP transcription factor is the target of miR319 which is implicated in growth control. miR390 exerts its action through the biogenesis of trans-acting small interference RNAs that, in turn, regulate auxin-responsive factors and miR393 targets the auxin receptors TIR1/AFBs and a bHLH transcription factor [161]. It is clear from the above findings that microRNAs are crucial components of the gene regulatory network through their negative regulation of target genes. Now question arises that whether these identified metal-regulated miRNAs are specifically altered in their gene expression for adjustment and tolerance to the metal stress or these alterations in miRNA expression are secondary consequences of a disturbed cellular homeostasis due to the metal stress, remains to be uncovered in future studies. Further studies with miRNAs and their role in metal accumulation and tolerance will enhance our understanding of plant responses to metal stress.

8.10 Plant Improvement Strategies for Enhanced Phytoextraction

A worldwide search is ongoing for identifying the perfect hyperaccumulator plant/s; currently known hyperaccumulators are plagued by many different limiting factors that prevent their efficient use for phytoextraction such as slow growth rate, small biomass, metal selectivity and being usable only in their natural habitats [162].

The genetic studies with slow growing hyperaccumulator plants has given us the greater understanding of the heavy metal accumulation mechanisms and tremendous knowledge about the genes suitable for overexpression and transfer between species. Now plant breeders and geneticists can utilize different bioengineering tools to enhance the metal phytoextraction potential of non-accumulator plants by transfer of metal hyperaccumulator genes to high biomass species.

8.11 Conclusion

Many genes are involved in the hyperaccumulation pathway, whether it is in metal uptake, translocation, sequestration, or detoxification. Various studies have been done where the overexpression of phytochelatin synthase, metallothionein genes, and transporter genes involved in hyperaccumulation was performed with mixed results. A number of strategies however have emerged for creating transgenics with suitable phytoremediation properties, the most simple and direct one is the overexpression of the genes that are involved in the metabolism, uptake and transport of heavy metals. These genes can be introduced to plants by using *Agrobacterium tumefaciens*-mediated plant transformation. *B. juncea* and *B. nigra* will be the suitable candidates for creating a high-biomass hyperaccumulator plants by introducing metal transport, and accumulator genes through current biotechnological methods. For example, the members of the ZIP family genes would make a good target for modification of root uptake of Cd, Zn, and Ni [30]. Modification of enzymes involved in the PC production is another promising approach to increasing metal tolerance and accumulation, and this approach has already been tried with mixed results [163, 164].

To enhance the root-to-shoot translocation (a hallmark of efficient hyperaccumulators), HMA4 is one of the most important candidate genes for hyperaccumulation, as multiple lines of research have provided valid evidence for its role in xylem loading of heavy metals, and shown its crucial role in metal hyperaccumulation in *A. halleri* [67]. MTPs can also be used for the development of transgenic crops that are more tolerant of metal deficient soils, producing more resistant crops. All the genes involved in the metal accumulation process make good targets for future research and development of transgenic lines for phytoextraction. However, it is clear that the introduction of a single gene will not result in a perfect hyperaccumulator and further research into the metal accumulation pathway is necessary to understand the common mechanisms in all hyperaccumulators.

MicroRNAs (miRNAs) are a distinct class of small RNAs in plants which play a crucial role in several biological processes including environmental stresses in several plant species. The identification and analysis of miRNAs responsive to different metal toxicities has provided us sufficient information to understand the regulation of several enzymes and proteins involved in metal tolerances. For example, by combining computational and several laboratory approaches sufficient evidences have been produced concerning active involvement of miRNAs during toxicity response by regulating various transcription factors and protein coding genes involved in plant growth and development. Further research is needed to elucidate the role of miRNAs and their targets, mainly transcription factors in signaling pathways of plant responses to environmental changes. A better understanding of the role of miRNAs during metal stress will contribute to the better design of strategies aimed at improving stress tolerance of crop plants.

The metal biofortification of metals has significant implications in the nutrition of humans as well as plants. The human mineral deficiencies are spread worldwide and most common form of human micronutrient malnutrition is iron deficiency affecting more than two billion people, predominantly in countries where cereals are the staple food [165]. Through modern biotechnology edible crops such as rice, wheat, and barley or vegetables can be made to contain more of the essential nutrients, like iron and zinc. This process can have a serious impact on improving nutrition throughout the developing world (e.g., golden rice) [166]. Introducing the selective MTP and HMA genes can potentially give rise to cereals with an increased nutritional value, by the production of transgenic seeds with an elevated micronutrient uptake.

References

1. Kabata-Pedias A (2010) Trace elements in soils and plants. CRC Press, Boca Raton
2. Baker A, Brooks R (1989) Terrestrial higher plants which hyperaccumulate metallic elements—a review of their distribution, ecology and phytochemistry. Biorecovery 1:81
3. Krämer U (2010) Metal hyperaccumulation in plants. Annu Rev Plant Biol 61(1):517–534
4. Verbruggen N, Hermans C, Schat H (2009) Molecular mechanisms of metal hyperaccumulation in plants. New Phytol 181(4):759–776
5. Assunção AGL, Schat H, Aarts MGM (2003) Thlaspi caerulescens, an attractive model species to study heavy metal hyperaccumulation in plants. New Phytol 159(2):351–360
6. Reeves RD, Kruckeberg AR, Adıgüzel N et al (2001) Studies on the flora of serpentine and other metalliferous areas of western Turkey. South Afr J Sci 97:513
7. Baker A, McGrath SP, Reeves RD, Smith JAC (2000) Metal hyperaccumulator plants: a review of the ecology and physiologyof a biochemical resource for phytoremediation of metal polluted soils. In: Terry N, Banuelos G, Vangronsveld J (eds) Phytoremediation of contaminated soil and water. Lewis, Boca Raton
8. Lombi E, Zhao FJ, Dunham SJ, McGrath SP (2001) Phytoremediation of heavy metal-contaminated soils: natural hyperaccumulation versus chemically enhanced phytoextraction. J Environ Qual 30(6):1919–1926, Epub 2002/01/16. eng
9. Salt DE, Blaylock M, Kumar NP, Dushenkov V, Ensley BD, Chet I et al (1995) Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants. Biotechnology (N Y) 13(5):468–474, Epub 1995/05/01. eng
10. Raskin II, Smith RD, Salt DE (1997) Phytoremediation of metals: using plants to remove pollutants from the environment. Curr Opin Biotechnol 8(2):221–226, Epub 1997/04/01. Eng
11. Reeves RD, Baker AJM (2000) Metal accumulating plants. In: Raskin I, Ensley BD (eds) Phytoremediation of toxic metals: using plants to clean up the environment. Wiley, New York
12. Memon AR, Ito S, Yatazawa M (1979) Absorbtion and accumulation of iron, manganese and copper in plants in the temperate forest of central Japan. Soil Sci Plant Nutr 25:611–620
13. Memon AR, Ito S, Yatazawa D (1980) Distribution of zinc and cadmium in temperate forest taxa of central Japan. Soil Sci Plant Nutr 26:281–290

14. Memon AR, Ito S, Yatazawa M (1980) Taxonomic characteristics in accumulating cobalt and nickle in temperate forest vegetation of central Japan. Soil Sci Plant Nutr 26:271–280
15. Memon AR, Schroder P (2009) Implications of metal accumulation mechanisms to phytoremediation. Environ Sci Pollut Res Int 16(2):162–175, Epub 2008/12/11. eng
16. Ent A, Baker AM, Reeves R, Pollard AJ, Schat H (2013) Hyperaccumulators of metal and metalloid trace elements: facts and fiction. Plant Soil 362(1–2):319–334
17. Pollard AJ, Powell KD, Harper FA, Smith JAC (2002) The genetic basis of metal hyperaccumulation in plants. Crit Rev Plant Sci 21(6):539–566
18. Wu Y, Wu G, Xiao L, Lu C (2007) Event-specific qualitative and quantitative PCR detection methods for transgenic rapeseed hybrids MS1xRF1 and MS1xRF2. J Agric Food Chem 55(21):8380–8389, Epub 2007/09/26. eng
19. Grispen VMJ, Hakvoort HWJ, Bliek T, Verkleij JAC, Schat H (2011) Combined expression of the Arabidopsis metallothionein MT2b and the heavy metal transporting ATPase HMA4 enhances cadmium tolerance and the root to shoot translocation of cadmium and zinc in tobacco. Environ Exp Bot 72(1):71–76
20. Lang M, Hao M, Fan Q, Wang W, Mo S, Zhao W et al (2011) Functional characterization of BjCET3 and BjCET4, two new cation-efflux transporters from Brassica juncea L. J Exp Bot 62(13):4467–4480, Pubmed Central PMCID: 3170545, Epub 2011/06/10. eng
21. National center for biotechnology information
22. Landberg T, Greger M (1996) Differences in uptake and tolerance to heavy metals in Salix from unpolluted and polluted areas. Appl Geochem 11(1–2):175–180
23. Rascio N, Navari-Izzo F (2011) Heavy metal hyperaccumulating plants: how and why do they do it? And what makes them so interesting? Plant Sci 180(2):169–181, Epub 2011/03/23. eng
24. Memon AR, Aktoprakligil D, Özdemir A, Vertii A (2001) Heavy metal accumulation and detoxification mechanisms in plants. Turk J Bot 25(3):111–121
25. Cai X, Lytton J (2004) The cation/Ca(2+) exchanger superfamily: phylogenetic analysis and structural implications. Mol Biol Evol 21(9):1692–1703
26. Blaylock MJ, Huang JW (2000) Phytoextraction of metals. In: Raskin I, Ensley BD (eds) Phytoremediation of toxic metals: using plants to clean up the environment. Wiley, New York
27. Marschner P (2012) Marschner's mineral nutrition of higher plants. Elsevier, Amsterdam
28. Lin Y-F, Aarts MM (2012) The molecular mechanism of zinc and cadmium stress response in plants. Cell Mol Life Sci 69(19):3187–3206
29. Gaither LA, Eide D (2001) Eukaryotic zinc transporters and their regulation. Biometals 14(3–4):251–270
30. Hammond JP, Bowen HC, White PJ, Mills V, Pyke KA, Baker AJM et al (2006) A comparison of the Thlaspi caerulescens and Thlaspi arvense shoot transcriptomes. New Phytol 170(2):239–260
31. Weber M, Harada E, Vess C, Roepenack-Lahaye E, Clemens S (2004) Comparative microarray analysis of Arabidopsis thaliana and Arabidopsis halleri roots identifies nicotianamine synthase, a ZIP transporter and other genes as potential metal hyperaccumulation factors. Plant J 37(2):269–281
32. Lasat MM, Pence NS, Garvin DF, Ebbs SD, Kochian LV (2000) Molecular physiology of zinc transport in the Zn hyperaccumulator Thlaspi caerulescens. J Exp Bot 51(342):71–79, Epub 2000/08/12. eng
33. Plaza S, Tearall KL, Zhao F-J, Buchner P, McGrath SP, Hawkesford MJ (2007) Expression and functional analysis of metal transporter genes in two contrasting ecotypes of the hyperaccumulator Thlaspi caerulescens. J Exp Bot 58(7):1717–1728
34. Hall JL, Williams LE (2003) Transition metal transporters in plants. J Exp Bot 54(393):2601–2613
35. Sánchez-Fernández R, Davies TGE, Coleman JOD, Rea PA (2001) The Arabidopsis thaliana ABC protein superfamily, a complete inventory. J Biol Chem 276(32):30231–30244
36. Noh B, Murphy AS, Spalding EP (2001) Multidrug resistance–like genes of Arabidopsis required for Auxin transport and Auxin-mediated development. Plant Cell 13(11):2441–2454

37. Campbell EJ, Schenk PM, Kazan K, Penninckx IAMA, Anderson JP, Maclean DJ et al (2003) Pathogen-responsive expression of a putative ATP-binding cassette transporter gene conferring resistance to the Diterpenoid Sclareol is regulated by multiple defense signaling pathways in Arabidopsis. Plant Physiol 133(3):1272–1284
38. Geisler M, Blakeslee JJ, Bouchard R, Lee OR, Vincenzetti V, Bandyopadhyay A et al (2005) Cellular efflux of auxin catalyzed by the Arabidopsis MDR/PGP transporter AtPGP1. Plant J 44(2):179–194
39. Kim D-Y, Bovet L, Kushnir S, Noh EW, Martinoia E, Lee Y (2006) AtATM3 is involved in heavy metal resistance in Arabidopsis. Plant Physiol 140(3):922–932
40. David-Assael O, Berezin I, Shoshani-Knaani N, Saul H, Mizrachy-Dagri T, Chen J et al (2006) AtMHX is an auxin and ABA-regulated transporter whose expression pattern suggests a role in metal homeostasis in tissues with photosynthetic potential. Funct Plant Biol 33(7):661–672
41. Robinson B, Fernández J-E, Madejón P, Marañón T, Murillo J, Green S et al (2003) Phytoextraction: an assessment of biogeochemical and economic viability. Plant Soil 249(1):117–125
42. Salt DE, Smith RD, Raskin I (1998) Phytoremediation. Annu Rev Plant Physiol Plant Mol Biol 49:643–668, Epub 2004/03/12. Eng
43. Ueno D, Koyama E, Kono I, Ando T, Yano M, Ma JF (2009) Identification of a novel major quantitative trait locus controlling distribution of Cd between roots and shoots in rice. Plant Cell Physiol 50(12):2223–2233
44. Lasat MM (2002) Phytoextraction of toxic metals: a review of biological mechanisms. J Environ Qual 31(1):109–120, Epub 2002/02/12. eng
45. Yang XE, Li TQ, Long XX, Xiong YH, He ZL, Stoffella PJ (2006) Dynamics of zinc uptake and accumulation in the hyperaccumulating and non-hyperaccumulating ecotypes of Sedum alfredii Hance. Plant Soil 284(1–2):109–119
46. Hanikenne M, Nouet C (2011) Metal hyperaccumulation and hypertolerance: a model for plant evolutionary genomics. Curr Opin Plant Biol 14(3):252–259
47. Mills RF, Krijger GC, Baccarini PJ, Hall JL, Williams LE (2003) Functional expression of AtHMA4, a P1B-type ATPase of the Zn/Co/Cd/Pb subclass. Plant J 35(2):164–176
48. Siemianowski O, Mills RF, Williams LE, Antosiewicz DM (2011) Expression of the P(B)-type ATPase AtHMA4 in tobacco modifies Zn and Cd root to shoot partitioning and metal tolerance. Plant Biotechnol J 9(1):64–74, Epub 2010/05/25. eng
49. Kuhlbrandt W (2004) Biology, structure and mechanism of P-type ATPases. Nat Rev Mol Cell Biol 5(4):282–295
50. Argüello J, Eren E, González-Guerrero M (2007) The structure and function of heavy metal transport P1B-ATPases. BioMetals 20(3–4):233–248
51. Moreno I, Norambuena L, Maturana D, Toro M, Vergara C, Orellana A et al (2008) AtHMA1 is a Thapsigargin-sensitive Ca2+/heavy metal pump. J Biol Chem 283(15):9633–9641
52. Kim YY, Choi H, Segami S, Cho HT, Martinoia E, Maeshima M et al (2009) AtHMA1 contributes to the detoxification of excess Zn(II) in Arabidopsis. Plant J 58(5):737–753
53. Hussain D, Haydon MJ, Wang Y, Wong E, Sherson SM, Young J et al (2004) P-type ATPase heavy metal transporters with roles in essential zinc homeostasis in Arabidopsis. Plant Cell 16(5):1327–1339, Pubmed Central PMCID: 423219, Epub 2004/04/22. eng
54. Morel M, Crouzet J, Gravot A, Auroy P, Leonhardt N, Vavasseur A et al (2009) AtHMA3, a P1B-ATPase allowing Cd/Zn/Co/Pb vacuolar storage in Arabidopsis. Plant Physiol 149(2):894–904
55. Ueno D, Milner MJ, Yamaji N, Yokosho K, Koyama E, Clemencia Zambrano M et al (2011) Elevated expression of TcHMA3 plays a key role in the extreme Cd tolerance in a Cd-hyperaccumulating ecotype of Thlaspi caerulescens. Plant J 66(5):852–862
56. Willems G, Dräger DB, Courbot M, Godé C, Verbruggen N, Saumitou-Laprade P (2007) The genetic basis of zinc tolerance in the metallophyte Arabidopsis halleri ssp. halleri (Brassicaceae): an analysis of quantitative trait loci. Genetics 176(1):659–674

57. Zorrig W, Abdelly C, Berthomieu P (2011) The phylogenetic tree gathering the plant Zn/Cd/Pb/Co P1B-ATPases appears to be structured according to the botanical families. C R Biol 334(12):863–871
58. Yamaji N, Xia J, Mitani-Ueno N, Yokosho K, Feng Ma J (2013) Preferential delivery of zinc to developing tissues in rice is mediated by P-type heavy metal ATPase OsHMA2. Plant Physiol 162(2):927–939
59. Miyadate H, Adachi S, Hiraizumi A, Tezuka K, Nakazawa N, Kawamoto T et al (2011) OsHMA3, a P1B-type of ATPase affects root-to-shoot cadmium translocation in rice by mediating efflux into vacuoles. New Phytol 189(1):190–199
60. Tehseen M, Cairns N, Sherson S, Cobbett CS (2010) Metallochaperone-like genes in Arabidopsis thaliana. Metallomics 2(8):556–564
61. Andrés-Colás N, Perea-García A, Puig S, Peñarrubia L (2010) Deregulated copper transport affects Arabidopsis development especially in the absence of environmental cycles. Plant Physiol 153(1):170–184
62. Luhua S, Hegie A, Suzuki N, Shulaev E, Luo X, Cenariu D et al (2013) Linking genes of unknown function with abiotic stress responses by high-throughput phenotype screening. Physiol Plant 148(3):322–333
63. de Abreu-Neto JB, Turchetto-Zolet AC, de Oliveira LFV, Bodanese Zanettini MH, Margis-Pinheiro M (2013) Heavy metal-associated isoprenylated plant protein (HIPP): characterization of a family of proteins exclusive to plants. FEBS J 280(7):1604–1616
64. Tian H, Baxter IR, Lahner B, Reinders A, Salt DE, Ward JM (2010) Arabidopsis NPCC6/NaKR1 is a phloem mobile metal binding protein necessary for phloem function and root Meristem maintenance. Plant Cell 22(12):3963–3979
65. Wu G, Kang H, Zhang X, Shao H, Chu L, Ruan C (2010) A critical review on the bio-removal of hazardous heavy metals from contaminated soils: issues, progress, eco-environmental concerns and opportunities. J Hazard Mater 174(1–3):1–8, Epub 2009/10/30. eng
66. Talke IN, Hanikenne M, Krämer U (2006) Zinc-dependent global transcriptional control, transcriptional deregulation, and higher gene copy number for genes in metal homeostasis of the hyperaccumulator Arabidopsis halleri. Plant Physiol 142(1):148–167
67. Hanikenne M, Baurain D (2014) Origin and evolution of metal p-Type ATPases in Plantae (Archaeplastida). Front Plant Sci 4:544
68. Sinclair SA, Krämer U (2012) The zinc homeostasis network of land plants. Biochim Biophys Acta 1823(9):1553–1567
69. Richau KH, Kozhevnikova AD, Seregin IV, Vooijs R, Koevoets PL, Smith JA et al (2009) Chelation by histidine inhibits the vacuolar sequestration of nickel in roots of the hyperaccumulator Thlaspi caerulescens. New Phytol 183(1):106–116
70. Deinlein U, Weber M, Schmidt H, Rensch S, Trampczynska A, Hansen TH et al (2012) Elevated nicotianamine levels in Arabidopsis halleri roots play a key role in zinc hyperaccumulation. Plant Cell 24(2):708–723
71. Bailey TL, Williams N, Misleh C, Li WW (2006) MEME: discovering and analyzing DNA and protein sequence motifs. Nucleic Acids Res 34(Suppl 2):W369–W373
72. Bailey TL, Boden M, Buske FA, Frith M, Grant CE, Clementi L et al (2009) MEME SUITE: tools for motif discovery and searching. Nucleic Acids Res 37(Web Server issue):W202–W208, Pubmed Central PMCID: 2703892
73. Marchler-Bauer A, Lu S, Anderson JB, Chitsaz F, Derbyshire MK, DeWeese-Scott C et al (2011) CDD: a Conserved Domain Database for the functional annotation of proteins. Nucleic Acids Res 39(Database issue):D225–D229, Pubmed Central PMCID: 3013737
74. Lytle CM, Lytle FW, Yang N, Qian J-H, Hansen D, Zayed A et al (1998) Reduction of Cr(VI) to Cr(III) by wetland plants: potential for in situ heavy metal detoxification. Environ Sci Technol 32(20):3087–3093
75. Pickering IJ, Prince RC, George MJ, Smith RD, George GN, Salt DE (2000) Reduction and coordination of arsenic in Indian mustard. Plant Physiol 122(4):1171–1177, Pubmed Central PMCID: 58951. Epub 2000/04/12. eng

76. Cobbett CS (2000) Phytochelatins and their roles in heavy metal detoxification. Plant Physiol 123(3):825–832
77. Yadav SK (2010) Heavy metals toxicity in plants: an overview on the role of glutathione and phytochelatins in heavy metal stress tolerance of plants. South Afr J Bot 76(2):167–179
78. Pomponi M, Censi V, Di Girolamo V, De Paolis A, di Toppi LS, Aromolo R et al (2006) Overexpression of Arabidopsis phytochelatin synthase in tobacco plants enhances Cd(2+) tolerance and accumulation but not translocation to the shoot. Planta 223(2):180–190, Epub 2005/09/01. eng
79. Gong J-M, Lee DA, Schroeder JI (2003) Long-distance root-to-shoot transport of phytochelatins and cadmium in Arabidopsis. Proc Natl Acad Sci 100(17):10118–10123
80. Guo W-J, Meetam M, Goldsbrough PB (2008) Examining the specific contributions of individual Arabidopsis metallothioneins to copper distribution and metal tolerance. Plant Physiol 146(4):1697–1706
81. Cobbett C, Goldsbrough P (2002) Phytochelatins and metallothioneins: roles in heavy metal detoxification and homeostasis. Annu Rev Plant Biol 53:159–182
82. Lee J, Bae H, Jeong J, Lee JY, Yang YY, Hwang I et al (2003) Functional expression of a bacterial heavy metal transporter in Arabidopsis enhances resistance to and decreases uptake of heavy metals. Plant Physiol 133(2):589–596, Pubmed Central PMCID: 219035, Epub 2003/09/27. eng
83. Li Y, Dhankher OP, Carreira L, Lee D, Chen A, Schroeder JI et al (2004) Overexpression of phytochelatin synthase in Arabidopsis leads to enhanced arsenic tolerance and cadmium hypersensitivity. Plant Cell Physiol 45(12):1787–1797
84. Schmöger MEV, Oven M, Grill E (2000) Detoxification of Arsenic by phytochelatins in plants. Plant Physiol 122(3):793–802
85. Gasic K, Korban S (2007) Transgenic Indian mustard (Brassica juncea) plants expressing an Arabidopsis phytochelatin synthase (AtPCS1) exhibit enhanced As and Cd tolerance. Plant Mol Biol 64(4):361–369
86. Leszczyszyn OI, Imam HT, Blindauer CA (2013) Diversity and distribution of plant metallothioneins: a review of structure, properties and functions. Metallomics 5(9):1146–1169
87. Murphy A, Taiz L (1995) Comparison of metallothionein gene expression and nonprotein thiols in ten Arabidopsis ecotypes. Correlation with copper tolerance. Plant Physiol 109(3):945–954, Pubmed Central PMCID: 161396
88. Roosens NH, Bernard C, Leplae R, Verbruggen N (2004) Evidence for copper homeostasis function of metallothionein (MT3) in the hyperaccumulator Thlaspi caerulescens. FEBS Lett 577(1–2):9–16
89. Roosens NH, Leplae R, Bernard C, Verbruggen N (2005) Variations in plant metallothioneins: the heavy metal hyperaccumulator Thlaspi caerulescens as a study case. Planta 222(4):716–729
90. Roosens NH, Bernard C, Leplae R, Verbruggen N (2005) Adaptative evolution of metallothionein 3 in the Cd/Zn hyperaccumulator Thlaspi caerulescens. Z Naturforsch C 60(3–4):224–228
91. Zhigang A, Cuijie L, Yuangang Z, Yejie D, Wachter A, Gromes R et al (2006) Expression of BjMT2, a metallothionein 2 from Brassica juncea, increases copper and cadmium tolerance in Escherichia coli and Arabidopsis thaliana, but inhibits root elongation in Arabidopsis thaliana seedlings. J Exp Bot 57(14):3575–3582
92. Yang Z, Wu Y, Li Y, Ling HQ, Chu C (2009) OsMT1a, a type 1 metallothionein, plays the pivotal role in zinc homeostasis and drought tolerance in rice. Plant Mol Biol 70(1–2):219–229
93. Stepansky A, Leustek T (2006) Histidine biosynthesis in plants. Amino Acids 30(2):127–142
94. Persans MW, Yan X, Patnoe JM, Kramer U, Salt DE (1999) Molecular dissection of the role of histidine in nickel hyperaccumulation in Thlaspi goesingense (Halacsy). Plant Physiol 121(4):1117–1126, Pubmed Central PMCID: 59479
95. Callahan DL, Baker AJ, Kolev SD, Wedd AG (2006) Metal ion ligands in hyperaccumulating plants. J Biol Inorg Chem 11(1):2–12

96. Kerkeb L, Kramer U (2003) The role of free histidine in xylem loading of nickel in Alyssum lesbiacum and Brassica juncea. Plant Physiol 131(2):716–724, Pubmed Central PMCID: 166847
97. Wycisk K, Kim EJ, Schroeder JI, Kramer U (2004) Enhancing the first enzymatic step in the histidine biosynthesis pathway increases the free histidine pool and nickel tolerance in Arabidopsis thaliana. FEBS Lett 578(1–2):128–134
98. Alves S, Nabais C, Simoes Goncalves Mde L, Correia Dos Santos MM (2011) Nickel speciation in the xylem sap of the hyperaccumulator Alyssum serpyllifolium ssp. lusitanicum growing on serpentine soils of northeast Portugal. J Plant Physiol 168(15):1715–1722
99. Centofanti T, Sayers Z, Cabello-Conejo M, Kidd P, Nishizawa N, Kakei Y et al (2013) Xylem exudate composition and root-to-shoot nickel translocation in Alyssum species. Plant Soil 373(1–2):59–75
100. Mandakova T, Singh V, Kramer U, Lysak MA (2015) Genome structure of the heavy metal hyper accumulator Noccaea caerulescens and its ability on metalliferous and nonmetalliferous soils. Plant Physiol 169(1):674–689
101. Mari S, Gendre D, Pianelli K, Ouerdane L, Lobinski R, Briat J-F et al (2006) Root-to-shoot long-distance circulation of nicotianamine and nicotianamine–nickel chelates in the metal hyperaccumulator Thlaspi caerulescens. J Exp Bot 57(15):4111–4122
102. Colangelo EP, Guerinot ML (2006) Put the metal to the petal: metal uptake and transport throughout plants. Curr Opin Plant Biol 9(3):322–330
103. van de Mortel JE, Almar Villanueva L, Schat H, Kwekkeboom J, Coughlan S, Moerland PD et al (2006) Large expression differences in genes for iron and zinc homeostasis, stress response, and lignin biosynthesis distinguish roots of Arabidopsis thaliana and the related metal hyperaccumulator Thlaspi caerulescens. Plant Physiol 142(3):1127–1147
104. Kramer U, Talke IN, Hanikenne M (2007) Transition metal transport. FEBS Lett 581(12):2263–2272
105. Peiter E, Montanini B, Gobert A, Pedas P, Husted S, Maathuis FJ et al (2007) A secretory pathway-localized cation diffusion facilitator confers plant manganese tolerance. Proc Natl Acad Sci U S A 104(20):8532–8537, Pubmed Central PMCID: 1895984, Epub 2007/05/15. eng
106. Mäser P, Thomine S, Schroeder JI, Ward JM, Hirschi K, Sze H et al (2001) Phylogenetic relationships within cation transporter families of Arabidopsis. Plant Physiol 126(4):1646–1667
107. Anton A, Weltrowski A, Haney CJ, Franke S, Grass G, Rensing C et al (2004) Characteristics of zinc transport by two bacterial cation diffusion facilitators from Ralstonia metallidurans CH34 and Escherichia coli. J Bacteriol 186(22):7499–7507
108. Grass G, Franke S, Taudte N, Nies DH, Kucharski LM, Maguire ME et al (2005) The metal permease ZupT from Escherichia coli is a transporter with a broad substrate spectrum. J Bacteriol 187(5):1604–1611
109. Argüello JM, Raimunda D, González-Guerrero M (2012) Metal transport across biomembranes: emerging models for a distinct chemistry. J Biol Chem 287(17):13510–13517
110. Montanini B, Blaudez D, Jeandroz S, Sanders D, Chalot M (2007) Phylogenetic and functional analysis of the Cation Diffusion Facilitator (CDF) family: improved signature and prediction of substrate specificity. BMC Genomics 8:107, Pubmed Central PMCID: 1868760
111. Ricachenevsky FK, Menguer PK, Sperotto RA, Williams LE, Fett JP (2013) Roles of plant Metal Tolerance Proteins (MTP) in metal storage and potential use in biofortification strategies. Front Plant Sci 4:144
112. Yuan L, Yang S, Liu B, Zhang M, Wu K (2012) Molecular characterization of a rice metal tolerance protein, OsMTP1. Plant Cell Rep 31(1):67–79
113. Persans MW, Nieman K, Salt DE (2001) Functional activity and role of cation-efflux family members in Ni hyperaccumulation in Thlaspi goesingense. Proc Natl Acad Sci U S A 98(17):9995–10000, Pubmed Central PMCID: 55566
114. Kawachi M, Kobae Y, Mimura T, Maeshima M (2008) Deletion of a histidine-rich loop of AtMTP1, a vacuolar Zn(2+)/H(+) antiporter of Arabidopsis thaliana, stimulates the transport activity. J Biol Chem 283(13):8374–8383, Pubmed Central PMCID: 2417167

115. Haney CJ, Grass G, Franke S, Rensing C (2005) New developments in the understanding of the cation diffusion facilitator family. J Ind Microbiol Biotechnol 32(6):215–226, Epub 2005/05/13. eng
116. Desbrosses-Fonrouge AG, Voigt K, Schroder A, Arrivault S, Thomine S, Kramer U (2005) Arabidopsis thaliana MTP1 is a Zn transporter in the vacuolar membrane which mediates Zn detoxification and drives leaf Zn accumulation. FEBS Lett 579(19):4165–4174
117. Arrivault S, Senger T, Kramer U (2006) The Arabidopsis metal tolerance protein AtMTP3 maintains metal homeostasis by mediating Zn exclusion from the shoot under Fe deficiency and Zn oversupply. Plant J 46(5):861–879
118. Becher M, Talke IN, Krall L, Krämer U (2004) Cross-species microarray transcript profiling reveals high constitutive expression of metal homeostasis genes in shoots of the zinc hyperaccumulator Arabidopsis halleri. Plant J 37(2):251–268
119. Dräger DB, Desbrosses-Fonrouge A-G, Krach C, Chardonnens AN, Meyer RC, Saumitou-Laprade P et al (2004) Two genes encoding Arabidopsis halleri MTP1 metal transport proteins co-segregate with zinc tolerance and account for high MTP1 transcript levels. Plant J 39(3):425–439
120. Shahzad Z, Gosti F, Frérot H, Lacombe E, Roosens N, Saumitou-Laprade P et al (2010) The Five *AhMTP1* zinc transporters undergo different evolutionary fates towards adaptive evolution to zinc tolerance in *Arabidopsis halleri*. PLoS Genet 6(4), e1000911
121. Elbaz B, Shoshani-Knaani NOA, David-Assael ORA, Mizrachy-Dagri T, Mizrahi K, Saul H et al (2006) High expression in leaves of the zinc hyperaccumulator Arabidopsis halleri of AhMHX, a homolog of an Arabidopsis thaliana vacuolar metal/proton exchanger. Plant Cell Environ 29(6):1179–1190
122. Cheng N-H, Pittman JK, Shigaki T, Lachmansingh J, LeClere S, Lahner B et al (2005) Functional association of Arabidopsis CAX1 and CAX3 is required for normal growth and ion homeostasis. Plant Physiol 138(4):2048–2060
123. Korenkov V, Hirschi K, Crutchfield JD, Wagner GJ (2007) Enhancing tonoplast Cd/H antiport activity increases Cd, Zn, and Mn tolerance, and impacts root/shoot Cd partitioning in Nicotiana tabacum L. Planta 226(6):1379–1387
124. Henriques R, Jasik J, Klein M, Martinoia E, Feller U, Schell J et al (2002) Knock-out of Arabidopsis metal transporter gene IRT1 results in iron deficiency accompanied by cell differentiation defects. Plant Mol Biol 50(4–5):587–597
125. Haydon MJ, Cobbett CS (2007) A novel major facilitator superfamily protein at the tonoplast influences zinc tolerance and accumulation in Arabidopsis. Plant Physiol 143(4):1705–1719
126. Kramer U, Pickering IJ, Prince RC, Raskin I, Salt DE (2000) Subcellular localization and speciation of nickel in hyperaccumulator and non-accumulator Thlaspi species. Plant Physiol 122(4):1343–1353, Pubmed Central PMCID: 58970
127. Gustin JL, Loureiro ME, Kim D, Na G, Tikhonova M, Salt DE (2009) MTP1-dependent Zn sequestration into shoot vacuoles suggests dual roles in Zn tolerance and accumulation in Zn-hyperaccumulating plants. Plant J 57(6):1116–1127
128. Lakshmanasenthil S, Vinothkumar T, Ajithkumar TT, Marudhupandi T, Veettil DK, Ganeshamurthy R et al (2013) Harmful metals concentration in sediments and fishes of biologically important estuary, Bay of Bengal. J Environ Health Sci Eng 11(1):33, Pubmed Central PMCID: 3880047
129. van der Zaal BJ, Neuteboom LW, Pinas JE, Chardonnens AN, Schat H, Verkleij JAC et al (1999) Overexpression of a novel Arabidopsis gene related to putative zinc-transporter genes from animals can lead to enhanced zinc resistance and accumulation. Plant Physiol 119(3):1047–1056
130. Reinhart B, Weinstein E, Rhoades M (2002) MicroRNAs in plants. Genes Dev 16:1616–1626. doi:10.1101/gad.1004402
131. Bartel D (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. Nucleic Acids Res 116:281–297
132. Carrington JC, Ambros V (2003) Role of MicroRNAs in plant and animal development. Science 301(5631):336–338

133. Jones-Rhoades M (2012) Conservation and divergence in plant microRNAs. Plant Mol Biol 80(1):3–16
134. Mallory AC, Bouché N (2008) MicroRNA-directed regulation: to cleave or not to cleave. Trends Plant Sci 13(7):359–367
135. Tian X, Chen L, Wang J, Qiao J, Zhang W (2013) Quantitative proteomics reveals dynamic responses of Synechocystis sp. PCC 6803 to next-generation biofuel butanol. J Proteomics 78:326–345
136. Chen X (2009) Small RNAs and their roles in plant development. Annu Rev Cell Dev Biol 25(1):21–44
137. Graves P, Zeng Y (2012) Biogenesis of Mammalian microRNAs: a global view. Genom Proteom Bioinform 10(5):239–245
138. Allen E, Xie Z, Gustafson AM, Carrington JC (2005) microRNA-directed phasing during trans-acting siRNA biogenesis in plants. Cell 121(2):207–221
139. Axtell MJ, Jan C, Rajagopalan R, Bartel DP (2006) A two-hit trigger for siRNA biogenesis in plants. Cell 127(3):565–577
140. Liu H, Jin T, Liao R, Wan L, Xu B, Zhou S et al (2012) miRFANs: an integrated database for Arabidopsis thaliana microRNA function annotations. BMC Plant Biol 12(1):68. doi:10.1186/1471-2229-12-68
141. Zhang S, Yue Y, Sheng L, Wu Y, Fan G, Li A et al (2013) PASmiR: a literature-curated database for miRNA molecular regulation in plant response to abiotic stress. BMC Plant Biol 13(1):33. doi:10.1186/1471-2229-13-33
142. Zhou L, Liu Y, Liu Z, Kong D, Duan M, Luo L (2010) Genome-wide identification and analysis of drought-responsive microRNAs in Oryza sativa. J Exp Bot 61(15):4157–4168. doi:10.1093/jxb/erq237
143. Wang T, Chen L, Zhao M, Tian Q, Zhang WH (2011) Identification of drought-responsive microRNAs in Medicago truncatula by genome-wide high-throughput sequencing. BMC Genomics 12:367, Pubmed Central PMCID: 3160423, Epub 2011/07/19. eng
144. Li B, Duan H, Li J, Deng X, Yin W, Xia X (2013) Global identification of miRNAs and targets in Populus euphratica under salt stress. Plant Mol Biol 81(6):525–539
145. Macovei A, Vaid N, Tula S, Tuteja N (2012) A new DEAD-box helicase ATP binding protein (OsABP) from rice is responsive to abiotic stress. Plant Signal Behav 7:1–6. doi:10.4161/psb.7.1.18574
146. Zhang J, Xu Y, Huan Q, Chong K (2009) Deep sequencing of Brachypodium small RNAs at the global genome level identifies microRNAs involved in cold stress response. BMC Genomics 10(1):449. doi:10.1186/1471-2164-10-449
147. Barakat A, Sriram A, Park J, Zhebentyayeva T, Main D, Abbott A (2012) Genome wide identification of chilling responsive microRNAs in Prunus persica. BMC Genomics 13(1):481. doi:10.1186/1471-2164-13-481
148. Chen L, Ren Y, Zhang Y, Xu J, Sun F, Zhang Z et al (2012) Genome-wide identification and expression analysis of heat-responsive and novel microRNAs in Populus tomentosa. Gene 504(2):160–165
149. Yu X, Wang H, Lu Y, de Ruiter M, Cariaso M, Prins M et al (2012) Identification of conserved and novel microRNAs that are responsive to heat stress in Brassica rapa. J Exp Bot 63(2):1025–1038
150. Liang G, Yang F, Yu D (2010) MicroRNA395 mediates regulation of sulfate accumulation and allocation in Arabidopsis thaliana. Plant J 62(6):1046–1057
151. Sunkar R, Kapoor A, Zhu J-K (2006) Posttranscriptional induction of two Cu/Zn superoxide dismutase genes in Arabidopsis is mediated by downregulation of miR398 and important for oxidative stress tolerance. Plant Cell 18(8):2051–2065
152. Ding Y, Chen Z, Zhu C (2011) Microarray-based analysis of cadmium-responsive microRNAs in rice (Oryza sativa). J Exp Bot 62(10):3563–3573
153. Chen L, Wang T, Zhao M, Tian Q, Zhang W-H (2012) Identification of aluminum-responsive microRNAs in Medicago truncatula by genome-wide high-throughput sequencing. Planta 235(2):375–386

154. Zhou ZS, Song JB, Yang ZM (2012) Genome-wide identification of Brassica napus microRNAs and their targets in response to cadmium. J Exp Bot 63(12):4597–4613
155. Zeng Q-Y, Yang C-Y, Ma Q-B, Li X-P, Dong W-W, Nian H (2012) Identification of wild soybean miRNAs and their target genes responsive to aluminum stress. BMC Plant Biol 12(1):182. doi:10.1186/1471-2229-12-182
156. Gupta OP, Sharma P, Gupta RK, Sharma I (2014) MicroRNA mediated regulation of metal toxicity in plants: present status and future perspectives. Plant Mol Biol 84(1–2):1–18
157. Xu L, Wang Y, Zhai L, Xu Y, Wang L, Zhu X et al (2013) Genome-wide identification and characterization of cadmium-responsive microRNAs and their target genes in radish (Raphanus sativus L.) roots. J Exp Bot 64(14):4271–4287
158. Huang S, Peng J, Qiu C, Yang Z (2009) Heavy metal-regulated new microRNAs from rice. J Inorg Biochem 103(2):282–287. doi:10.1016/j.jinorgbio.2008.10.019
159. Huang SQ, Xiang AL, Che LL, Chen S, Li H, Song JB et al (2010) A set of miRNAs from Brassica napus in response to sulphate deficiency and cadmium stress. Plant Biotechnol J 8(8):887–899
160. Shimo H, Ishimaru Y, An G, Yamakawa T, Nakanishi H, Nishizawa NK (2011) Low cadmium (LCD), a novel gene related to cadmium tolerance and accumulation in rice. J Exp Bot 62(15):5727–5734
161. Mendoza-Soto AB, Sanchez F, Hernandez G (2012) MicroRNAs as regulators in plant metal toxicity response. Front Plant Sci 3:105
162. Kamnev AA, van der Lelie D (2000) Chemical and biological parameters as tools to evaluate and improve heavy metal phytoremediation. Biosci Rep 20(4):239–258, Epub 2000/11/25. eng
163. Zhu YL, Pilon-Smits EAH, Tarun AS, Weber SU, Jouanin L, Terry N (1999) Cadmium tolerance and accumulation in Indian mustard is enhanced by overexpressing γ-glutamylcysteine synthetase. Plant Physiol 121(4):1169–1177
164. Lee S, Moon JS, Ko T-S, Petros D, Goldsbrough PB, Korban SS (2003) Overexpression of Arabidopsis phytochelatin synthase paradoxically leads to hypersensitivity to cadmium stress. Plant Physiol 131(2):656–663
165. Mandelbaum-Schmid J (2004) Vitamin and mineral deficiencies in human population. Bull World Health Organ 82:230–231
166. Ye X, Al-Babili S, Klöti A, Zhang J, Lucca P, Beyer P et al (2000) Engineering the provitamin A (β-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. Science 287(5451):303–305

Chapter 9
Current Technical Perspective and Application of Aquatic Weeds in Phytoremediation

Anand Mohan, S.M. Bhatt, Madhuri Girdhar, Geetanshu Goyal, Abid Ali Ansari, and Hasibur Rehman

9.1 Introduction

Soil and water resources are being contaminated in the last few decades due to the addition of huge loads of pollutants, primarily various toxic compounds [1]. The pollution is on rise due to various anthropogenic inputs such as rapid growth of population, modern agriculture technologies, and heavy industrialization [2]. In developing countries like India and China, industrial effluents are regularly discharged into various open water sources, which further grave the problem. Disposal of industrial wastes into the water bodies adversely affects the aquatic flora and fauna along with human health. As per environment regulation, the permitted concentration of metal ions should not exceed 5 ppm. There are various processed chemical additives like catalysts and other chemicals which are produced as corrosion product during distillation of downstream processing along with metallic contaminants that are commonly produced during such industrial processes and finally end up in water bodies leading to water pollution. Rational behind the surge of these challenges are (a): poor economical situation of the countries, (b): lack of proper techniques, and (c): delay in strict implementation of environment policies [3–6].

The level of pollution and toxicity due to heavy metals varies from place to place and is a global problem. Their presence in wastewater both at low and high level proves to be phytotoxic and poses risk for both the environment and human health [7]. The sediments of metals when reaches food chain, in limited concentration, prove nutritionally beneficial for human health but at higher ranges, could cause acute or chronic poisoning. The researchers all over the world are attracted by the aquatic ecosystems and major causes of its pollution because water is an essential

---

A. Mohan (✉) • S.M. Bhatt • M. Girdhar • G. Goyal
Department of Biotechnology, Lovely Professional University, Phagwara, Punjab, India
e-mail: anandmohan77@gmail.com

A.A. Ansari • H. Rehman
Department of Biology, Faculty of Science, University of Tabuk, Tabuk, Saudi Arabia

A.A. Ansari et al. (eds.), *Phytoremediation*, DOI 10.1007/978-3-319-40148-5_9

part of the earth's ecosystem which is necessary for survival of mankind. The awareness for protection of this precious resource is of major concern for improving the quality of water; hence there is an urgent requirement of economical and sustainable technology without interfering with the environment. Phytoremediation technology is an eco-friendly and effective way to reduce the unprecedented pollution in aquatic ecosystems.

For developing countries, the eco-friendly technique of phytoremediation which utilizes the plants and associated microorganisms for natural cleanup becomes highly effective, economical, sustainable and acts as biological means to reduce the pollution [8–11]. Specific plantation arrangements, artificially constructed wetlands, plant-floating systems, and other configurations are designed as remediation techniques. Certain studies have been carried out on aerobic fixed film biological systems for the treatment of petroleum refinery effluent [12]. According to the report of Wuyep et al. [13], some mycelia cells of *Polyporus squamosus* have capacity to absorb various toxic metals originated from petroleum refineries in the immobilized form. Further, it has been observed that these toxic metals have severe health effects in human beings in the form of respiratory issues, cardiac failure, renal dysfunction, dehydration, initiation of cancer cells, etc. [14]. Beyond permitted limit of Cd and Cr, living organism develops symptom of cancer in vital organs such as lungs, kidney, and liver [15]. Thus, the sensitive issue is the treatment of water at the point of discharge, so that during supply of water for domestic use the concentration of toxic metal may be minimized [16] (Fig. 9.1).

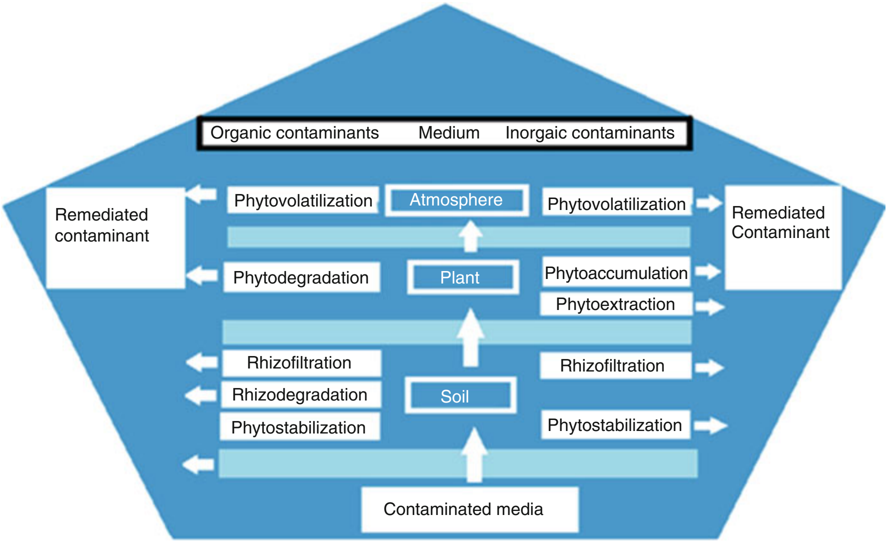


Fig. 9.1 Representation of the processes involved in the technology of phytoremediation

9.2 Phytoremediation Technology

In order to improve the quality of polluted water from the hazardous contaminants, the emerging technology, i.e., phytoremediation, uses selected plants for the remediation of contaminated water. This technology utilizes the uptake mechanisms of both organic and inorganic contaminants. The processes such as phytostabilization, rhizodegradation, rhizofiltration, phytodegradation and phytovolatilization are well known and are utilized for organic contaminants, where organic contaminants which are volatile in nature are not absorbed into the plant tissue, but precipitate out into the environment. The processes i.e., phytostabilization, rhizofiltration, and phytoaccumulation are utilized for inorganic contaminants [17–19]. The bioavailability of contaminants into the soil matrix is reduced by the exudates of the plant roots which stabilize, demobilize, and bind to the contaminants and are known as phytostabilization process. The roots of certain species accumulate, adsorb, and precipitate contaminants in the soil and groundwater through immobilization process. This process is basically used for the organic and metal contaminants present in soil, sediments, and sludge medium [17–19]. Another technique is known as phytoextraction, in which metals, metalloids, radionuclides, nonmetals, and organic contaminants present in the soil are absorbed and hyperaccumulated into the harvestable root and shoot tissues of certain plant species from the growth substrate [20–22].

Another mechanism of decontamination is phytovolatilization in which contaminants are volatilized and released in the atmosphere. These processes are operative in contaminated aquifers effected soil and released sludge [8, 23]. The metabolic processes occur within the plants helping in the breakdown of contaminants through the process of phytotransformation/phytodegradation. In this process, detoxification of metals is used for the metabolic processes, where contaminants are degraded via process of phytodegradation [24, 25]. Rhizofiltration is another mechanism which promotes uptake of toxicants from contaminated water [20, 26–28]. Microbes attached to the plants are further helpful in degradation of contaminants releasing chelators and chemicals which attract these microbes and hence rapid uptake is possible, resulting in decontamination of large quantity of water. Further, aquatic plants especially free-floating water hyacinth, nitrogen fixing pteridophytes for example azolla may provide better alternative solution in managing detoxification of these contaminants from the ecosystem [29, 30].

9.3 Aspects of Metal Accumulation and Phytoremediation

Many works have been carried out by the researchers on different metals by the technology of phytoremediation such as mercury and arsenic removal [31]; cadmium [32, 33]; and trace elements in soils and water [34]. A very good option for application of phytoremediation technique is in the area of metal recovery [35]. The scientists have also reported that the branch of genetic engineering could also develop certain genetically modified plants, which can be resistant to heavy metal in terms of their growth and could accumulate high levels of metal as compared to their wild varieties. Other techniques such as selective breeding enhance the applicability of phytoremediation technology, which has been reported by certain workers [34, 36, 37]. It was estimated by the scientists that there are 400 species which are identified as metal hyperaccumulators [38].

Recent trends in application of hyperaccumulator species along with some other aquatic weeds such as hydrilla, duckweed, and lily are involved in high absorption of selenium, cadmium, chromium, cobalt, nickel, and lead [39]. Growth of these hyperaccumulator species requires specific conditions, therefore success of phytoremediation using hyperaccumulators has limited scope in different geographical regions [40]. In developing countries like India, water hyacinth may prove to be a good hyperaccumulators of many metal species and also not required specific conditions for growth. In developing countries, application of such weeds in bioremediation has dual benefits, where not only waste can be eliminated but also pollutant load can be minimized at varying economical rate [40].

The basic principle of phytoremediation technology is to remove the contaminants, which includes the identification and implementation of most efficient aquatic plants. The aquatic plants are very efficient for the uptake of dissolved nutrients and metals from the contaminated water [41]. The selection of appropriate plants should be done on the basis of their ability to uptake high organic and inorganic pollutants, and it should be fast growing and easily controlled in quantitatively propagated dispersion [42, 43]. The uptake and accumulation mechanism of different plants varies from plants to plants and species to species within a genus [44]. The factors such as phytosynthetic activity and growth rate of the plants lead to the economic success of phytoremediation technology [45]. The economic success also depends on other factor related with the low to moderate amount of pollution [46]. Certain workers worked on the removal of contaminants with the help of aquatic weeds and concluded that species like Water Lettuce (*Pistia stratiotes L.*), Water Hyacinth (*Eichhornia crassipes* (Mart.) Solms), Bulrush (*Typha*), Common Reed (*Phragmites Australis*) are useful.

Duckweed (*Water Lemna*) and Vetiver Grass (*Chrysopogon zizanioides*) are very efficient for the removal of moderate to high level of contaminants present in the water. The duckweed plants have found use as an aquatic weed for the removal of metal contaminant and further, it was concluded by the workers that stress conditions can stunt the growth above 34 °C and has pH sensitivity [47]. The bulrush plant has been studied by many workers with the outcome that electrical conductivity concentration and total dissolved solids (TDS) would increase after exposing the plants in polluted environment [48]. An experiment was conducted on the kitchen wastewater and was found that only 4 % TDS was removed by the common weed [49]. The current analysis focuses on two important aquatic weeds i.e., water hyacinth and duckweed which were selected on the basis of their efficiency to remove the heavy metals and other pollutants with high reproduction rate, efficiency, and tolerance towards ecological factors (Fig. 9.2).

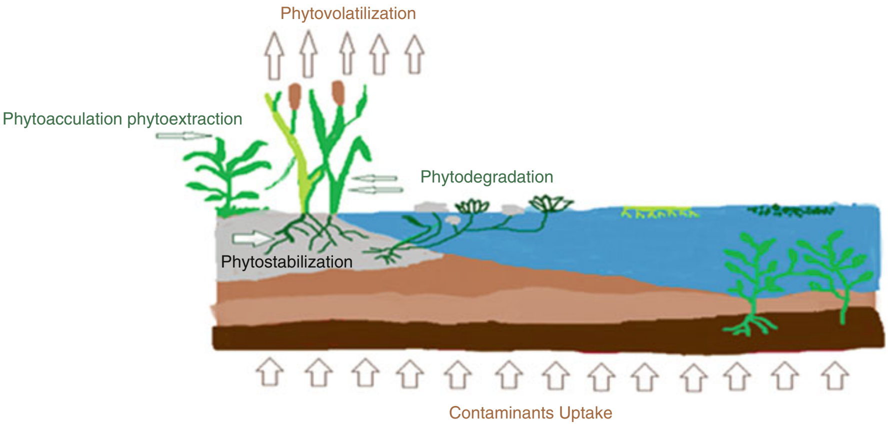


Fig. 9.2 Conceptual presentation of various phytoremediation techniques

9.4 Mechanisms of Heavy Metal Uptake by Plant

Different plants have different capability of metal uptake which has been widely explored. The performance of plant uptake could be improved by optimizing the factors responsible for metal uptake [50]. The plants act as both "accumulators" and "excluders," depending upon their metal uptake mechanism [50]. Accumulators are those plants which have the ability to concentrate the contaminants in the aerial tissues. In the tissues the contaminants get biodegraded or biotransformed into their inert forms. Excluders are those plant species, which restrict the contaminants to get accumulated in their biomass. Essential micronutrients are obtained from the environment by the plants through highly specific and efficient mechanisms, even in the condition when these micronutrients are present in low ppm in the surrounding matrix. Most of the plants secrete chelating agents which induce sequestering of metal ions and further, change in pH leads to solubilization of micronutrients. This facilitates high uptake and transport of nutrients.

For the translocation and storage of micronutrients, plants have highly specific mechanisms. Technically, this is an important step in phytoremediation of metal ions [51]. Further, most of the plants are equipped with specialized proteins linked with cell membrane that are also involved in rapid uptake and translocation viz. (a) Various transporters, (b) pumps, and (c) channels. A range of ions are specific for each transport mechanism. During the uptake of metal contaminants by the plant species, there are certain basic problems involved at the time of interaction of ionic species with the metal. Further generally translocation of heavy metal from the roots to the shoots is desirable, as it is generally not feasible to harvest the root biomass [52]. Plant uptake-translocation mechanisms are likely to be closely regulated. The accumulation of trace elements by the plants is generally restricted at a particular point of their metabolic needs. Beyond that point, plants do not accumulate trace elements, which are embedded in their root zones. The basic requirement of trace elements needed for growth of most of the plant species is in the small range of 10–15 ppm revealed by the workers [52].

Researchers also observed that the "hyperaccumulator" plants can take up toxic metal ions in their biomass up to thousands of ppm. Particularly in hyperaccumulating plants, the storage form of toxic metal ions and the toxicity effect of these metal ions is a topic of interest for various workers in the field of phytoremediation. Some hyperaccumulatory species are different from the normal plants based on multiple mechanism of storage [51]. Some operating mechanism linked with the plants root is presence of pumps and water evaporation, which serve as a catalyst for high nutritional uptake and thus increase phytoremediation as compared to others. Beside, this hyperaccumulation has powerful capability to thrive well in toxic environment and thus is capable of maintaining high biomass [53]. This is in accordance with the requirement of bioremediation of toxic metals such as Cd, Zn, Co, Mn, Ni, and Pb where these metals can be accumulated from 100 to 1000 times more than normal plants. Certain microbes living in close association with roots help in rapid mobilization and thus make them powerful plant excluders [54].

9.5 Diversity of Aquatic Plants and Comparative Assessment of Water Purification Capability

Several papers have referred about the aquatic plants, thriving in their natural habitat, having the capability to extract nutrient for their growth. The reason which has prompted the spate of papers within the last 10 years on the nutrient extraction possibilities of aquatic plants is the increasing awareness of the problems of water pollution, both fresh and salt water, as a consequence of population growth and industrial development, and the disposal of human, animal, and industrial wastes into inland waters and into the sea [55]. Many examples of the devastating consequences of the wastewater on formerly clean and useful rivers and lakes have aroused public and scientific awareness of the need not only to arrest the practice of direct dumping but to try and reverse it by extracting the pollutants. As compared to the floating and emergent vascular plants, small plants like phytoplankton, or submerged plants are more difficult and expensive to harvest. Problem of increased water pollution due to heavy solid waste releases resulted in the extinction of various aquatic species.

Workers have considered four aquatic species important for phytoremediation purposes: *Justicia Americana, Eichhornia crassipes* (water hyacinth), *Alternanthera philoxeroides*, and *Typha latifolia*. As compared to other aquatic plants, water hyacinth has several ideal properties such as: (a) They can float easily since they have no root system and (b) This property is beneficial in terms of translocation of high organic load and thus, BOD can easily be reduced in any conventional sewage holding ponds, before their final release in any water bodies. Similarly, *Alternanthera philoxeroides* has been used in removal of phosphorous from polluted water bodies. Another aquatic species *Typha latifolia* has been used to remove phosphorous from

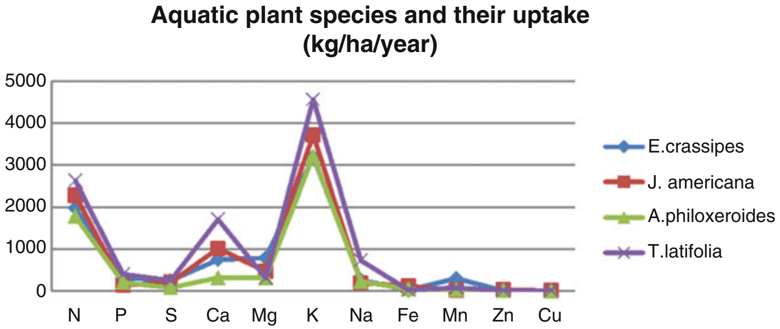


Fig. 9.3 The graphical representation of different aquatic weeds, hyperaccumulating nature with different metals [28, 56]

soil in anaerobic condition even up to 15–20 cm depth. At a stage when *P* equilibrium had been reached then the ponds could be dried and used for conventional crops until the *P* levels are reduced. The scientists have observed that aquatic plants have the ability, particularly water hyacinth and have become well recognized in the extraction of compounds and elements from the water efficiently [56, 57] (Fig. 9.3).

9.6 Analysis of Capability of Water Hyacinth (*Eichhornia crassipes*) for Phytoremediation Capability

Eichhornia crassipes is a prolific free-floating aquatic weed found in tropical and subtropical areas of the world and recognized to be very useful in domestic wastewater treatment [58, 59]. It belongs to the family of pickerelweed—*Pontederiaceae* and Genus—*Eichhornia* [60]. In the late nineteenth and early twentieth century, this tropical plant spread throughout the world [61]. It is lavishly found along India, Bangladesh, and South East Asia [62]. The morphological characteristics of the plant include rounded structure, upright with shiny green leaves, lavender flowers with root system dark blue in color [63, 64]. The reproduction potential of the plant is very high as it grows double in the time period of 5–15 days [63, 65–67]. Rapid reproductive potential and complex root system form dense interlocking mats, which is a common characteristic of water hyacinth. It carries out reproduction in both sexual and asexual forms [68]. Its natural growth region varies from 33°N to 33°S of equator and it has a growth capacity of 220 kg/ha-day to 600 kg/ha-day seasonally in water bodies achieving 224–412 t/ha density [69].

Inflorescence contains 6–10 lily-like flowers, each 4–7 cm in diameter [70] with flowering period varying over 15 days. Release of seeds directly occurs in the water at the end of flowering season, with bending of flower stalks reaching under the water surface [65, 71]. The usual height of water hyacinth is almost 1.5 m and more from flower top to root bottom [72]. Individual plant height ranges from 500 to 1175 mm from the flower top to root tips.

*9.6.1 Growth Capability of Water Hyacinth (***Eichhornia crassipes** *(Mart.) Solms.) and Metal Removal*

Optimal growth of the water hyacinth is the key parameter for a phytoremediation system to work efficiently and this has been concluded and observed by various scientists. For phytoremediation purpose, water hyacinth should have minimal optimal growth. pH, temperature, and salinity of water decides the overall size of the plant [65, 73]. Nitrogen and phosphorous rich water induces high growth of water hyacinth [74–76]. In experimental conditions, it was observed that total nitrogen at 28 mg/L and total phosphorous at 7.7 mg/L produced maximum growth [77]. Basic survival of water hyacinth requires 5.5 mg of N/L and 1.06 mg of P/L investigated by Reddy et al. [78]. Data analysis shows that maximum growth was achieved in the presence of 20 mg N/L, 3 mg P/L, and 52 mg K/L, respectively. There was a close relation existing between total plant biomass and evapotranspiration rate [79].

9.6.2 Commercial Growth of Water Hyacinth

Various experiments have been conducted at pilot scale to observe the growth rate and productivity of plant in different nutritional conditions. It has been observed by Wolverton and McDonald [80] that water hyacinth productivity was around 40–88 million ton (dry weight/h/year). In 2004, Sooknah and Wilkie reported [81], addition of dairy manure for 31 days gives rise to yield of water hyacinth to 1608 g/m^2. Addition of secondary treated municipal wastewater gives rise to productivity, 16 g/m^2 per day. Ayyasamy et al. [82] reported 37 % increase in biomass in duration of 10 days. The average coverage area of proliferation for ten plants was one acre pond with duration of 8 months. Small leaves with inflated petioles were observed in plants grown in distilled water [83]. Water hyacinth cannot grow in high salt concentration above 2 ppm [84]. Various pilot projects have demonstrated that water hyacinth is an important functional unit for the wastewater treatment systems [85, 86].

9.6.3 Potential for Metal Removal

Studies show that water hyacinth has high potential of multiple metal removals such as As, Cd, Pb, and Hg from wastewater [87]. Certain studies utilizing water hyacinth were carried on effluents from the rubber industry for the metals and organic sulfur compounds [88], and the palm oil industry for the removal of metals and lipid. Therefore, water hyacinth has been exhaustively tested for its capability to remove effluent from rubber industry and palm oil industry. At low metal concentration (5 mg/L), water hyacinth works well but at higher concentration of metal, water hyacinth starts wilting due to high toxicity on plants [89]. Similar results were investigated in case of cadmium metal by O’Keefe et al. in 1984 [90]. Water hyacinth can absorb high amount of Zn, Fe, Cu, Ni, Cd, Hg, Cr, and As [87, 91–93]. In addition they absorb a very good amount of acid and other chemicals such as phenol, formaldehyde, formic, acetic, and oxalic acid [87, 92, 93]. Thus, water hyacinth can absorb cadmium up to 0.24 kg/ha in Erh-Chung wetlands in Taiwan [93].

Previous data shows that water hyacinth has different absorption capability for different metals i.e., 5.24 kg/ha for lead, 26.17 kg/ha for Zn, 13.46 kg/ha for Ni, and 0.24 kg/ha for Cd [93]. Morphological deformities were observed in plant if the metal concentration was exceeding the saturation point reported by Valipour et al. [84]. Water hyacinth was reported to be a best known species for Cd accumulation [28, 94, 95]. In maximizing the adsorption efficiency for different metals, toxicity is a major hurdle. Therefore, few studies have been conducted for its survival capacity [96]. A lot of sludge is produced when water hyacinth has been used for bioremediation of industrial effluents, which finally can be treated using chemical reaction and filtration. One of the interesting examples can be quoted here regarding application of water hyacinth in pretreatment of industrial effluents at Port Dickson refineries in Malaysia. It requires purposeful cultivation at specific site, which can beneficially adsorb most of toxic metals released from industries before this effluent finally gets mixed with water bodies [97, 98]. Genetic engineering-based work has been conducted by some workers [37, 99].

9.6.4 Water Hyacinth in Improving Water Qualities Such as BOD, COD, and TDS

There was a reduction in electrochemical conductivity by 18.1 % in 5 days and it was observed that total dissolved solid (TDS) was removed by 39.1 % in time period of 20 days [100]. For water purity, there are few important parameters which define overall water quality i.e., TDS, BOD, COD, and DO. This can be improved by using suitable adsorbent such as water hyacinth. State of growth of water hyacinth decides overall potential to improve water quality. Under live conditions, during photosynthesis, TDS in some cases has observed to be increased [65] which may be due to some adsorb metal or clay present in the root nodules of the plants. It was observed that TDS was reduced with the passage of accumulation time. Live cell helps in reduction of carbon dioxide and thus helpful in increasing dissolved oxygen of water [78]. Live cells of water hyacinth also induce growth of favorable bacterial species and thus, reduction in BOD and COD was observed [66, 101]. Opposite to this, dead mats of water hyacinth reduce in dissolved oxygen concentration in water [102, 103].

Shallow depth systems are highly efficient in eliminating total suspended solids and nitrogen along with phosphorous reported by Trivedy and Pattanshetty in 2002 [104]. Water hyacinth at shallow depth in tank is helpful in removing solid waste viz. suspended particles, BOD, COD, N, and P. In high pond depth, an anaerobic zone was developed, which may result in slow degradation of organic component escalating problem of foul odor emission [84, 105]. Regular harvesting of old plants is recommended, as it was observed that the young plants are efficient in the removal of nutrients as compared to old ones [106, 107].

The anaerobic condition was caused by the leaching back of nutrients from old plants into the water if they are not harvested on time observed by Ayyasamy et al. [82]. Monitoring was carried out in textile mill by the utilization of water hyacinth reported by Gamage and Yapa in 2001 [108]. It was observed that biological oxygen demand was reduced to 75 % and chemical oxygen demand was 81.4 %, respectively. In case of rubber factory effluents, BOD was reduced to 99 % and COD was 80 % reported by Kulatillake and Yapa in 1984 [109]. Increase in the Hydraulic Retention Time was responsible for decrease in COD whereas reduction in ammonium concentration significantly depends on the type of plant species but not on the HRT factor investigated by Snow and Ghaly in 2008 [110].

It was concluded by the scientists that both the factors i.e., plant type and HRT were responsible for nitrate reduction. There was a reduction in COD and it was observed that 71 % reduced in 1 day and 83 % in duration of 5 days [111]. Interestingly when water hyacinth applied to the polluted river, 13–51 % COD removed was observed [112] in 4 days and up to 83 % in 5 days [111]. In this way, 87 % NH_4 can be reduced [113]. Further, 84–86 % N_2 could be removed in 8 weeks. In addition, nitrate was also reduced with 19 % efficiency in 14 days and 30 % efficiency in 70 days [114]. In duration of 10 months, 8.4 % of nitrate was removed, investigated by Cornwell et al. [115]. Ingersoll and Baker [116] observed 90 % nitrate (conc. 30 mg/L) could be removed. Inorganic nitrogen level reduced by 94 % along with orthophosphate reduction by 40–55 % [117] and 80 % reduction of total inorganic nitrogen [118]. It was measured that up to 32 % reduction in total phosphorous [118] with removal rate 80 % reported by Ornes and Sutton [119]. At pilot scale, Bramwell and Devi Prasad observed that 27.6 % N and P 4.48 % was removed. On introduction of air stripping unit, flocculation and settling unit removes 99 % total P, 99 % total nitrate, and 99 % total NH_3 [117].

According to estimation, 170 kg of N and 60 kg of P can be removed by one hectare of water hyacinth cultivation which can remove 2500 kg of N/year and as high as 7629 kg of N/ha/year [120–122]. Ayyasamy et al. [82] reported that 64–83 % of nitrate was removed, when initial concentration was up to 300 mg/L and beyond 400 mg/L concentration, nitrate concentration was decreased because of high osmotic pressure. In groundwater sample, nitrate removal was dependent on sulfate and phosphate concentration. In textile effluent, application of water hyacinth reduces volatile solid level by 72.6 %, phosphate to 52.9 %, suspended solids to 46.6 %, dissolved solids to 61.07 %, total solids to 59.4 %, chloride reduction to 36 %, and total nitrogen to 83.5 % [108].

9.6.5 Hypothesis for Treatment

Phytoremediation via using aquatic weeds is a suitable and eco-friendly option. Different reports show that water hyacinth can be efficiently used for treatment of different industrial effluents. In addition, it has unique capability to alter different

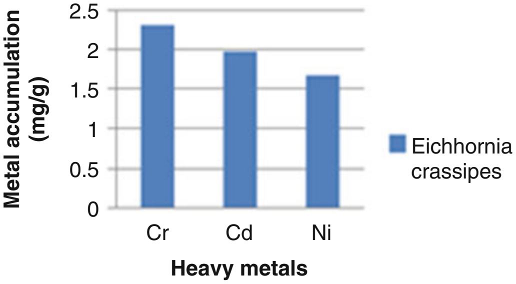


Fig. 9.4 Heavy metal accumulation in *Eichhornia crassipes* [157]

components present in effluent. Therefore, they are one of the best bioaccumulators. Mechanisms can be explained under following heads:

(a) *Root absorption*: Root is involved in heavy metal uptake via active adsorption by cell membrane. Aerobic microbes further are helpful in inducing metal uptake along with nutrients. Beside this, uptakes of various toxic chemicals are also involved effectively.
(b) *Foliar absorption*: Plants like water hyacinth can absorb various aerial contaminants via stroma cells and cracks in cuticle.
(c) *Adsorption*: Root architectural composition is helpful in trapping suspended solids beside providing shelter for growth (microbes) (Fig. 9.4).

9.7 Duckweed and Its General Properties

Duckweed is a native aquatic species of United States, with high relative growth rate (RGR) of 0.006–0.31. It is easy to harvest as it floats on water [123]. There are 40 species of duckweed known all over the world. *Lemna*, *Spirodela*, *wolffia*, and *Wolffiella* are the four genera to which duckweed belongs [124]. The leaves of all these species are minute and flattened in structure, with 1 mm to 1 cm fronds, oval to round in shape. Root-like structures are observed in some of these species, which help either in plant stabilization or in absorbing nutrients, which are present in less concentration in aquatic environment. Duckweed can grow in all seasons due to its property of tolerating cold, whereas, compared to it, other aquatic plants such as water hyacinth can grow only in summer. It can also grow over wide pH range. The biomass production of duckweed is rapid as compared to other aquatic plants. Due to these properties of duckweed, its use in phytoremediation is exploited and is turning out to be beneficial.

9.7.1 Duckweed as Sentinel Species

The American Public Health Association (APHA) and Environment Canada have completed duckweed toxicity test protocol. Most emphasis is given on its capability for phytoremediation and comparatively less importance is given towards its use as a toxicity indicator. Duckweeds can also be used towards the identification of sentinel species which are utilized for identification of hazards to human health. In an important work, when cadmium-containing duckweed was fed to crayfish, its acetylcholinesterase activity reduced as a significant sign for bioaccumulation effect [125]. In this specific work, crayfish is acting as a sentinel species as they are just one step away from humans, in the food chain. According to a report, out of 80,000 chemicals used in United States, only few hundred are tested for safe human consumption. Duckweed can provide quick indication of these chemical for their potential toxicological impacts on humans such as in the case of synthesized pharmaceuticals [126] and polyaromatic hydrocarbons [127].

9.7.2 Duckweed and Its Phytoremediation Capability

Duckweed has been employed in phytoremediation, a plant-based eco-friendly technology which is much better than older physical and chemical methods. It is also cheap and easily maneuverable technique of remediation [128–130]. In the field of phytoremediation and ecotoxicology, the model plants of duckweed class are basically taken from Lemna genus [131, 132]. The most studied *Lemnaceae* family species are the *Lemna gibba L.* and the *Lemna minor L.* [133, 134]. In *Lemna* species investigation was carried out for the removal of Zn metal from water, at the concentration of 1, 5, 10, 15, and 20 mg/L, percentage removal was obtained at 97 % for *L. trisulca*, 89 % for *L. minuta*, and 83 % for *L. minor. Lemna minor* can remediate up to 13 and 22 μM concentration of cadmium-polluted water which states its potential as a cadmium bioaccumulator [135]. *Lemna polyrrhiza* accumulated 27.0, 10.0, and 5.5 μg/mg of Zn, Pd, and Ni, respectively, when exposed to 10 mg/L concentration of each and was found that *L. polyrrhiza* was quite effective in phytoremediation capability [136]. *Lemna minor* when exposed to municipal wastewater reduced the copper concentration to 55 % by its remediation effects. Donganlar et al. [137] observed that *Lemna gibba* is very effective in removing Mn from polluted water.15 mg/g dry weight of Mn was accumulated at already prevailing copper concentration of 16 mg/L by phytoremediation.

9.7.3 Phytoremediation Activity of Duckweed for the removal of Remediation of Various Heavy Metals

Axtell et al. [138] stated that 82 % nickel and 76 % lead can be removed by duckweed, which proves its potential for phytoremediation. *Lemna minor* (duckweed), when exposed to wastewater, the order of metal toxicity in reducing order of damage obtained was Zn > Ni > Fe > Cu > Cr > Pd [139]. Kaur et al. [140] reported that after 28 days of exposure, at the pH of 5–6, duckweed can remove 99.99 % Pb while 99.3 % Ni was removed at pH6. It was reported that duckweed has high capacity of removing Pb, when exposure to industrial waste occurs, with maximum bioconcentration potential obtained as 0.900 at the pH of 6. The textile wastewater with Cr and Zn elements, present in dilute concentration, can be treated with duckweed. Uysal [141] analyzed a study on pilot plant, where duckweeds reduced chromium concentration as in pond and wetlands. At pH of 4.0, the chromium concentration was accumulated up to 4.423 mg Cr/g. Khellaf and Zerdaoui [142] observed that heavy metal tolerance of duckweed is quite high. Concentration of 0.4, 3.0, 0.4, and 15.0 mg/L of Cu, Ni, Cd, and Zn respectively were tolerated without any harmful effects. The toxicity detection via *Lemna* test is better than other algal test, hence, in polluted water duckweed can be utilized to provide checkpoints for heavy metal accumulation [143]. Minute size and fast growth rate at wide pH are the physiological factors, which make these plants more capable for heavy metal detection and remediation [131].

It has been reported that duckweed is very efficient for reducing the biochemical oxygen demand, percentage of heavy metals, chemical oxygen demand, orthophosphate, nitrate, and ammonia during its exposure to wastewater. Loveson et al. [144] studied that the quality of two wetlands improved by Spirodela polyrrhiza duckweed, when exposed for 8 days. The percentage reduction of Pb, Cu, Zn, Co, Mn, and Ni was observed at sufficiently high rates. Similar results were obtained when *Lemna gibba* duckweed was used by Hegazy et al. [145]. The remediation by *Lemna* is the result of metal salt precipitation, adsorption, and sequestration [146]. The use of *Lemna* species is also reported for sewage water treatment that is further used for fish rearing and agricultural practices. The study depicts that duckweed (*S. polyrrhiza*) is useful in phytofiltration as well, where arsenic is reduced by the process of chemical adsorption and by the pathway of phosphate uptake, when treated with dilute concentration of DMAA (dimethylarsinic acid). The priority of remediation by *Lemna gibba* is in a sequence, Arsenic > Boron > Uranium, when exposed to secondary effluents containing them [147].

Oil refinery effluent was used by some workers for remediation and was observed that duckweed has heavy metal removal capacity up to 99.8 %, 99.6 %, 98.7 %, and 72 % of copper, cadmium, lead, and zinc, respectively. Zayed [95] experimentally derived the order Cu > Se > Pb > Cd > Ni > Cr, according to the effect of these heavy metals towards the growth of the duckweed plant. This study suggests that the plant is more effective for the accumulation of Cd, Se, and Cu than to Ni, Pb, and Cr. Teixeira et al. [148] reported the use of *L. minor* in mine effluent treatment as it can accumulate iron effectively. Duckweed is researched for phytoremediation from last 40 years and recently it has been further reported for removing organic chemicals in the field of pharmaceuticals-based pollutions [149, 150].

9.7.4 Duckweed for the Removal of Toxic Arsenic from Aquatic Environment

The arsenic contamination in food crops and household water causes adverse effects on the health of large mass of human population. Remediation of water polluted with arsenic can be achieved by aquatic macrophytes [134, 151]. The bioavailable forms of arsenic in aquatic water are arsenate and arsenite [152]. The dynamics of arsenate and phosphate is analogous with respect to the exchange of elements among water and adsorbents. However, the site favors phosphate over arsenate [153]. Mkandawire and Dudel [134] worked on arsenic accumulation at field and laboratory scale. It was concluded that the field water has twofold less arsenic concentrations as compared to laboratory conditions. The reason can be the time period for exposure. In laboratory condition it was 21 days while it was undefined time period in field. Rahman et al. [154, 155] studied *S. polyrhiza* for arsenic accumulation and found that more arsenic was accumulated from As (V) than that of DMAA solution. The arsenic uptake was favored by iron uptake but not by phosphate uptake. In DMAA solution, the accumulation was affected neither by iron nor by phosphate [154]. Therefore, *S. polyrhiza* has potential of removing heavy metals. Zhang et al. [156] investigated that *Wolffia globosa*, a duckweed, can also remove arsenic and has tolerance for 400 mg arsenic per kg of its dry weight. Hence it can be concluded that different species of duckweed are useful for the removal of various heavy metals. Their way of phytoremediation is cheap as well as eco-friendly which adds up to their advantages.

9.8 Conclusion

Water is an essential medium and requirement for the growth and support of all kinds of life forms. The continuous contamination of this essential medium through various kinds of anthropogenic inputs is going to pose a direct threat to the survival of all kinds of species along with humans in near future. Immediate intervention is required by the regulatory bodies and authorities for rising up to this challenging situation. A cost-effective technique and viable alternative to conventional remediation methods could be phytoremediation technology, which itself takes the help of weeds and wild aquatic plant for treatment of this contaminated water. There are several known species which could be of much help in the current situation. The successful remediation method requires the plants should have the ability of fast growth along with high biomass production in their earlier phase. Certain hardy, tolerant wild weed species can act as a strong barrier towards stopping of entering of contaminants into the food chain. The review views towards inclusion of duckweed and water hyacinth species in the current scheme of things due to their high bioaccumulation capabilities for metals along with capabilities for lowering of other essential parameters such as BOD. Various workers have researched in this area and several government-supported treatment systems have proven the worth of these weeds. These plants have been even successful in surviving very high metal concentrations in the effluents along with their potential for high level of absorption and accumulation. Their inclusion is highly recommended for treatment of contaminated water effluents.

References

1. Alluri HK, Ronda SR, Settalluri VS, Bondili JS, Suryanarayana V, Venkateshwar P (2007) Biosorption: an eco-friendly alternative for heavy metal removal. Afr J Biotechnol 6(25):2924–2931
2. Central Pollution Control Board (2008) Status of water quality in India 2007. CPCB, New Delhi, India
3. Rai LC, Gaur JP, Kumar HD (1981) Phycology and heavy metal pollution. Biol Rev Camb Philos Soc 56:99–151
4. Lokeshwari H, Chandrappa GT (2007) Effects of heavy metal contamination from anthropogenic sources on Dasarahalli tank, India. Lakes and Reservoirs. Res Manage 12:121–128
5. Chang JS, Yoon IH, Kim KW (2009) Heavy metal and arsenic accumulating fern species as potential ecological indicators in As-contaminated abandoned mines. Ecol Indic 9:1275–1279
6. Yadav SK, Juwarkar AA, Kumar GP, Thawale PR, Singh SK, Chakrabarti T (2009) Bioaccumulation and phyto-translocation of arsenic, chromium and zinc by Jatropha curcas L.: impact of dairy sludge and biofertilizer. Bioresour Technol 100:4616–4622
7. Akpor OB, Muchie M (2010) Remediation of heavy metals in drinking water and wastewater treatment systems: processes and applications. Int J Phys Sci 5(12):1807–1817
8. Ghosh M, Singh SP (2005) A review on phytoremediation of heavy metals and utilization of its byproducts. Appl Ecol Environ Res 3(1):1–18
9. Macek T, Mackova M, Kas J (2000) Exploitation of plants for the removal of organics in environmental remediation. Biotech Adv 18:23–34
10. Susarla S, Medina VF, McCutcheon SC (2002) Pytoremediation: an ecological solution to organic chemical contamination. Ecol Eng 1818:647–658
11. Xia H, Wu L, Tao Q (2003) A review on phytoremediation of organic contaminants. Chi J Appl Ecol 14:457–460
12. Hamoda MD, AL-Haddad AA (1987) Investigation of petroleum refinery effluent treatment in an aerobic fixed. Water Air Soil Pollut 199:57–65
13. Wuyep PA, Chuma AG, Awodi S, Nok AJ (2007) Biosorption of Cr, Mn, Fe, Ni, Cu and Pb metals from petroleum refinery effluent by calcium alginate immobilized mycelia of Polyporus squamosus. Sci Res Essay 2(7):217–221
14. World Health Organization (WHO) (1984) Guideline for drinking water quality recommendations, (vol. 1). World Health Organization, Geneva
15. Department of Environment, Ministry of Natural Resources and Environment, Government of Malaysia (DoE) (1974) Environmental Quality Act 1974–Act 127, Environment Quality (Sewage and Industrial Effluents) Regulations 1979-12/79
16. Cunningham SD, William RB, Jianwei WH (1995) Phytoremediation of contaminated soils. Tibtech 13:393–397
17. Cunningham SD, Ow DW (1996) Promises and prospects of phytoremediation. Plant Physiol 110(3):715–719
18. Baker AJM, McGrath SP, Sidoli CMD, Reeves RD (1994) The possibility of in situ heavy metal decontamination of polluted soils using crops of metal-accumulating plants. Resour Conserv Recycl 11:41–49
19. Brooks RR (1994) Plants and chemical elements: biochemistry, uptake, tolerance and toxicity. In: Gargo ME (ed) VCH Verlagsgesellsschaft, Weinheim, pp 88–105
20. Salt DE, Blaylock M, Kumar PBAN, Dushenkov V, Ensley BD, Chet I, Raskin I (1995) Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants. Biotechnology 13:468–474
21. Salt DE, Pickering IJ, Prince RC, Gleba D, Dushenkov S, Smith RD, Raskin I (1997) Metal accumulation by aquacultured seedlings of Indian Mustard. Environ Sci Technol 31(6):1636–1644

22. Rulkens WH, Tichy R, Grotenhuis JTC (1998) Remediation of polluted soil and sediment: perspectives and failures. Water Sci Technol 37:27–35
23. Girdhar M, Sharma NR, Rehman H, Kumar A, Mohan A (2014) Comparative assessment for hyperaccumulatory and phytoremediation capability of three wild weeds. Biotechnology 4(6):579–589
24. Black H (1995) Absorbing possibilities: phytoremediation. Environ Health Perspect 103(12):1106–1108
25. Chaudhry TM, Hayes WJ, Khan AG, Khoo CS (1998) Phytoremediation—focusing on accumulator plants that remediate metal contaminated soils. Australasian J Ecotox 4:37–51
26. Dushenkov V, Kumar PBAN, Motto H, Raskin I (1995) Rhizofiltration: the use of plants to remove heavy metals from aqueous streams. Environ Sci Technol 29:1239–1245
27. Flathman PE, Lanza GR (1998) Phytoremediation: current views on an emerging green technology. J Soil Contam 7(4):415–432
28. Zhu YL, Zayed AM, Qian JH, Souza M, Terry N (1999) Phytoaccumulation of trace elements by wetland plants, II. Water hyacinth. J Environ Qual 28:339–344
29. Arora A, Saxena S, Sharma DK (2006) Tolerance and phytoaccumulation of chromium by three Azolla species. World J Microb Biot 22:97–100
30. Umali LJ, Duncan JR, Burgess JE (2006) Performance of dead Azolla filiculoides biomass in biosorption of Au from wastewater. Biotechnol Lett 28:45–49
31. Meagher RB, Heaton AC (2005) Strategies for the engineered phytoremediation of toxic element pollution: mercury and arsenic. J Ind Microb Biot 32(11–12):502–513
32. Kapoor A, Viraraghavan T, Cullimore DR (1999) Removal of heavy metals using the fungus Aspergillus niger. Bioresour Technol 70:95–104
33. Kapoor A, Viraraghavan T (1998) Application of immobilized Aspergillus niger biomass in the removal of heavy metals from an industrial wastewater. J Environ Sci Health 33:1507–1514
34. LeDuc DL, Terry N (2005) Phytoremediation of toxic trace elements in soil and water. J Ind Microb Biot 32(11–12):514–520
35. Volesky B (1987) Biosorbent for metal recovery. Trends Biotechnol 5:96
36. Macek T, Francova K, Kochankova L, Lovecka P, Ryslava E, Rezek J et al (2004) Phytoremediation: biological cleaning of a polluted environment. Rev Environ Health 19(1):63–82
37. Lang ML, Zhang YX, Chai TY (2004) Advances in the research of genetic engineering of heavy metal resistance and accumulation in plants. Chin J Biotech 20(2):157–164
38. Prasad MNV, Freitash H (2000) Removal of toxic metals from the aqueous solution by the leaf, stem and root phytomass of Quercus ilex L. (Holly Oak). Environ Pollut 110(2):277–283
39. Upadhyay AR, Tripathi BD (2007) Principle and process of biofiltration of Cd, Cr, Co, Ni & Pb from tropical opencast coalmine effluent. Water Air Soil Pollut 180(1–4):213–223
40. Xia H, Ma X (2005) Phytoremediation of ethion by water hyacinth (Eichhornia crassipes) from water. Biores Technol 97:1050–1054
41. Lu Q (2009) Evaluation of aquatic plants for phytoremediation of eutrophic stormwaters. PhD Thesis, University of Florida, Florida
42. Roongtanakiat N, Tangruangkiat S, Meesat R (2007) Utilization of vetiver grass (Vetiveria zizanioides) for removal of heavy metals from industrial wastewaters. Sci Asia 33:397–403
43. Stefani GD, Tocchetto D, Salvato M, Borin M (2011) Performance of a floating treatment wetland for in-stream water amelioration in NE Italy. Hydrobiologia 674:157–167
44. Singh OV, Labana S, Pandey G, Budhiraja R, Jain RK (2003) Phytoremediation: an overview of metallic ion decontamination from soil. Appl Microbiol Biotechnol 61:405–412
45. Xia H, Ma X (2006) Phytoremediation of ethion by water hyacinth (Eichhornia crassipes) from water. Biores Technol 97:1050–1054
46. Jamuna S, Noorjahan CM (2009) Treatment of sewage waste water using water hyacinth—Eichhornia sp and its reuse for fish culture. Toxicol Int 16(2):103–106
47. Mkandawire M, Dudel EG (2007) Are lemna spp. effective phytoremediation agents. Bioreme Biodiv Bioavail 1(1):56–71

48. Mashauri DA, Mulungu DMM, Abdulhussein BS (2000) Constructed wetland at the university of Dar es salaam. Water Res 34(4):1135–1144
49. Baskar G, Deeptha VT, Rahman AA (2009) Treatment of wastewater from kitchen in an institution hostel mess using constructed wetland. Int J Recent Trends Eng 1(6):54–58
50. Sinha RK, Herat S, Tandon PK (2004) 14 phytoremediation: role of plants in contaminated site management. In: Book of environmental bioremediation technologies, Springer, Berlin, pp 315–330
51. U. S. Department of Energy (1994) Mechanisms of plant uptake, translocation, and storage of toxic elements. Plume Focus Area, December. Summary Report of a workshop on phytoremediation research needs
52. Tangahu BV, Abdullah SRS, Basri H, Idris M, Anuar N, Mukhlisin M (2011) A review on heavy metals (As, Pb, and Hg) uptake by plants through phytoremediation. Int J Chem Eng 1–31
53. Salido AL, Hasty KL, Lim JM, Butcher DJ (2003) Phytoremediation of arsenic and lead in contaminated soil using Chinese Brake ferns (Pteris vittata) and Indian mustard (Brassica juncea). Int J Phytoremediat 5(2):89–103
54. Erdei L, Mezosi G, Mecs I, Vass I, Foglein F, Bulik L (2005) Phytoremediation as a program for decontamination of heavy-metal polluted environment. In: Proceedings of the eighth Hungarian congress on plant physiology and the sixth Hungarian conference on photosynthesis
55. Abbasi SA (1987) Aquatic plant based water treatment systems in Asia. In: Reddy KR (ed) Aquatic plants for water treatment and resource recovery. Magnolia Publishers, Orlando
56. Kay SH, Haller WT, Garrad LA (1984) Effect of heavy metals on water hyacinths [Eichhornia crassipes (Mart.) Solms. Aquat Toxicol 5:117–128
57. Zhu YL, Zayed AM, Quian JH, De Souza M, Terry N (1999) Phytoaccumulation of trace elements by wetland plants: II. Water hyacinth. J Environ Qual 28:339–344
58. Dinges R (1976) Water hyacinth culture for wastewater treatment. Texas Department of Health Resources, Austin
59. Wolverton BC, McDonald RC (1979) The water hyacinth: from prolific pest to potential provider. Ambio 8:1–12
60. Aquatics (2005) PSS 123 Garden Flowers. http://pss.uvm.edu/pss123/aquatics.html
61. Wilson JR, Holst N, Rees M (2005) Determinants and patterns of population growth in water hyacinth. Aquat Bot 81:51–67
62. Bolenz S, Omran H, Gierschner K (1990) Treatments of water hyacinth tissue to obtain useful products. Biol Waste 33(4):263–274
63. APIRIS (2005) Invasive non indigenous plants in Florida. http://plants.ifas.ufl.edu/hyacin2.html
64. Dhote S, Dixit S (2009) Water quality improvement through macrophytes—a review. Environ Monit Assess 152:149–153
65. Lissy AMPN, Madhu BG (2010) Removal of heavy metals from waste water using water hyacinth. In: Proceedings of the international conference on advances in civil engineering, pp 42–47
66. Dar SH, Kumawat DM, Singh N, Wani KA (2011) Sewage treatment potential of water hyacinth (Eichhornia crassipes). Res J Environ Sci 5(4):377–385
67. Patil JH, Raj MLA, Bhargav S, Sowmya SR (2011) Anaerobic co-digestion of water hyacinth with primary sludge. Res J Chem Sci 1(3):72–77
68. Villamagna AM (2009) Ecological effects of water hyacinth (Eichhornia crassipes) on lake Chapala, Mexico, PhD thesis. Fisheries and Wildlife Sciences, Virginia Polytechnic Institute and State University, Blacksburg
69. O’Brien WJ (1981) Use of aquatic macrophytes for wastewater treatment. Amer Soc Civ Engineers (ASCE) Env Eng Div 107:681–698
70. Hasan MR, Chakrabarti R (2009) Use of algae and aquatic macrophytes as feed in small-scale aquaculture: a review. FAO Fisheries and Aquaculture Technical Paper, p 531
71. Center TD, Hill MP, Cordo H, Julien MH (2002) Water hyacinth. In: Van Driesche R et al (eds) Biological control of invasive plants in the Eastern United States. USDA Forest Service Publication, Morgantown, FHTET-2002-04, 41-64

72. Center TD, Van TK, Dray FA Jr, Franks SJ, Rebelo MT, Pratt PD, Rayamajhi MB (2005) Herbivory alters competitive interactions between two invasive aquatic plants. Biol Control 33:173–185
73. USEPA (1988) Design Manual—constructed wetlands and aquatic systems for municipal wastewater treatment, United States Environmental Protection Agency, Report no. EPA/625/1-88/022, Office of Research and Development, Cincinnati, p 83
74. Debusk TA, Dierberg FE (1989) Effects of nutrient availability on water hyacinth standing crop and detritus deposition. Hydrobiologia 174:151–159
75. Moorhead KK, Reddy FR, Graetz DA (1988) Water hyacinth productivity and detritus accumulation. Hydrobiologia 157:179–185
76. Reddy KR, Agami M, Tucker JC (1989) Influence of nitrogen supply rates on growth and nitrogen storage by water hyacinth (Eichhornia crassipes) plants. Aquat Bot 36:33–43
77. Sato M, Kondo S (1981) Biomass production of water hyacinth and its ability to remove inorganic minerals from water. I. Effect of the concentration of culture solution on the rates of plant growth and nutrient uptake. Jap J Ecol 31:257–267
78. Reddy KR (1981) Diel variations in physio-chemical parameters of water in selected aquatic systems. Hydrobiologia 85(3):201–207
79. Beath JM (2000) Consider phytoremediation for waste site cleanup. Chem Eng Progr 96(7):61–69
80. Wolverton BC, McDonald RC (1979) Water hyacinth (Eichhornia crassipes) productivity and harvesting studies. Econ Bot 33:1–10
81. Sooknah RD, Wilkie AC (2004) Nutrient removal by floating aquatic macrophytes cultured in anaerobically digested flushed dairy manure wastewater. Ecol Eng 22:27–42
82. Ayyasamy PM, Rajakumar S, Sathishkumar M, Swaminathan K, Shanthi K, Lakshmanaperumalsamy P, Lee S (2009) Nitrate removal from synthetic medium and groundwater with aquatic macrophytes. Desalination 242:286–296
83. Richards JH (1982) Development potentials of axillary birds of water hyacinth (Eichhornia crassipes). Amer J Bot 69:615–622
84. Valipour A, Raman VK, Motallebi P (2010) Application of shallow pond system using water hyacinth for domestic wastewater treatment in the presence of high total dissolved solids (TDS) and heavy metal salts. Environ Eng Manage J 9(6):853–860
85. Brix H, Shierup HH (1989) The use of aquatic macrophytes in water pollution control. Ambio 18:100–107
86. Mandi L (1994) Marrakesh wastewater purification experiment using vascular aquatic plants Eichhornia crassipes and Lemna gibba. Water Sci Technol 29:283–287
87. Chigbo FE, Smith RW, Shore FL (1982) Uptake of arsenic, cadmium, lead and mercury from polluted waters by the water hyacinth. Environ Poll A27:31–36
88. John CK (1985) Treatment of agro-industrial wastes using water hyacinth. Water Sci Technol 17(4–5):781–790
89. Mane AV, Saratale GD, Karadge BA, Samant JS (2011) Studies on the effects of salinity on growth, polyphenol content and photosynthetic response in Vetiveria zizanioides (L.) Nash. Emir J Food Agric 23(1):59–70
90. O'Keefe DH, Hardy JK, Rao RA (1984) Cadmium uptake by water hyacinth: effect of solution factors. Environ Pollut Series A 34:133–147
91. Mishra VK, Tripathi BD (2008) Concurrent removal and accumulation of heavy metals by the three aquatic macrophytes. Biores Technol 99:7091–7097
92. Haider SZ, Malik KMA, Rahman MN, Wadsten T (1984) Proceedings of international conference on water hyacinth. UNEP, Nairobi, p 351
93. Liao SW, Chang WL (2004) Heavy metal phytoremediation by water hyacinth at constructed wetlands in Taiwan. J Aquat Plant Manage 42:60–68
94. Wang Q, Cui Y, Dong Y (2002) Phytoremediation of polluted waters: potentials and prospects of wetland plants. Acta Biotechnol 22:199–208
95. Zayed A, Gowthaman S, Terry N (1998) Phytoaccumulation of traces elements by wetland plants: I. Duckweed J Environ Qual 27:715–721

96. Soltan ME, Rashed MN (2003) Laboratory study on the survival of water hyacinth under several conditions of heavy metal concentrations. Adv Environ Res 7:82–91
97. Amin SM (2006) Environmental safety of petroleum refinery operation: Malaysian case studies. Graduate thesis, Faculty of Engineering, University of Malaya
98. Beddri AM (2006) Removal of heavy metals in refinery effluents using water hyacinth. Graduate Thesis, Civil Eng Department, University of Malaya
99. Eapen S, D' Souza SF (2005) Prospects of genetic engineering of plants for phytoremediation of toxic metals. Biotech Adv 23(2):97–114
100. Borges AKP, Tauk-Tornisielo SM, Domingos RN, Angelis DF (2008) Performance of the constructed wetland system for the treatment of water from the Corumbatai river. Braz Arch Biol Technol 51(6):1279–1286
101. Shah RA, Kumawat DM, Singh N, Wani KA (2010) Water hyacinth (Eichhornia crassipes) as a remediation tool for dye-effluent pollution. Int J Sci Nature 1(2):172–178
102. Mangas-Ramirez E, Elias-Gutierrez M (2004) Effect of mechanical removal of water hyacinth (Eichhornia crassipes) on the water quality and biological communities in a Mexican reservoir. J Aquat Ecosys Health Manage 7:161–168
103. Perna C, Burrows D (2005) Improved dissolved oxygen status following removal of exotic weed mats in important fish habitat lagoons of the tropical Burdekin river floodplain, Australia. Marine Poll Bull 51:138–148
104. Trivedy RK, Pattanshetty SM (2002) Treatment of dairy waste by using water hyacinth. Water Sci Technol 45(12):329–334
105. Sooknah R (2000) A review of the mechanisms of pollutant removal in water hyacinth systems. Sci Tech Res J 6:49–57
106. Lehn H, Bopp M (1987) Prediction of heavy-metal concentrations in mature plants by chemical analysis of seedlings. Plant Soil 101:9–14
107. EL-Leboudi AE, Abd-Elmoniem EM, Soliman EM, El-Sayed OF (2008) Removal of some heavy metals from treated waste water by aquatic plants. In: 3rd international conference on water resources and arid environments and the 1st Arab Water Forum, Riyadh, Saudi Arabia
108. Gamage NS, Yapa PAJ (2001) Use of water hyacinth [Eichhornia crassipes (Mart) solms] in treatment systems for textile mill effluents—a case study. J Natn Sci Foundation Sri Lanka 29(1–2):15–28
109. Kulatillake N, Yapa PAJ (1984) A study on the use of water hyacinth in rubber effluent treatment systems. In: Proceedings of the Malaysian Chemical Congress, Kuala Lumpur
110. Snow AM, Ghaly AE (2008) A comparative study of the purification of aquaculture wastewater using water hyacinth, water lettuce and parrot's feather. Am J Appl Sci 5(4):440–453
111. Koottatep T, Polprasert C (1997) Role of plant uptake on nitrogen removal in constructed wetlands located in the tropics. Water Sci Technol 36:1–8
112. Jing SR, Lin YF, Lee DY, Wang TW (2001) Nutrient removal from polluted river water by using constructed wetlands. Bioresour Technol 76:131–135
113. Elias JM, Salati FE, Salati E (2001) Performance of constructed wetland system for public water supply. Water Sci Technol 44:579–584
114. Schulz C, Gelbrecht J, Rennert B (2004) Constructed wetlands with free water surface for treatment of aquaculture effluents. J Appl Ichthyol 20:64–70
115. Cornwell DA, Zoltek J, Patrinely CD (1977) Nutrient removal by water hyacinths. J Water Poll Control Fed 49:57–65
116. Ingersoll T, Baker LA (1998) Nitrate removal in wetland microcosms. Water Res 32:677–684
117. Sheffield CW (1967) Water hyacinth for nutrient removal. Hyacinth Cont J 6:27–30
118. Reddy KR, Campbell KL, Graetz DA, Portier KM (1982) Use of biological filters for treating agricultural drainage effluents. J Environ Qual 11:591–595
119. Ornes WH, Sutton DL (1975) Removal of phosphorus from static sewage effluent by water hyacinth. Hyacinth Cont J 13:56–61
120. Knipling EB, West SH, Haller WT (1970) Growth characteristics, yield potential and nutritive content of water hyacinths. Hyacinth Control J 8:34–35
121. Rogers HH, Davis DE (1972) Nutrient removal by water hyacinth. Weed Sci 20:423–428

122. Reddy KR, Tucker JC (1983) Productivity and nutrient uptake of water hyacinth, Eichhornia crassipes I. Effect of nitrogen source. Econ Bot 37:237–247
123. Chaiprapat S, Cheng JJ, Classen JJ, Liehr SK (2005) Role of internal nutrient storage in duckweed growth for swine wastewater treatment. Trans ASAE 48(6):2247–2258
124. Jain SK, Gujral GS, Jha NK, Vasudevan P (1988) Heavy metal uptake by pleurotus Sajor-Caju from metal enriched Duckweed substrate. Biol Waste 24:275–282
125. Devi PU, Akagi K, Ostapenko V, Tanaka Y, Sugahara T (1996) Withaferin A: a new radiosensitizer from Indian medicinal plant Withania somnifera. Int J Radiat Biol 69(2):193–197
126. Brain AR, Johnson DJ, Richards SM, Hanson ML, Sanderson H, Lam MW, Young C, Mabury SA, Sibley PK, Solomon KR (2004) Microcosm evaluation of the effects of an eight pharmaceutical mixture to the aquatic macrophytes Lemna gibba and Myriophyllum sibiricum. Aquat Toxicol 70:23–40
127. Kummerova M, Zezulka S et al (2007) Photoinduced toxicity of fluoranthene on primary processes of photosynthesis in lichens. Lichenologist 39:91–100
128. Cunningham SD, Berti WR (1993) Remediation of contaminated soil with green plants: an overview. In vitro Cell Dev Biol 29:207–212
129. Raskin I, Nanda-Kumar PBA, Dushenkov S, Salt DE (1994) Bio-concentration of heavy metals by plants. Curr Opin Biotechnol 5:285–290
130. Salt DE, Smith RD, Raskin I (1998) Phytoremediation. Annu Rev Plant Physiol Plant Mol Biol 49:643–668
131. Sandra R, Drazenka S, Petra C, Lvanka ML, Marija RM, Sinisa S, Branka KP, Mirjana P (2010) Ecotoxicological assessment of industrial effluent using Duckweed (Lemna minor L.) as a test organism. Ecotoxicology 19(1):216–222
132. Chaudhary E, Sharma P (2014) Duckweed plant: a better future option for phytoremediation. Int J Emerg Sci Eng (IJESE) 2(7):39–41
133. Mkandawire M, Tauert B et al (2004) Capacity of Lemna gibba L. (Duckweed) for uranium and arsenic phytoremediation in mine tailing waters. Int J Phytoremediation 6(4):347–362
134. Mkandawire M, Dudel EG (2005) Accumulation of arsenic in Lemna gibba L. (duckweed) in tailing waters of two abandoned uranium mining sites in Saxony, Germany. Sci Total Environ 336(1–3):81–89
135. Bianconi D, Pietrini F, Massacci A, Iannelli MA (2013). Uptake of Cadmium by Lemna minor, a (hyper?-) accumulator plant involved in phytoremediation applications. E3S Web of conferences 1 13002. doi:http://dx.doi.org/10.1051/e3sconf/20130113002
136. Sharma SS, Gaur JP (1995) Potential of Lemna polyrrhiza for the removal of heavy metal. Ecol Eng 4:37–43
137. Donganlar BZ, Seher C, Telat Y (2012) Metal uptake and physiological changes in Lemna gibba exposed to manganese and nickel. Int J Biol 4:148–151
138. Axtell NR, Sternberg SPK, Claussen K (2003) Lead and nickel removal using Microspora and Lemna minor. Biores Technol 89:41–48
139. Horvat T, Vidakovic Cifrek Z, Orescanin V, Tkalec M, Pevalek Kozlina B (2007) Toxicity assessment of heavy metal mixtures by *Lemna minor* L. Sci Total Environ 384:229–238
140. Kaur L, Kasturi G, Sharma S (2012) Role of pH in the accumulation of lead and nickel by common Duckweed (Lemna minor). Inter J Bioassays 1(12):191–195
141. Uysal Y (2013) Removal of chromium ions from wastewater by Duckweed, Lemna minor L by using a pilot system with continuous flow. J Hazard Mater 263:486–492
142. Khellaf N, Zerdaoui M (2009) Growth response of the Duckweed Lemna minor to heavy metal pollution. Iranian J Environ Health Sci Eng 6(3):161–166
143. Stefan G, Christoph H, Christoph H, Kerstin K, Albrecht P (2010) Whole effluent assessment of industrial wastewater for determination of bat compliance. Environ Sci Pollut R 17:856–865
144. Loveson A, Sivalingam R (2013) Phyto toxicological assessment of two backwater wetlands in Kannamaly, Ernakulam using aquatic macrophyte—Spirodela polyrhiza. J Environ Anal Toxicol 3(180)

145. Hegazy AK, Kabiel HF, Fawzy M (2009) Duckweed as heavy metal accumulator and pollution indicator in industrial wastewater ponds. Desalin Water Treat 12(1):400–406
146. Smain M, Saida S, Michel C (2009) Toxicity and removal of heavy metals (Cadmium, Copper, and Zinc) by Lemna gibba. Ecotox Environ Safe 72:774–780
147. Ahmet S, Erdal O (2009) The accumulation of Arsenic, Uranium, and Boron in Lemna gibba L. Exposed to secondary effluents. Ecol Eng 35:1564–1567
148. Teixeira S, Vieira MN, Espinha Marques J et al (2014) Bioremediation of an iron-rich mine effluent by Lemna Minor. Int J Phytoremediation 16:1228–1240
149. Clatworthy JN, Harper JL (1960) The comparative biology of closely related species living in the same area. Comparative Biol 13:307–324
150. Culley DD, Rejmankova E, Kvet J, Frye JB (1981) Production, chemical quality and use of duckweeds (Lemnaceae) in aquaculture, wastewater management, and animal feeds. J World Mariculture Soc 12(1):27–49
151. Robinson B, Duwig C, Bolan N, Kannathasan M, Saravanan A (2003) Uptake of arsenic by New Zealand watercress (*Lepidium sativum*). Sci Total Environ 301:67–73
152. Sizova OI, Kochetkov VV, Validov SZ, Boronin AM, Kosterin PV, Lyubun YV (2002) Arsenic-contaminated soils: genetically modified Pseudomonas spp. and their arsenic-phytoremediation potential. Soils Sediments 2:19–23
153. Mkandawire M, Lyubun YV, Kosterin PV, Dudel EG (2004) Toxicity of arsenic species to Lemna gibba L. and the influence of phosphate on arsenic bioavailability. Environ Toxicol 19:26–35
154. Rahman MA, Hasegawa H, Ueda K, Maki T, Okumura C, Rahman MM (2007) Arsenic accumulation in duckweed (Spirodela polyrhiza L.): a good option for phytoremediation. Chemosphere 69:493–499
155. Rahman MA, Hasegawa H, Kitahara K, Maki T, Ueda K, Rahman MM (2008) The effects of phosphorous on the accumulation of arsenic in water fern (Azolla pinnata L.). J Eco Technol Res 14:21–24
156. Zhang X, Zhao FJ, Huang Q, Williams PN, Sun GX, Zhu YG (2009) Arsenic uptake and speciation in the rootless duckweed Wolffia globosa. New Phytol 182:421–428
157. Verma VK, Tewari S, Rai JPN (2008) Ion exchange during heavy metal bio-sorption from aqueous solution by dried biomass of macrophytes. Bioresour Technol 99:1932–1938

Chapter 10
Constructed Wetlands: Role in Phytoremediation of Heavy Metals

Syed Shakeel Ahmad, Zafar A. Reshi, Manzoor A. Shah, and Irfan Rashid

10.1 Constructed Wetlands: Importance and Types

Constructed wetlands (CWs) are engineered systems that are designed and constructed to utilize the natural processes, involving wetland vegetation, soils, and their associated microbial assemblages to assist in treating wastewater [1]. According to the Interstate Technology Regulatory Council Wetlands Team, USA (ITRC) [2], constructed wetlands (CWs) are "*engineered systems, designed and constructed to utilize the natural functions of wetland vegetation, soils and their microbial populations to treat contaminants in surface water, groundwater or waste streams.*" Synonymous terms for constructed wetlands include man-made, engineered, and artificial wetlands. The first full-scale constructed wetland (CW) for wastewater treatment was built at Petrov near Prague in May 1989. Constructed wetlands are a cost-effective and technically feasible approach to treating wastewater and runoff. The constructed wetland provides a natural environment of warm climate, high water table, and high organic matter for microbes to break down contaminants [3].

The use of constructed wetlands for wastewater treatment is becoming more and more popular in many parts of the world. Today, subsurface flow constructed wetlands are quite common in many developed countries, such as Germany, the UK, France, Denmark, Austria, Poland, and Italy [4]. Constructed wetlands are also appropriate for developing countries but due to lack of awareness their use is not widespread [5–7]. Constructed wetlands can be less expensive to build than other treatment options. Operation and maintenance expenses (energy and supplies) are low and require only periodic, rather than continuous monitoring. Constructed wetlands are primarily used to treat domestic municipal wastewaters, but their use for other types of wastewaters such as agricultural and industrial

---

S.S. Ahmad (✉) • Z.A. Reshi • M.A. Shah • I. Rashid
Department of Botany, University of Kashmir, Srinagar, J&K 190 006, India
e-mail: ssahmad900@gmail.com

A.A. Ansari et al. (eds.), *Phytoremediation*, DOI 10.1007/978-3-319-40148-5_10

wastewaters, various runoff waters, and landfill leachate have become more frequent [8–10].

Mitsch [11] suggests the following guidelines for creating successful constructed wetlands:

1. Keep the design simple. Complex technological approaches often invite failure.
2. Design for minimal maintenance.
3. Design the system to use natural energies, such as gravity flow.
4. Design for the extremes of weather and climate.
5. Design the wetland with the landscape, not against it.
6. Integrate the design with the natural topography of the site.
7. Avoid over-engineering the design with rectangular basins, rigid structures and channels, and regular morphology. Mimic natural systems.

10.1.1 Types of Constructed Wetlands

The classification of constructed wetlands is based on various factors, such as the vegetation type, hydrology, and flow of direction (vertical or horizontal) [12]. There are mainly three types of constructed wetlands: surface flow wetlands, subsurface flow wetlands, and hybrid systems.

10.1.1.1 Surface Flow Wetlands

In case of surface flow wetlands, water level is above the ground surface; vegetation is rooted and emerges above the water surface: water flow is primarily above ground. The different macrophytes that are used in this type of constructed wetlands include *Phragmites australis, Typha ungustifolia, Sparganium erectum* etc.

10.1.1.2 Free Floating Macrophyte-Based Wetlands

In this type of constructed wetlands, floating macrophytes are used. The main floating macrophytes used in these systems are *Azolla cristata, Salvinia natans*, water hyacinth *Eichhornia crassipes and* duckweeds [13]. The different submerged species used in these constructed wetlands prevent entry of light into the system thereby inhibiting the growth of different algal groups. Hence the macrophytes need to be periodically removed from the wetland. The floating plant mat blocks out sunlight, thereby preventing photosynthesis and inhibiting algae growth hence the macrophytes need to be periodically removed from the system [14]. Duckweeds are extremely invasive and grow in most environments [15].

10.1.1.3 Submerged Macrophyte-Based Wetlands

In these constructed wetlands there are used different kinds of submerged macrophytes. The main submerged species used in these constructed wetlands include *Ceratophyllum demersum, Hydrilla* sp., *Potamogeton* sp. etc. They have been proposed as final polishing steps following primary and secondary treatment [16].

10.1.1.4 Emergent Macrophyte-Based Wetlands

In this type of constructed wetlands, emergent macrophytes are used. Emergent macrophyte-based wetlands are the most common type of constructed wetlands. The different emergents used in these constructed wetlands include *Juncus effusus, Phregmites australis, Typha ungustifolia, Sparganium erectum* etc. [17, 18, 19]. A slow flow rate is applied so that a shallow depth is maintained [20].

10.1.1.5 Subsurface Flow Wetlands

They are also called vegetated submerged beds, or plant-rock filter systems. They have below ground water level. The basin mainly consists of sand or gravel. The different macophytes used in this type of constructed wetlands include *Glyceria maxima, Iris pseudacorus, Phragmites australis, Typha ungustifolia, Sparganium erectum* etc.

10.1.1.6 Horizontal Flow Constructed Wetland (HF CWs)

This type of constructed wetland was developed in the 1950s in Germany by Käthe Seidel [12]. This type of constructed wetland consists of rock or gravel beds, impermeable layer and wetland vegetation. Waste water afer entering through the inlet passes through the horizontal path before it is discharged through the outlet. Hence the name horizontal flow constructed wetland.

10.1.1.7 Vertical Flow Constructed Wetlands (VF CWs)

In this type of constructed wetland water percolated down through sand medium. Among different kinds of constructed wetlands this type of constructed wetland has very high operational costs.

10.1.1.8 Free Water Surface Constructed Wetland (FWS CW)

This type of constructed wetland consists of a series of impermeable bains about 20–40 cms deep. In this type of constructed wetland the main macrophytes planted include emergents like *Phragmites australis, Typha ungustifolia, Sparganium erectum* etc.

10.1.1.9 Constructed Wetlands with Floating Leaved Macrophytes

Constructed wetlands with floating leaved macrophytes are very rare, and there are no guidelines to design, operate, and maintain these systems [1]. In these systems, the different plants used are *Nelumbo nucifera* [21, 22] and *Nuphur lutea* [23].

10.1.1.10 Hybrid Systems

Different types of constructed wetlands may be combined with each other in order to exploit the specific advantages of the different systems. In hybrid or multistage systems, different cells are designed for different types of reactions. During the 1990s, HF-VF and VF-HF hybrid systems were introduced [24]. Hybrid systems are used especially when removal of ammonia-N and total-N is required [1].

10.1.2 How Constructed Wetlands Work

Constructed wetlands are made up of a series of ponds each designed to perform a particular function. Solids are allowed to settle in the primary storage ponds. The water then enters another pond containing vegetation. Here physical, chemical, and biological reactions reduce contaminants. Nitrogen and phosphorus are used by aquatic vegetation. Heavy metals are also removed by different plants which show tolerance for these metals. Water then enters the tertiary cell. It serves as a habitat for wildlife.

10.1.3 Costs for Creating Constructed Wetlands

The main requirements for establishing constructed wetlands include land, design, vegetation, hydraulic control system and fencing. The total investment costs for establishing constructed wetlands vary from county to country and could be as low as 29 USD per m^2 in India [25] or 33 USD per m^2 in Costa Rica [26], or as high as 257 EUR per m^2 in Belgium [27].

For the development of constructed wetlands, the basic requirements are containers, plant species, sand, and gravel media in certain ratio. In different types of constructed wetlands microbes and other invertebrates develop naturally [28]. The three types of macrophytes that are used in constructed wetlands are floating macrophyte (i.e., *Azolla sp., Salvinia natans*, *Lemna* spp. or *Eichhornia crassipes*), submerged macrophyte (i.e., *Ceratophyllum demersum, Potamogeton* sp. *Elodea canadensis*) and rooted emergent macrophyte (i.e., *Phragmites australis, Typha* spp. *Sparganium erectum* [29]). Plants (free-floating, emergent or submergent vegetation) are the part of constructed ecosystem to remediate contaminants from municipal, industrial wastewater, metals, and acid mine drainage [30]. The different macrophytes that are used in subsurface flow CWs in warm climates are Papyrus sedge (*Cyperus papyrus*), Umbrella sedge (*Cyperus albostriatus* and *Cyperus alternifolius*), Dwarf papyrus (*Cyperus haspens*), Bamboo, smaller ornamental species, Broad-leaved cattail (*Typha latifolia),* Species of genus—*Heliconia:* lobster-claws, wild plantains—*Canna:* Canna lily—*Zantedeschia:* Calla lily Napier grass or Elephant grass (*Pennisetum purpureum*).

10.2 Heavy Metals: Sources and Impacts

Constructed wetlands are designed for the removal of different kinds of pollutants including heavy metals from the wastewater. Heavy metals released from different sources enter into the water bodies and pose serious threats to different trophic levels of the food chain including human beings. Heavy metals are metals having a density of 5 g/cm^3 [31]. Heavy metals include a category of 53 elements with specific weight higher than 5 g/cm^3 [32, 33]. Heavy metals are elements with metallic properties and an atomic number >20. Heavy metals mainly include the transition metals, some metalloids, lanthanides, and actinides. The most common heavy metal contaminants are As, Cd, Cr, Cu, Hg, Pb, and Zn.

Over population, industrialization, rapid urbanization, overuse of pesticides, detergent and agricultural chemicals, liquid and solid waste products, and discharge of municipal wastes resulted in heavy metal pollution of natural water resources [34]. Man-made activities such as mining and smelting of metal ores, industrial, commercial, and domestic applications of insecticides and fertilizers have all contributed to elevated levels of heavy metals in the environment [35, 36].

The primary sources of metal pollution are the burning of fossil fuels, mining and smelting of metalliferous ores, municipal wastes, sewage, pesticides, and fertilizers [37] oil, gasoline and coal combustion, smelting, and refuse incineration [38]. In uncontaminated soil, the average concentrations of heavy metals vary in orders of magnitudes, but on average the concentrations are, e.g., Zn: 80 ppm, Cd: 0.1–0.5 ppm and Pb: 15 ppm. However, in polluted soil dramatically higher concentrations are found, e.g., Zn: >20,000 ppm, Cd: >14,000 ppm and Pb: >7000 ppm (http://www.speclab.com/elements/). Heavy metals are ubiquitous environmental pollutants that arise from a variety of industrial, commercial, and domestic activities

[36]. Increasing industrial activities have led to an increase in environmental pollution and the degradation of several aquatic ecosystems with the accumulation of metals in biota and flora [39]. According to Phuong et al. [40], most heavy metal contaminants originate from anthropogenic sources such as long-term discharge of untreated domestic and industrial wastewater runoff, accidental spills, and direct soil waste dumping. In addition, heavy metals can enter the water bodies through atmospheric sources [41] and nearby rice fields [42]. Due to innovations in mining and metal-working techniques during ancient times, the close relationship between metals, metal pollution, and human history was formed [43]. Energy intensive and chlor-alkali industries for the manufacture of agrochemicals deteriorate the water quality of lakes and reservoirs due to the discharge of various pollutants, especially a range of heavy metals [44]. Coal mining [45] and its allied/dependent industries (thermal power plants) are major sources of heavy metals in the industrial belts of developing countries such as India [44, 46]. Metals are natural components in soil [47]. Lead is a common pollutant from road runoff. Zinc is a common metal present in variable amounts and if found in appreciable amounts can be an indicator of industrial pollution. While copper is also an indicator of industrial contamination of urban waters [48, 49]. The different macrophytes have a potential to sequester heavy metals from the soils contaminated with these metals. Colonization of macrophytes on the sediments polluted with heavy metals and the role of these plants in transportation of metals in shallow coastal areas are very important [50].

Contamination of aquatic environment by heavy metals is a serious environmental problem, which threatens aquatic ecosystems, agriculture, and human health [51]. Accumulation of metals and their toxic effects through the food chain can lead to serious ecological and health problems [52]. Heavy metals are the most dangerous contaminants since they are persistent and accumulate in water, sediments and in tissues of the living organisms, through two mechanisms, namely "bioconcentration" (uptake from the ambient environment) and "biomagnification" (uptake through the food chain) [53]. Trace elements such as Cu, Fe, Mn, Ni, and Zn are essential for normal growth and development of plants. They are required in numerous enzyme catalyzed or redox reactions, in electron transfer and have structural function in nucleic acid metabolism [54]. Metals like Cd, Pb, Hg, and As are not essential [55]. High levels of Cd, Cu, Pb, and Fe can act as ecological toxins in aquatic and terrestrial ecosystems [56, 57]. Excess metal levels in surface water may pose a health risk to humans and to the environment [50]. Since HM are not biodegradable and may enter the food chain, they are a long-term threat to both the environment and human health [58]. Some of these metals are micronutrients necessary for plant growth, such as Zn, Cu, Mn, Ni, and Co, while others have unknown biological function, such as Cd, Pb, and Hg [59]. Metal pollution has harmful effect on biological systems and does not undergo biodegradation. Toxic heavy metals such as Pb, Co, and Cd can be differentiated from other pollutants, since they cannot be biodegraded but can be accumulated in living organisms, thus causing various diseases and disorders even in relatively lower concentrations [60]. Heavy metals, with soil residence times of thousands of years, pose numerous health dangers to higher organisms. They are also known to have effect to plant growth, ground cover, and have a negative impact on soil microflora [61]. It is well known that heavy metals cannot be chemically degraded and need to be physically removed or be transformed into nontoxic compounds [59]. Table 10.1 shows the harmful effects of different heavy metals on living organisms.

10.3 Role of Constructed Wetlands in Phytoremediation

The use of wetlands for quality improvement of wastewater, referred to as rhizofiltration, is the best known and most researched application of constructed wetlands. Flooding of wetland sediments leads to rapid denitrification because of anoxic conditions; therefore, wetland soils contain low levels of nitrate [62]. CWs have proven successful for remediating a variety of water quality issues, with advantages over the natural wetland, Constructed wetland (CWs) thus designed to take advantage of natural wetland systems, but do so within a more controlled way. The plants most often used in CWs are persistent emergent plants, such as bulrushes (*Scirpus*), spikerush (*Eleocharis*), and other sedges (*Cyperus*), Rushes (*Juncus*), common reed (*Phragmites*), and cattails (*Typha*). Plants for CWs must be able to tolerate continuous flooding and exposure to waste streams containing relatively high and often variable concentrations of pollutants. The functions of wetland plants make them an important component of CWs. Plants contribute to contaminant removal by altering hydrology, sequestering particulates, and accumulating pollutants [63]. These processes can be utilized to design CWs with a number of treatment approaches, which are mainly phytoextraction, rhizofiltration, and phytostabilization.

Some other macrophytes that are used in wetlands for the removal of heavy metals include *Acorus calamus, Carex* spp. (sedges), *Cyperus* (sweet manna grass), *Juncus* sp. (Rushes)*, Phalaris arundinacea* (reed canary grass), *Phragmites australis* (common reed), *Sagittaria* (arrow heads), *Scirpus* sp. (Balrushes), *Sparganium* sp. (bur reeds), *Spartina* spp. (cordgrasses), *Typha* sp. (cattails), *Ziznia aquatic* (wild rice), *Ceratophyllum* sp. (coontails), *Eggeria densa* (Brazilian waterweed), *Hydrilla verticillata* (Hydrilla), *Isoetes* sp. (Quillworts), *Myriophyllum* spp. (water milfoils), *Najas* spp. (water nymphs), *Potamogeton* sp. (pond weeds), *Urticularia* spp. (bladderworts), *Lemna* spp. (duckweed), *Azolla* (aquatic fern) and *Hydrocharis* (frog bit). These macrophytes are highly beneficial to aquatic ecosystems because they provide food and shelter for fish and aquatic invertebrates, wildlife also produce oxygen, which helps in overall lake functioning [50]. Macrophytes are considered as important components of the aquatic ecosystems not only as food source for aquatic invertebrates, but they also act as an efficient accumulator of heavy metals [64, 65]. Aquatic plants sequester large quantities of metals [66–68]. Trace element removal by wetland vegetation can be greatly enhanced by the judicious selection of appropriate wetland plant species. Selection is based on the type of elements to be removed, the geographical location, environmental conditions, and the known accumulation capacities of the species.

Table 10.1 Harmful effect of different heavy metals on living organisms

Heavy metal	Harmful effects	References
As	It interferes with oxidative phosphorylation and ATP synthesis	Tripathi et al. [79]
Cd	Inhaling Cd leads to respiratory and renal problems. It also interferes with calcium regulation in biological systems; causes chronic anemia. It is also carcinogenic, mutagenic, and teratogenic; endocrine disruptor	Salem et al. [80] and Awofolu [81]
Cr	It can result in gastritis, nephrotoxicity, and hepatotoxicity. Chromium toxicity causes hair loss	Salem et al. [80] and Paustenbach et al. [82]
Cu	Excessive free copper impairs zinc homeostasis, and vice versa, which in turn impairs antioxidant enzyme function, increasing oxidative stress. It causes brain and kidney damage, liver cirrhosis and chronic anemia, stomach and intestinal irritation	Salem et al. [80], Wuana and Okieimen [83], and Sandstead [84]
Hg	Anxiety, autoimmune diseases, depression, difficulty with balance, drowsiness, fatigue, hair loss, insomnia, irritability, memory loss, recurrent infections, restlessness, vision disturbances, tremors, temper outbursts, ulcers and damage to brain, kidney, and lungs. Toxic effects include damage to the brain, kidneys, and lungs. Mercury poisoning can result in several diseases, including acrodynia (pink disease), Hunter-Russell syndrome, and Minamata disease	Neustadt and Pieczenik [85], Ainza et al. [86], and Gulati et al. [87], Clifton [88], Bjørklund [89], Tokuomi [90], and Davidson [91]
Pb	Exposure to lead produces deleterious effects on the hematopoietic, renal, reproductive, and central nervous system, mainly through increased oxidation. Its poisoning causes problems in children such as impaired development, reduced intelligence, loss of short-term memory, learning disabilities, and coordination problems; causes renal failure; increased risk for development of cardiovascular disease	Flora et al. [92], Salem et al. [80], Padmavathiamma and Li [93], Wuana and Okieimen [83] and Iqbal [94]
Zn	Long-term excessive zinc intakes (ranging from 150 mg/day to 1–2 g/day) have included sideroblastic anemia, hypochromic microcytic anemia, leukopenia, lymphadenopathy, neutropenia, hypocupremia, and hypoferremia. Over dosage can cause dizziness, nausea, vomiting, epigastric pain, lethargy, and fatigue	Hess and Schmid [95] and Fosmire [96]
Mn	Neurological effects in humans and animals and causes disabling syndrome called *manganism*. It also causes lethargy, increased muscle tonus, tremor, and mental disturbances	USEPA [97] and Kawamura [98]

Macrophytes are unchangeable biological filters and play an important role in the maintenance of the aquatic ecosystem. Aquatic macrophytes are taxonomically closely related to terrestrial plants, but are aquatic phanerogams, which live in a completely different environment. Their characteristics to accumulate metals make them an interesting research objects for testing and modeling ecological theories on evolution and plant succession, as well as on nutrient and metal cycling [69]. Many industrial and mining processes cause heavy metal pollution, which can contaminate natural water systems and become a hazard to human health. Therefore, colonization of macrophytes on the sediments polluted with heavy metals and the role of these plants in transportation of metals in shallow coastal areas are very important. [50]. Despite this, roots of wetland plants may accumulate heavy metals and transport them to aboveground portions of plants [70, 71].

The extent of metal accumulation within aquatic macrophytes is known to vary significantly between species. For example, the emergent aquatic plants usually accumulate lower amount of metals than submerged aquatic vegetation [72]. The emergent macrophytes growing in constructed wetlands designed for wastewater treatment have several properties in relation to the treatment processes that make them an essential component of the design. Several of the submerged, emergent, and free-floating aquatic macrophytes are known to accumulate and bioconcentrate heavy metals [73, 74]. Aquatic macrophytes take up metals from the water, producing an internal concentration several fold greater than their surroundings. Many of the aquatic macrophytes are found to be the potential scavengers of heavy metals from water and wetlands [75]. Yet research has focused mainly on the interaction between biological factors such as competition, coexistence, grazing, life cycles, adaptation, and environmental factors (salinity, depth, wave exposure) of importance for structuring brackish water macrophytes and algal communities [76].

10.4 Heavy Metal Pollution in Kashmir Himalayan Wetlands

Though a number of studies pertaining to the ecology of Kashmir Himalayan wetlands have been carried out, there are only a few attempts related to heavy metal analysis in these ecosystems. Of these studies, worth mentioning are the attempts by Ahmad et al. [77] in recent past. According to Ahmad et al. [77], the main source of heavy metals in the Kashmir Himalayan wetlands is use of pesticides in the rice fields and orchards of Kashmir and use of lead shots for hunting/poaching of birds.

In a series of studies, Ahmad et al. [77, 78] and other unpublished data heavy metal dynamics in different components of the wetland systems including water, sediments, and macrophytes have been worked out. In *Phragmites australis*, the accumulation of the different heavy metals was in order of Al > Mn > Ba > Zn > Cu > Pb > Mo > Co > Cr > Cd > Ni. Translocation factor, i.e., ratio of shoot to root metal concentration revealed that metals were largely retained in the roots of *P. australis*, thus reducing the supply of metals to avifauna and preventing their bioaccumulation. Moreover, the higher retention of heavy metals in the belowground parts of *P.*

australis reduces the supply of metals to avifauna, which mainly feed on aboveground parts of the plant, thereby preventing bioaccumulation of heavy metals in higher trophic levels. This further adds to the desirability of *P. Australis* as a phytoremediation species [77]. Ahmad et al. [78] also assessed the heavy metal accumulation capability of two dominant species (*Ceratophyllum demersum* and *Potamogeton natans*) in a Kashmir Himalayan Ramsar site. The accumulation of the different metals in *P. natans* was in the order of Al > Mn > Pb > Cu > Zn > Ni > Co > Cr > Cd, while in *C. demersum* it was Al > Mn > Zn > Co > Cu > Pb > Cr > Ni > Cd. In *C. demersum*, the highest bioconcentration factor (BCF) was obtained for Co (3616) and Mn (3589) while in *P. natans* the highest BCF corresponded to Cd (1027). Overall *Potamogeton–Ceratophyllum* combination provided a useful mix for Co, Mn, and Cd removal from contaminated sites. Beside *Phragmites australis* some other macrophytes that showed good phytoremediation potential were *Azolla cristata, Hydrocharis dubia, Myriophyllum spicatum, Nymphaea alba, Nymphoides peltata, Salvinia natans, Typha angustata, Sparganium erectum, and Trapa natans.* Ahmad et al. [77] reported that Hokersar an important Ramsar site of Kashmir Himalayas filters 73 % of Co, 88.24 % of Cu, 65.13 % of Pb, 51.98 % of Zn, 40.93 % of Mn, 58.36 % of Fe, 41.02 % of Cd, 75.07 % of Cr, and 86.59 % of Ni.

10.5 Knowledge Gaps and Future Directions

Kashmir Himalayas are gifted with a number of wetlands like Hokersar wetland, Haigam wetland, Malangpora wetland, Mirgund wetland, Narkura wetland, etc. These Kashmir Himalayan wetlands are presently subjected to various anthropogenic pressures like encroachment, rapid urbanization and industrialization, dumping of solid waste, sites of gunshots for hunting/poaching, etc. There have been scanty studies of heavy metals in Kashmir Himalayan wetlands except a few attempts in recent past by Ahmad et al. [77, 78]. Constructed wetlands are not common in Kashmir Himalayas. Realizing the important role played by the constructed wetlands, it is expected that constructed wetlands will also become popular in Kashmir Himalayas.

References

1. Vymazal J, Kropfelova L (2008) Is concentration of dissolved oxygen a good indicator of processes in filtration beds of horizontal-flow constructed wetlands? In: Vymazal J (ed) Waste water treatment, plant dynamics and management. Springer, Dordrecht, pp 311–317
2. ITRC (2003) Technical and regulatory guidance document for constructed treatment wetlands. The Interstate Technology Regulatory Council Wetlands Team, Washington, DC
3. TOOLBASE (2001) Constructed wetlands for wastewater treatment. http://www.toolbase.org/Technology-Inventory/Sitework/constructed-wetlands. Accessed 30 Jan 2012

4. Hoffmann H, Platzer C, Winker M, Muench EV (2011) Technology review of constructed wetlands: subsurface flow constructed wetlands for greywater and domestic wastewater treatment. Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) GmbH, Germany
5. Mohamed A (2004) Planung, Bauund Betriebeiner Pflanzenkläranlage in Syrien (Planning, construction and operation of a constructed wetland in Syria, in German). PhD thesis, University Flensburg, Germany
6. Heers M (2006) Constructed wetlands under different geographic conditions: evaluation of the suitability and criteria for the choice of plants including productive species. Master thesis, Faculty of Life Sciences, Hamburg University of Applied Sciences, Germany
7. Kamau C (2009) Constructed wetlands: potential for their use in treatment of grey water in Kenya. M.Sc. thesis, Christian-Albrechts University, Kiel, Germany
8. Kadlec RH, Knight RL (1996) Treatment wetlands. CRC Press, Boca Raton
9. Kadlec RH, Wallace SD (2008) Treatment wetlands, 2nd edn. CRC Press, Boca Raton
10. Vymazal J, Brix H, Cooper PF, Green MB, Haberl R (eds) (1998) Constructed wetlands for wastewater treatment in Europe. Backhuys Publishers, Leiden
11. Mitsch WJ (1992) Landscape design and the role of created, restored and natural riparian wetlands in controlling nonpoint source pollution. Ecol Eng 1:27–47
12. Vymazal J (2010) Constructed wetlands for wastewater treatment. Water 2:530–549
13. USEPA (1988) Constructed wetlands and aquatic plant systems for municipal wastewater treatment. EPA/625/1-88/022
14. Lemna Corporation (1994) Innovations in Lagoon-based treatment. Retention Times, Spring/Summer (Promotional literature)
15. USEPA (2000) Manual, Constructed wetlands treatment of municipal wastewaters. EPA/625/R-99/010. United States Environment Protection Agency. Office of Research and Development, Cincinnati
16. Brix H (1994) Use of wetlands in water pollution control: historical development, present status, and future perspectives. J Water Sci Technol 30(8):209–223
17. Stottmeister U, Weibner A, Kuschk P (2003) Effect of plants and microorganisms on constructed wetlands for waste water treatment. Biotechnol Adv 22(1–2):93–117
18. Brix H (2003) Plants used in constructed wetlands and their functions. 11th international seminar on the use of aquatic macrophytes for wastewater treatment in constructed wetlands
19. Shtemenko NI, Shepelenko VN, Richnow H, Kuschk P (2005) Surface lipid composition of two emergent water plants used in constructed wetlands, vol 48, Modern tools and methods of water treatment for improving living standards NATO science series. Springer, The Netherlands, pp 325–330
20. Hamilton H, Nix PG, Sobolewski Y (1993) An overview of constructed wetlands as alternatives to conventional waste treatment systems. Water Pollut Res J Canada 28(3):529–548
21. Wang J, Cai X, Chen Y, Liang M, Zhang Y, Wang Z, Li Q, Liao X (1994) Analysis of the configuration and treatment effect of constructed wetland waste water treatment system for different waste waters in China. In: Proceedings of fourth international conference on wetland systems for water pollution control. ICWS ($ Secreteriat Guangzhou, P. R. China, pp 114–120)
22. Yang Z, Goldman N, Friday A (1994) Comparison of models for nucleotide substitution used in maximum likelihood phylogenetic estimation. Mol Biol Evol 11:316–324
23. Twilley RR, Brindson MH, Davis GJ (1977) Phosphorus absorption, translocation and secretion in *Nuphar luteum*. Limnol Oceanogr 22:1022–1032
24. Vymazal J (2013) The use of hybrid constructed wetlands for wastewater treatment with special attention to nitrogen removal: a review of a recent development. Water Res 47(14):4795–4811
25. Billore SK, Singh N, Sharma JK, Dass P, Nelson RM (1999) Horizontal subsurface flow gravel bed constructed wetland with *Phragmites karka* in central India. Water Sci Technol 40:171–173
26. Dallas S, Scheffe B, Ho G (2004) Reedbeds for greywater treatment—case study in Santa Elena—Monteverde, Costa Rica, Central America. Ecol Eng 23:55–61

27. Rousseau DPL, Vanrolleghem PA, De Pauw N (2004) Constructed wetlands in Flanders: a performance analysis. Ecol Eng 23:151–163
28. American Public Health Association (APHA) (1998) Standard methods for examination of water and wastewater. 20th ed. American Public Health Association, Washington, DC
29. Masi F, Martinuzzi N (2007) Constructed wetlands for the Mediterranean countries: hybrid systems for water reuse and sustainable sanitation. Desalination 215:44–55
30. Schroder P, Avino JN, Azaizeh H, Goldhirsh AG, Gregorio SD, Komives T, Langergraber G, Lenz A, Maestri E, Memon AR, Ranalli A, Sebastiani L, Smrcek S, Vanek T, Vuilleumier S, Wissing F (2007) Using phytoremediation technologies to upgrade waste water treatment in Europe. Environ Sci Pollut Res 14(7):495
31. Nies DH (1999) Microbial heavy-metal resistance. Appl Microbiol Biotechnol 51:730–750
32. Holleman D, Wiberg E (1987) Lehrbuch deranorganischen chemie. Springer, Berlin
33. Weast RC (1984) CRC Handbook of chemistry and physics, 64th edn. CRC Press, Boca Raton
34. Baruah S, Hazarika K, Sarma PK (2011) Uptake and localization of lead in *Eichorniacrassipes* grown within a hydroponic system. Adv Appl Sci Res 3(1):51–59
35. Alloway BJ (1997) The mobilisation of trace elements in soils. In: Prost R (ed) Contaminated soils. Proceedings of the third international conference on the biogeochemistry of trace elements, Paris 15–19 May 1995. INRA, Paris, pp 133–145
36. Gorsberg RM, Elkins BV, Igon SR, Goldman CR (1984) The removal of heavy metals by artificial wetlands. In: Proceedings of water reuse symposium III, future of water reuse, San Diego, vol. 2, pp 639–648
37. Kabata-Pendias A, Pendias H (2001) Trace elements in soils and plants. CRC Press, Boca Raton
38. Nriagu JO (1989) A global assessment of natural sources of atmospheric trace metals. Nature 338:47–49
39. Espinoza-Quiñones FR, Zacarkim CE, Palacio SM, Obregón CLD, Zenatti C, Galante RM, Rossi N, Rossi FL, Pereira IRA, Welter RA, Rizzutto MA (2005) Removal of heavy metal from polluted river water using aquatic macrophyte *Salvinia* sp. Brazilian J Phys 35:44–746
40. Phuong PK, Son CPN, Sauvian JJ, Terradellas J (1998) Contamination of PCB’s, DDT’, and heavy metals in sediments of Ho Chi Minh city’s canals, Vietnam. Bull Environ Contam Toxicol 60:115–131
41. Batty J, Pain D, Caurant F (1996) Metal concentrations in eels 471 *Anguilla Anguilla* from the Camargue region of France. Biol Conserv 76:17–23
42. Tavecchia G, Pradel R, Lebreton JD, Johnson AR, Mandain-Monval JY (2001) The effect of lead exposure on survival of adult mallards in the Camargue, southern France. J Appl Ecol 38:1197–1207
43. Nriagu JO (1996) A history of global metal pollution. Science 272:223–224
44. Rai PK, Sharma AP, Tripathi BD (2007) Urban environment status in Singrauli industrial region and its eco-sustainable management: a case study on heavy metal pollution. In: Lakshmi V (ed) Urban planning and environment, strategies and challenges. Macmillan Advanced Research Series, pp 213–217
45. Finkelman RB, Gross PMK (1999) The types of data needed for assessing the environmental and human health impacts of coal. Int J Coal Geol 40:91–101
46. Sharma PD (2003) Environmental pollution. In: Ecology and environment, 7th edn. Rastogi Publication, Meerut, India, pp 415–489
47. Lasat MM, Pence NS, Garvin DF, Ebbs SD, Kochian LV (2000) Molecular physiology of zinc transport in the Zn hyperaccumulator (*Thlaspicaerulescens*). J Exp Bot 51:71–79
48. Cardwell AJ, Hawker DW, Greenway M (2002) Metal accumulation in aquatic macrophytes from southeast Queensland, Australia. Chemosphere 48:653–663
49. Uka UN, Mohammed HA, Aina A (2013) Preliminary studies on the phytoremediation potential of *Phragmites karka* (Retz.) in Asa River. J Fisheries Aquatic Sci 8:87–93
50. Vardanyan LG, Ingol BS (2006) Studies on heavy metal accumulation in aquatic macrophytes from Sevan (Armenia) and Carambolim (India) lake systems. Environ Int 32:208–218

51. Sasmaza A, Obekb E, Hasarb H (2008) The accumulation of heavy metals in (*Typhalatifolia* L.) grown in a stream carrying secondary effluent. Ecol Eng 33:278–284
52. Malik A (2004) Metal bioremediation through growing cells. Chemosphere 30:261–278
53. Chaphekar SB (1991) An overview on bioindicators. J Environ Biol 12:163–168
54. Zenk MH (1996) Heavy metal detoxification in higher plants—a review. Gene 179:21–30
55. Mertz W (1981) The essential trace elements. Science 213:1332–1338
56. Balsberg-Pahlsson AM (1989) Toxicity of heavy metals (Zn, Cu, Cd, Pb) to vascular plants. A literature review. Water Air Soil Pollut 47:287–319
57. Gullizzoni P (1991) The role of heavy metals and toxic materials in the physiological ecology of submerged macrophytes. Aquatic Bot 41:87–109
58. Jarup L (2003) Hazards of heavy metal contamination. Br Med Bull 68:167–182
59. Gaur A, Adholeya A (2004) Prospects of arbuscular mycorrhizal fungi in phytoremediation of heavy metal contaminated soils. Curr Sci 86(4):528–534
60. Pehlivan E, Ozkan AM, Dinc S, Parlayici S (2009) Adsorption of Cu2+ and Pb2+ ion on dolomite powder. J Hazard Mater 167(1–3):1044–1049
61. Roy S, Labelle S, Mehta P (2005) Phytoremediation of heavy metal and PAH-contaminated brownfield sites. Plant and Soil 272(1–2):277–290
62. Otte ML, Jacob DL (2006) Constructed wetlands for phytoremediation. In: Mackova M (ed) Phytoremediation, rhizoremediation. Springer, Dordrecht, pp 57–67
63. Kadlec RH, Wallace SD (2009) Treatment wetlands. CRC Press, Boca Raton
64. Chung IH, Jeng SS (1974) Heavy metal pollution of Ta-Tu river. Bullet Inst Zool, Acad Sci 13:69–73
65. Devlin RMR (1967) Plant physiology. Reinhold, New York, p 564
66. Baldantoni D, Alfani A, Tomansi DI, Bartoli G, Santo AVD (2004) Assessment of macro and microelement accumulation capability of two aquatic plants. Environ Pollut 130:149–156
67. Mays PA, Edwards GS (2001) Comparison of heavy metal accumulation in natural wetland and constructed wetlands receiving acid mine drainage. Ecol Eng 16(4):487–500
68. Stoltz E, Greger M (2002) Accumulation properties of As, Cd, Cu, Pb and Zn by four wetland plant species growing on submerged mine tailings. Environ Exp Bot 47:271–280
69. Forstner U, Whittman GTW (1979) Metal pollution in the aquatic environment. Springer, Berlin, p 486
70. Dunstan WM, Windom HM (1975) The influence of environmental changes in heavy metal concentrations of *Spartina alterniflora*. In: Cronin LE (ed) Estuarine research, vol. II. Geology and engineering. Academic Press, New York, pp 393–404
71. Mudroch A, Capobianco J (1978) Study of selected metals in marshes on Lake St. Clair. Ontario Arch Hydrobiol 84:87–108
72. Albers PH, Camardese WN (1993) Effects of acidification on metal accumulation by aquatic plants and invertebrates in Constructed wetlands. Environ Toxicol Chem 12:959–967
73. Bryan GW (1971) The effects of heavy metals (other than mercury) on marine and estuarine organisms. Proc R Soc London B 177:389–410
74. Chow TJ, Snyder CB, Snyder HG, Earl JL (1976) Lead content of some marine organisms. J Environ Sci Health A11:33–44
75. Gulati KL, Nagpaul KK, Bukhari SS (1979) Uranium, boron, nitrogen, phosphorus and potassium in leaves of mangroves, Mahasagar. Bullet Natl Inst Oceanogr 12:183–186
76. Alloway BJ, Davis BE (1971) Heavy metal content of plants growing on soils contaminated by lead mining. J Agric Sci 76:321–323
77. Ahmad SS, Reshi ZA, Shah MA, Rashid I, Ara R, Andrabi SMA (2014) Phytoremediation potential of Phragmites australis in Hokersar wetland—a Ramsar site of Kashmir Himalaya. Int J Phytoremed 16:1183–1191
78. Ahmad SS, Reshi ZA, Shah MA, Rashid I, Ara R, Andrabi SMA (2015) Heavy metal accumulation in the leaves of *Potamogeton natans* and *Ceratophyllum demersum* in a Himalayan RAMSAR site: management implications. Wetland Ecol Manage doi:10.1007/s11273-015-9472-9

79. Tripathi RD, Srivastava S, Mishra S, Singh N, Tuli R, Gupta DK, Maathuis FJM (2007) Arsenic hazards: strategies for tolerance and remediation by plants. Trends Biotechnol 25:158–165
80. Salem HM, Eweida EA, Farag A (2000) Heavy metals in drinking water and their environmental impact on human health. ICEHM 2000, Cairo University, Egypt, pp 542–556
81. Awofolu O (2005) A survey of trace metals in vegetation, soil and lower animal along some selected major roads in metropolitan city of Lagos. Environ Monit Assess 105:431–447
82. Paustenbach DJ, Finley BL, Mowat FS, Kerger BD (2003) Human health risk and exposure assessment of chromium (VI) in tap water. J Toxicol Environ Health 66:1295–1339
83. Wuana RA, Okieimen FE (2011) Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation. ISRN Ecol, pp 1–20
84. Sandstead HH (1995) Requirements and toxicity of essential trace elements, illustrated by zinc and copper. Am J Clin Nutr 61(3):621–624
85. Neustadt J, Pieczenik S (2007) Toxic-metal contamination: mercury. Integr Med 6:36–37
86. Ainza C, Trevors J, Saier M (2010) Environmental mercury rising. Water Air Soil Pollut 205:47–48
87. Gulati K, Banerjee B, Bala Lall S, Ray A (2010) Effects of diesel exhaust, heavy metals and pesticides on various organ systems: possible mechanisms and strategies for prevention and treatment. Indian J Exp Biol 48:710–721
88. Clifton JC (2007) Mercury exposure and public health. Pediatr Clin North Am 54(2): 237–269
89. Bjørklund G (1995) Mercury and Acrodynia (PDF). J Orthomol Med 10(3&4):145–146
90. Tokuomi H, Kinoshita Y, Teramoto J, Imanishi K (1977) Hunter-Russell syndrome Nihon rinsho. Jpn J Clin Med 35(1):518–519
91. Davidson PW, Myers GJ, Weiss B (2004) Mercury exposure and child development outcomes. Pediatrics 113(4):1023–1029
92. Flora G, Gupta D, Tiwari A (2012) Toxicity of lead: a review with recent updates. Interdiscip Toxicol 5(2):47–58
93. Padmavathiamma PK, Li LY (2007) Phytoremediation technology: hyperaccumulation metals in plants. Water Air Soil Pollut 184:105–126
94. Iqbal MP (2012) Lead pollution—a risk factor for cardiovascular disease in Asian developing countries. Pakistani J Pharmaceut Sci 25:289–294
95. Hess R, Schmid B (2002) Zinc supplement overdose can have toxic effects. J Pediatr Hematol Oncol 24:582–584
96. Fosmire GJ (1990) Zinc toxicity. Am J Clin Nutr 51(2):225–227
97. US EPA (2004) Drinking water health advisory for Manganese. EPA-822-R-04-003. US Environmental Protection Agency, Office of Water, Health and Ecological Criteria Division, Washington, DC
98. Kawamura R, Ikuta HS, Fukuzumi S (1941) Intoxication by manganese in well water, Kitasato. Archiv Exp Med Biol 18:145–169

Chapter 11
Phytofiltration of Metal(loid)-Contaminated Water: The Potential of Native Aquatic Plants

Paulo J.C. Favas, João Pratas, Manoj S. Paul, Santosh Kumar Sarkar, and M.N.V. Prasad

11.1 Introduction

All biosphere compartments are vulnerable to pollution, including our freshwater sources—both lentic and lotic. These ecosystems are subjected to constant internal and external changes, both of natural or anthropogenic origin. Anthropogenic influences are often the cause of irreparable damage to some of these ecosystems like mountain streams which are very sensitive. For example, mining activities are well known for their potential deleterious effects on the environment, namely, the

---

P.J.C. Favas (✉)
School of Life Sciences and the Environment, University of Trás-os-Montes e Alto Douro, UTAD, Quinta de Prados 5000-801, Vila Real, Portugal

MARE—Marine and Environmental Sciences Centre, Faculty of Sciences and Technology, University of Coimbra, 3004-517 Coimbra, Portugal
e-mail: pjcf@utad.pt; http://www.utad.pt

J. Pratas
MARE—Marine and Environmental Sciences Centre, Faculty of Sciences and Technology, University of Coimbra, 3004-517 Coimbra, Portugal

Department of Earth Sciences, Faculty of Sciences and Technology, University of Coimbra, 3001-401 Coimbra, Portugal

Instituto de Geologia e Petróleo de Timor Leste, Díli, Timor-Leste

M.S. Paul
Department of Botany, St. John's College, Agra 282 002, Uttar Pradesh, India

S.K. Sarkar
Department of Marine Science, University of Calcutta,
35, Ballygunge Circular Road, Calcutta 700 019, West Bengal, India

M.N.V. Prasad
Department of Plant Sciences, University of Hyderabad,
Hyderabad 500 046, Telangana, India

A.A. Ansari et al. (eds.), *Phytoremediation*, DOI 10.1007/978-3-319-40148-5_11

contamination of soils, sediments, and waters due to uncontrolled runoff, leaching, and/or aeolian deposition. In fact, leading anthropogenic sources of heavy metals and metalloids pollution are mining and milling operations worldwide. Particularly in the case of abandoned mines where there is no control and monitoring. Lead (Pb), copper (Cu), zinc (Zn), uranium (U), and arsenic (As) are some of the metal(loid)s most frequently reported to have the highest impact on organisms.

Water management in mining areas requires a strategic use of technology, since they imply long-term treatment approaches. The high metal(loid)s content, and the pH/Eh variation of the permanent low flow rate seepage waters, creates major difficulties to the design of efficient and affordable remediation projects [1]. However, water remediation techniques, as well as public awareness campaigns about the risks of exposure to toxic heavy metals and metalloids, should be adopted in contaminated areas, especially where the population still uses private wells as a drinking water source.

Contaminated water can be treated by several methods. Currently, preference is being given to in situ and passive methods that are less environmentally disruptive and more economical. In this context, biotechnology offers phytoremediation techniques as a suitable alternative. In this chapter, results of the last two decades of investigations are presented in the light of phytotechnological potential evaluation, incorporating approaches like phytoremediation, phytoextraction, phytofiltration, biosorption, and bioindication, of the Portuguese native aquatic flora found in waters contaminated with metal(loid)s.

11.2 Phytoremediation Technology

Phytoremediation is the use of plants (trees, shrubs, grasses, and aquatic plants) and their associated microorganisms in order to remove, degrade, or isolate toxic substances from the environment (e.g., [2–12]). The word phytoremediation derives from the Greek *phyton*, meaning “plant,” and the Latin *remedium*, which means “to remedy” or “to correct.” Substances that may be subjected to phytoremediation include metals (Pb, Zn, Cd, Cu, Ni, Hg), metalloids (As, Sb), inorganic compounds (NO_3^-, NH_4^+, PO_4^{3-}), radioactive chemical elements (U, Cs, Sr), petroleum hydrocarbons (BTEX), pesticides and herbicides (atrazine, bentazon, chlorinated, and nitroaromatic compounds), explosives (TNT, DNT), chlorinated solvents (TCE, PCE) and industrial organic wastes (PCPs, PAHs), and others (e.g., [3, 9, 13–19]). Phytoremediation techniques include different modalities, depending on the chemical nature and properties of the contaminant (if it is inert, volatile, or subject to degradation in the plant or in the soil) and the plant characteristics. Thus, phytoremediation essentially comprises six different strategies, though more than one may be used by the plant simultaneously [8].

11.2.1 Phytodegradation (Phytotransformation)

Organic contaminants are degraded (metabolized) or mineralized inside plant cells by specific enzymes that include nitroreductases (degradation of nitroaromatic compounds), dehalogenases (degradation of chlorinated solvents and pesticides), and laccases (degradation of anilines). *Populus* species and *Myriophyllum spicatum* L. are examples of plants that have these enzymatic systems [20, 21].

11.2.2 Phytostabilization (Phytoimmobilization)

Contaminants, organic or inorganic, are incorporated into the lignin of the cell wall of roots cells or into humus. Metals are precipitated as insoluble forms by direct action of root exudates and subsequently trapped in the soil matrix. The main objective is to avoid mobilization of contaminants and limit their diffusion in the soil [5, 22–24]. Species of genera *Haumaniastrum*, *Eragrostis*, *Ascolepis*, *Gladiolus*, and *Alyssum* are examples of plants cultivated for this purpose.

11.2.3 Phytovolatilization

This technique relies on the ability of some plants to absorb and volatilize certain metal(loid)s. Some element ions of the groups IIB, VA, and VIA of the periodic table (specifically Hg, Se, and As) are absorbed by the roots, converted into nontoxic forms, and then released into the atmosphere. As example, the species *Astragalus bisulcatus* (Hook.) A. Gray and *Stanleya pinnata* (Pursh) Britton for Se or transgenic plants (with bacterial genes) of *Arabidopsis thaliana* (L.) Heynh., *Nicotiana tabacum* L., *Liriodendron tulipifera* L., or *Brassica napus* L. for Hg can be mentioned [24–28]. This technique can also be used for organic compounds.

11.2.4 Phytoextraction (Phytoaccumulation, Phytoabsorption, or Phytosequestration)

This involves the absorption of contaminants by roots followed by translocation and accumulation in the aerial parts. It is mainly applied to metals (Cd, Ni, Cu, Zn, Pb) but can also be used for other elements (Se, As) and organic compounds. This technique preferentially uses hyperaccumulator plants that have the ability to store high concentrations of specific metals in their aerial parts (0.01–1 % dry weight, depending on the metal). *Elsholtzia splendens* Nakai ex F. Maek., *Alyssum bertolonii* Desv., *Noccaea caerulescens* (J. Presl & C. Presl) F.K. Mey. (*Thlaspi caerulescens* J. Presl & C. Presl), and *Pteris vittata* L. are known examples of hyperaccumulator plants for Cu, Ni, Zn/Cd, and As, respectively [29–36].

11.2.5 Phytofiltration and Rhizofiltration

When plants absorb, concentrate, and/or precipitate contaminants, particularly heavy metals or radioactive elements, from an aqueous medium through their root system or other submerged organs (e.g., [4, 19, 24, 37–39]). The plants are kept in a hydroponic system, whereby the effluents pass and are "filtered" by the roots (rhizofiltration), or other organs that absorb and concentrate contaminants. Plants with high root biomass, or high absorption surface, with more accumulation capacity (aquatic hyperaccumulators) and tolerance to contaminants achieve the best results. Promising examples include *Helianthus annuus* L.; *Brassica juncea* (L.) Czern.; *Phragmites australis* (Cav.) Trin. ex Steud.; *Eichhornia crassipes* (Mart.) Solms; *Spirodela punctata* (G. Mey.) C.H. Thomps.; *Fontinalis antipyretica* Hedw.; and several species of *Salix*, *Populus*, *Lemna*, and *Callitriche* [5, 40–44].

11.2.6 Rhizodegradation and Phytostimulation

Growing roots promote the proliferation of degrading rhizosphere microorganisms which utilize exudates and metabolites of plants as a source of carbon and energy. In addition, plants may exude biodegrading enzymes themselves. The application of rhizodegradation/phytostimulation is limited to organic contaminants [5, 37]. The microbial community in the rhizosphere is heterogeneous due to variable spatial distribution of nutrients; however, species of the genus *Pseudomonas* are the predominant organisms associated with roots [24, 45, 46].

There are other strategies also, which are considered categories of phytoremediation by some authors, but actually, they are mixed techniques or variations of the earlier mentioned strategies [8].

11.2.7 Hydraulic Barriers

Some large trees, particularly those with deep roots (e.g., *Populus* sp.), remove large quantities of groundwater during transpiration. Contaminants in this water are metabolized by plant enzymes and vaporized together with water or simply sequestered in plant tissues [5, 47].

11.2.8 Vegetation Covers (Vegetative Caps or Phytocovers)

Herbs (usually grasses), eventually shrubs or trees, establish on landfills or tailings, are used to minimize the infiltration of rain water, and contain the spread of pollutants. The roots increase soil aeration thus, promoting biodegradation, evaporation, and transpiration [6, 12, 48–50].

11.2.9 Constructed Wetlands

These are ecosystems consisting of organic soils, microorganisms, algae, and vascular aquatic plants in areas where the water level is at/near the surface, at least part of the year. All the components work together in the treatment of effluents, through the combined actions of filtration, ion exchange, adsorption, and precipitation (e.g., [37, 51–54]). It is the oldest method of wastewater treatment and is not regarded as proper phytoremediation, since it is based on the contributions of the entire system [5, 55]. Depending on how it processes the water circulation, the wetland is classified as horizontal or surface flow and vertical or subsurface flow. In the latter case ensures a greater reactivity of the influent with the substrate. Good cleaning efficiency, low cost of construction along with easy operation and maintenance are the main advantages. It is widely applied in the treatment of domestic, agricultural, and industrial waste water (e.g., [56, 57]) but has proved to be suitable also for treating acid mine drainages (e.g., [58–60]).

11.2.10 Phytodesalination

This is a recently reported [24, 61] emerging technique that utilizes halophytes to remove excess salts from saline soils. The potential of *Suaeda maritima* (L.) Dumort and *Sesuvium portulacastrum* L. in removal and accumulation of NaCl, from highly saline soils, has been demonstrated [62]. Having own peculiarities, this technique is a modality of phytoextraction.

11.3 Phytofiltration of Contaminated Waters

As previously referred, there are two main divisions in the aquatic phytoremediation technology that involve [63, 64] (Fig. 11.1): 1—purely aquatic plants, which remove metals from water by roots, leaves, and stems (***phytofiltration***); 2—submersion of the roots of terrestrial plants in order to remove pollutants from the water (***rhizofiltration***). In addition, we may consider a third division [40]: 3—using young plant seedlings growing in aerated water (aquacultured) to remove toxic metals from water (***blastofiltration***).

In the present study dealing with phytofiltration potential of the Portuguese native aquatic flora, the term phytofiltration is used to describe the remediation of metal(loid)-contaminated water through uptake and bioaccumulation of metal(loid)s into organs of aquatic plants. Freshwater vascular plants (or simply aquatic plants) comprise mainly angiosperms with a few fern species, when combined with macroscopic algae are known collectively as macrophytes [63]. By definition, aquatic plants are those that complete their biological life cycle in aquatic environments and

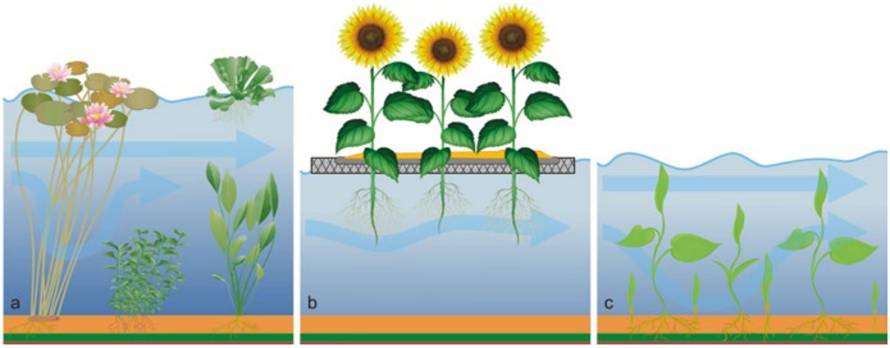


Fig. 11.1 Schematic representation of aquatic phytoremediation strategies: (**a**) phytofiltration, (**b**) rhizofiltration, and (**c**) blastofiltration

include diversified forms such as free-floating, submerged, and emergent life forms [38, 65, 66]. In a more specific way, the aquatic macrophytes can be classified as follows [57, 63, 67–70] (Fig. 11.2).

11.3.1 Rooted Emergent Plants

These macrophytes are rooted in bottom sediments/submerged soils or in aerial soils at which the water table is about 0.5 m below the soil. These are sometimes referred as semiaquatic plants. They are generally rhizomatous or cormous perennials (e.g., *Typha* spp., *Phragmites* spp., *Scirpus* spp.).

11.3.2 Rooted Submerged Plants

These are rooted in bottom sediments and with leaves under water (e.g., *Potamogeton pectinatus* L., *Myriophyllum spicatum* L., *Chara* spp.).

11.3.3 Rooted Floating-Leaved Plants

These are rooted in bottom sediments at water depths from about 0.5 to 3 m but with floating leaves. In heterophyllous species submerged leaves accompany the floating leaves. Floating leaves are on short petioles from long ascending stems (e.g., *Potamogeton natans* L.), or with long and flexible petioles (e.g., *Nymphaea* spp.) and reproductive organs are floating or aerial.

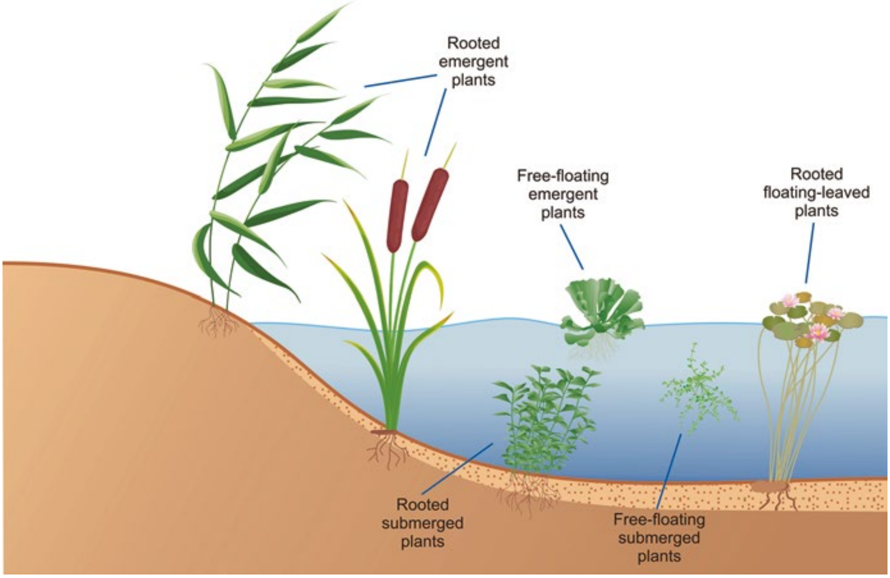


Fig. 11.2 Five major groups of aquatic macrophyte types

11.3.4 Free-Floating Emergent Plants

These are not rooted in sediments but live unattached in water. Forming a highly varied group, they range from long plants with rosettes of aerial and floating leaves and well-developed submerged roots (e.g., *Eichhornia crassipes*, *Pistia stratiotes* L.) to minute surface floating plants with few or no roots (e.g., *Lemna* spp., *Azolla* spp., *Salvinia* spp.).

11.3.5 Free-Floating Submerged Plants

These are submerged, nonrooted aquatic plants (e.g., *Ceratophyllum demersum* L., *Utricularia vulgaris* L., *Utricularia intermedia* Hayne, *Utricularia gibba* L.).

The first extensive review on the ability of aquatic plants to accumulate chemical elements from the aquatic environment was carried out by Hutchinson [71]. The author reported a set of aquatic plant species with the ability to accumulate cadmium (Cd), lead (Pb), and mercury (Hg) in concentrations that are many higher than the ambient environment. Since then, the number of studies involving aquatic macrophyte communities have been on the rise as a consequence of the growing importance of the water management. Phytofiltration of metal(loid)-contaminated waters involves both abiotic (water and sediments) and biotic (organisms living in the contaminated environment), and the specific process of phytofiltration depends not only

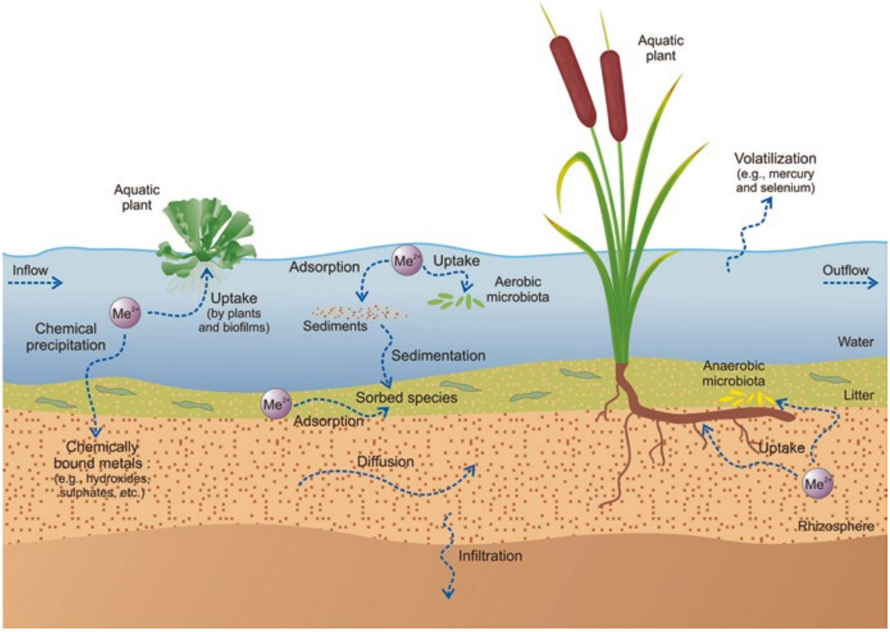


Fig. 11.3 Mechanisms of metal(loid)s removal in aquatic environments

on plant traits but also on certain physicochemical and geochemical processes. At natural sites, metal(loid)s removal also depends on factors that include microbial biofilms on abiotic substrates and the growth of periphyton [72–77]. Thus, the metal(loid)s removal from water in natural aquatic environments involves integrated physical, chemical, and biological processes, such as those reported by Hedin et al. [78], Sobolewski [58], and ITRC [51] in wetlands, namely the following (Fig. 11.3).

11.3.6 *Filtration of Suspended Solids*

Including adsorbed metal(loid)s.

11.3.7 *Sorption onto Organic Matter*

Several metals have a high affinity to bind to organic matter forming stable complexes.

11.3.8 Oxidation and Hydrolysis

Al, Fe, and Mn can form insoluble compounds—oxides, oxyhydroxides, and hydroxides—through hydrolysis and/or oxidation.

11.3.9 Formation of Carbonates

Some metals can form carbonates when concentrations of bicarbonate in water are high.

11.3.10 Formation of Insoluble Sulfides

Anaerobic conditions promote the growth of sulfate-reducing bacteria, which convert sulfates into hydrogen sulfide; metal(loid)s such Ag, As, Cd, Cu, Hg, Pb, and Zn react with hydrogen sulfide to form highly insoluble sulfides.

11.3.11 Binding to Iron and Manganese Oxides

Several metal(loid)s have a high affinity to bind to Fe and Mn oxides through the adsorption or coprecipitation phenomena.

11.3.12 Reduction to Nonmobile Forms by Bacterial Activity

Metal(loid)s such as Cr, Cu, Se, and U can be reduced into nonmobile forms—e.g., metallic forms—through processes governed by bacterial activity or physicochemical factors such as Eh–pH and hydrogen sulfide concentrations.

11.3.13 Biological Methylation and Volatilization

Some metal(loid)s (e.g., Hg, Se, and As) may be converted into nontoxic forms and then released into the atmosphere through volatilization by direct release from plants or by microbial or chemical activity in the water and sediments. These elements can also be biomethylated by plant roots and microorganisms — e.g., under anaerobic sediment conditions, Hg ions are biomethylated by anaerobic microorganisms to methyl mercury; methylation processes made these metal(loid)s highly toxic and available to the entire food chain through biomagnification, creating additional environmental problems.

11.3.14 Plant Uptake

Metal(loid)s are absorbed from water and/or sediment by roots or other submerged organs followed by translocation and accumulation in the tissues.

Metal(loid) uptake by aquatic plants depends on the type of plant, with direct absorption from the water column to the plant surface followed by passive or active transport across membranes and, to a lesser extent, root uptake [63, 79, 80]. These processes have been observed not only in submerged species (with poorly developed root systems) but also in free-floating plants [63]. However, root uptake in plants with developed root systems can also be effective, as endorsed by *Eleocharis dulcis* (Burm.f.) Trin. ex Hensch. with higher U levels in roots compared to stems. It has been suggested that the low accumulation in stems has an important advantage because metal cycling and resuspension following the decay of stems are minimized [81]. In another study, Entry et al. [82] demonstrated sunflower (*Helianthus annuus*) seems to be very effective in recovering U from contaminated water. Uranium accumulated mainly in the roots, with concentrations 5000–10,000 times greater than that of the ambient water.

Plants growing near mining sites or in trace element-rich substrates are able to accumulate or exclude toxic metal(loid)s and can therefore tolerate the imposed stress. Some of these accumulating plant species reveal the mineral composition of those substrates, for example, in the soil, sediment, and water. This ability can be used in mineral prospecting, in contamination bioindication or, if the biomass and bioproductivity are high, in phytoremediation. Certain aquatic plants, such as algae, bryophytes, and angiosperms, are considered to be indicators of trace element pollution and have been successfully used as diagnostic tools for monitoring environmental pollution [83, 84]. In fact, the ability of plants to accumulate metal(loid)s from water, which may not be essential for their growth and development, has been observed in several studies performed in natural wetlands where the metal(loid) concentration in aquatic plants is manifold higher than the ambient water. This evidence has led to the generalized idea that metal(loid) hyperaccumulation in aquatic plants is not as rare as in terrestrial plants and that suitable and sustainable remediation strategies could be developed based on this characteristic [63, 79, 80, 85, 86]. This function is of considerable significance in the emerging areas of wastewater treatment and in the establishment of constructed treatment wetlands (e.g., [70]). In recent years, several studies have been performed on metal(loid)s accumulation by aquatic plants, including possible phytofiltration applications.

The accumulation of metal(loid)s in aquatic plants may occur due to absorption, adsorption, and/or other retention mechanisms. Holistically, these physicochemical processes generally fall under the term “bioaccumulation”, when performed by living organisms. Another term, “biosorption”, is used to describe the set of mechanisms for the removal of substances from solution by biological materials (living or dead biomass), including absorption, adsorption, ion exchange, surface complexation, and precipitation (e.g., [64, 87–96]). However, some of these authors only consider the term “biosorption” in the case of dead biomass.

Several studies have shown that many factors affect the “bioaccumulation” and/or “biosorption” of metals in aquatic ecosystems. Among the physicochemical factors, pH is possibly the most important (e.g., [93, 97–101]). This can be attributed to the influence of pH on the solution chemistry of metals and the activity of the functional groups of the biomass [102]. Phytofiltration and rhizofiltration efficiencies are determined by the ability of plants to accumulate metal(loid)s and the biomass production. Thus, phytofiltration and rhizofiltration potential can be estimated by calculation of bioconcentration factor (or biological absorption coefficient) and translocation factor. The bioconcentration factor (BCF), defined as the ratio of the total concentration of elements in the plant tissue (C_{plant}) to its concentration in the water in which the plant was growing (C_{water}), is calculated as follows (e.g., [103–105]):

$$\mathrm{BCF} = \frac{C_{\text{plant}}}{C_{\text{water}}}$$

Translocation factor (TF), defined as the ratio of the total concentration of elements in the aerial parts of the plant (C_{shoot}) to the concentration in the root (C_{root}), is calculated as follows (e.g., [104, 106]):

$$\mathrm{TF} = \frac{C_{\text{shoot}}}{C_{\text{root}}}$$

Using both the BCF and the TF it is possible to assess the phytofiltration or rhizofiltration capacity of the plants. A high root-to-shoot translocation (TF) of metal(loid)s is a fundamental characteristic for a plants to be classified as effectively used in rhizofiltration. The commercial efficiency of phytofiltration and rhizofiltration can be estimated by the rate of metal(loid) accumulation and biomass production. After harvesting, biomass may be processed for extraction and recovery of metals with commercial value—phytomining [48, 49, 107, 108]. The commercial value of metals such as Ni, Zn, Cu, or Co may encourage the phytoremediation process. Alternatively, thermal, physical, chemical, or microbiological processes can be used to reduce the volume/weight of biomass.

The earlier referred metal(loid)s removal processes naturally occurring in aquatic ecosystems, constitute a form of natural attenuation of contamination (e.g., [109]). The natural attenuation can be defined as the natural processes of dilution, dispersion, precipitation, sorption, biodegradation, bioaccumulation, volatilization, and/or chemical and biochemical stabilization of contaminants occurring in aquatic environments that effectively reduce contaminant mobility, bioavailability, toxicity, or concentration to levels that are not too harmful on the human health and ecosystems

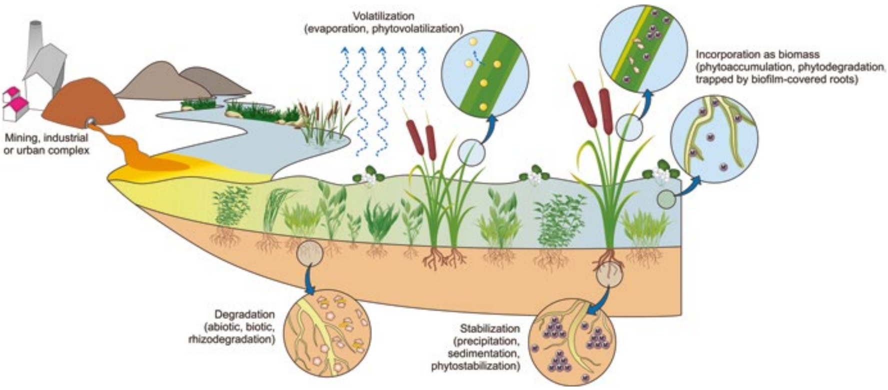


Fig. 11.4 Schematic representation of mechanisms of natural attenuation of contamination (including phytoremediation) in aquatic environments

(Fig. 11.4). Thus, human intervention to remediate waters and sediments and restoring aquatic ecosystems can be based on these natural processes. These processes can be replicated in a more complex manner in constructed wetlands, or in a more simply way in phytofiltration and rhizofiltration systems.

11.4 Phytofiltration Potential of Aquatic Plants

The ability of aquatic plants to accumulate metal(loid)s from water and/or from water–sediment interface has been observed in several studies performed both in field or laboratory conditions (e.g., [63, 80, 85, 86, 110–119]). It has been generally observed that the metal concentration in aquatic plants is several times higher than the concentration in surrounding water. Thus, several aquatic plant species have been identified as accumulators of metal(loid)s and as a result they might prove useful in biomonitoring and phytofiltration.

11.4.1 Natural Phytofiltration of Metal(loid)-Contaminated Water in Portugal

A few studies to assess the indigenous aquatic plant species of diverse contaminated areas and evaluate their potential for phytofiltration have been performed in Portugal (e.g., [41, 42, 112, 120–129]). In this chapter, important findings have been presented from several studies to evaluate the phytofiltration potential of native aquatic flora grown in waters enriched with metal(loid)s in distinct areas of Portugal.

11.4.1.1 Study Areas

The studied areas are located in Portugal (South Western Europe), including the uraniferous regions of Nisa (Southern Portugal), Beiras (Central Portugal), and Horta da Vilariça (Northern Portugal). Uraniferous deposits are located in the hercynian granites, in the metasediment enclaves and in the metamorphism contact haloes. In the Beiras region, several deposits have been exploited either by underground or surface mining. The main mineral processing method used was lixiviation, especially during the last active working phase (the last mine closed in 2001). Many of the sites were left in different stages of degradation. However, several programs for the environmental restoration of some of these old mines have been developed. Nisa and Horta da Vilariça regions were recognized for uranium deposits. However, these deposits have never been target for mining.

11.4.1.2 Material and Methods

Both plant and water samples were collected from selected sites in the vicinity of the uranium mineralized areas. The plants included submerged, free-floating, and rooted emergent species. Plant material was washed thoroughly with fresh water to remove sediment and other foreign objects. The preparation of plant material included, where appropriate, separation into roots and aerial tissues. After drying in an oven at 60 °C for 72 h, plant samples were ground into a homogeneous powder for further analysis. Water pH was determined using a pH meter (WQC-24, DKK-TOA) in the field. HCO_3 concentrations were also measured in situ using the titration method. The water samples were filtered through 0.45 μm Millipore cellulose membrane filters and cooled to 4 °C immediately after collection. For determination of metals and As, samples were acidified to $pH<2$ with 65 % HNO_3 (V/V).

To define the chemical characteristics of the stream water as well as the occurrence of heavy metal and other elemental contamination, several parameters were measured using current analytical methods, including Atomic Absorption Spectrometry (AAS, SOLAAR M Series equipment from Thermo–Unicam) for Ca, Mg, Na, and K; coupled graphite furnace AAS for Fe, Mn, Cu, Zn, Cd, Co, Ni, Bi, Cr, Pb, and As; and the High Performance Anion Exchange Chromatography method for Cl^-, NO_3^-, and PO_4^{3-}. Plants were prepared by microwave digestion with an HNO_3–H_2O_2 mixture in closed Teflon vessels (Multiwave 3000, Anton Paar). The analysis was performed in the same way as stated for water. Fluorometry was adopted for the determination of the U content in the water and plants using a "Fluorat-02-2 Manalyzer" (Lumex, Russia).

Water data quality control was performed by inserting reagent blanks and duplicate samples into each batch. Analytical precision, defined as the percent relative variation at the 95 % confidence level, ranged from 2 to 6 %, depending on the concentration levels. Certified reference material from the National Water Research Institute of Canada (reference TMDA-62) was also used to validate the results. For the plants, the analytical methods were assessed using a Polish certified reference material, Virginia Tobacco Leaves (CTA-VTL-2), which was included in the triplicate analyses. The agreement between the certified reference values and the values determined by the analytical method was in the range of 85.5–110.2 %.

11.4.1.3 Phytofiltration of Arsenic-Contaminated Water

Arsenic (As) is a metalloid that is ubiquitous in nature and is found in minerals and rocks, soils, natural waters, the atmosphere, and organisms (e.g., [130–132]). More than 245 minerals contain As, and the principal source of As is geological. However, human activities such as mining, pesticide application, and burning of fossil fuels also cause As pollution (e.g., [132]). Arsenic can occur in the environment in a variety of chemical forms with different mobility, bioavailability, and toxicity. Arsenic exists in inorganic and organic complexes such as arsenate [As(V)], arsenite [As(III)], monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), trimethylarsine (TMA), arsenocholine (AsC), arsenobetaine (AsB), arsenosugars, and others, and can occur in four oxidation states: +V (arsenate), +III (arsenite), 0 (arsenic), and −III (arsine) (e.g., [104]). However, in natural waters, As is found mostly in the inorganic form as oxyanions of trivalent arsenite [As(III)] or pentavalent arsenate [As(V)] (e.g., [131, 132]). These forms, which are also the most biologically important species, are interchangeable depending on the redox status of the environment [104].

In the natural environment, redox potential (Eh) and pH are the most important factors controlling As speciation. Under oxidizing conditions (high Eh values), $H_2AsO_4^-$ is dominant at low pH (less than approximately pH 6.9), while at higher pH, $HAsO_4^{2-}$ becomes dominant. In the pH range from 2 to 11, both $H_2AsO_4^-$ and $HAsO_4^{2-}$ species exist. The species $H_3AsO_4^0$ and AsO_4^{3-} may be present under extremely acidic and alkaline conditions, respectively. Under reducing conditions (low Eh values), H_3AsO_3 is the predominant inorganic As species (e.g., [131, 132]).

Concentrations of As in unpolluted surface water and groundwater typically vary from 1 to 10 μg/L (e.g., [132]). The highest concentrations of As are found in groundwater as a result of water–rock interactions and the tendency for favorable physical and geochemical conditions for As mobilization and accumulation (e.g., [131]). In fresh water, the variation is in the range of 0.15–0.45 μg/L [132] or 0.1–0.8 μg/L but can range up to 2 μg/L [131]. In this oxic water, arsenate is the predominant species, and both arsenate and arsenite are bioavailable to the plants in aquatic systems (e.g., [133]).

Although no evidence exists that As is essential for plant nutrition, As is naturally present in plants but in concentrations that rarely exceed 1 mg/kg (e.g., [130]). However, plants vary considerably in their tolerance of As and in the amount of As that they can take up from soils and water.

In the context of constructed wetlands, García et al. [134] reported that the direct uptake and accumulation of As in plants appears to play insignificant role in As removal. Similar findings were drawn by Singhakant et al. [135], who reported that only 0.5–1 % of the total As input was accumulated in plant tissues. There are also studies indicating that wetland plants have a remarkable effect on As retention [136, 137]. Several studies have shown that roots accumulate more As than shoots (e.g., [138, 139]). Except in hyperaccumulator plants, the typical ratios of shoot to root As concentrations are <1. The As distribution, in general, decreases from root to stem and leaf to fruit [130]. Different reasons may explain why As remains mostly in plant roots, such as limited translocation of As from roots to shoots (e.g., [140]), the presence of Fe and S (e.g., [141]), the effect of As speciation in the mechanism of translocation and its relationship to the phosphate transporter (e.g., [142]), and the formation of As(III)-phytochelation (PC) complexes in roots and subsequent sequestration in root vacuoles (e.g.,[105]). Arsenic speciation appears to play an important role in the uptake mechanism and further translocation. Due to the chemical similarity between arsenate and phosphate, arsenate is presumed to be taken up by the same transporters of phosphate in the roots. However, the form of As that is translocated to shoots is not known nor is how this translocation occurs [142, 143].

Reay [144] reported that the species *Ceratophyllum demersum*, a free-floating submergent plant, has been shown to accumulate As with a 20,000-fold concentration factor. Meanwhile, several studies have identified aquatic plants with high As content: *Lagarosiphon major* (Ridl.) Moss (300 mg/kg, in dry weight, DW [63]), *Egeria densa* Planch. (>1000 mg/kg DW [145]), *C. demersum* (>1000 mg/kg DW [145]), and *Lemna gibba* L. (1021.7 ± 250.8 mg/kg DW [146]). Some species of submerged macrophytes such as *Callitriche stagnalis* Scop. and *Myriophyllum propinquum* A. Cunn. have revealed high potential to accumulate As and therefore show potential for phytofiltration of As-contaminated water [147]. Other plants reported to accumulate As with some potential for phytofiltration of As-contaminated water are as follows: *Lepidium sativum* L. [148], *Rorippa nasturtium-aquaticum* (L.) Hayek [147], *Spirodela polyrrhiza* (L.) Schleid. [133], *Eichhornia crassipes* (Mart.) Solms and *Lemna minor* L. [149], *Hydrilla verticillata* (L.f.) Royle [105], *Eleocharis acicularis* (L.) Roem. & Schult. [103], and *Arundo donax* L. [104], *Callitriche lusitanica* Schotsman [42], *Micranthemum umbrosum* (J.F. Gmel.) S.F. Blake [150]; *Pistia stratiotes* [151], and *Vallisneria natans* (Lour.) H. Hara. [152].

The pH may affect the bioavailability of As and the consequent uptake by plants. For example, Wells and Richardson [153] reported a decrease in arsenate uptake in the moss *Hylocomium splendens* (Hedw.) Schimp. with increasing pH. In this moss, arsenate uptake was optimal at pH 5, where $H_2AsO_4^-$ was the dominant form in solution. As the pH increased to pH 8, where $HAsO_4^{2-}$ was the dominant anion, arsenate uptake decreased. Maximum As uptake rates occurring at pH 6.5 was observed by Mukherjee and Kumar [154] in aquatic plant *Pistia stratiotes*. The accumulation of As therefore depends on the type of plant (e.g., [141, 143]). The potential of some aquatic plants to accumulate As has been well demonstrated, and thus strongly supports their possible use in phytofiltration of As-contaminated water (e.g., [105, 155]).

In the studied areas As was detected in the surface waters at a range of concentrations between 0.15 and 40.2 μg/L (Fig. 11.5). The pH of the water ranged between 4.9 and 8.6. According to Smedley and Kinniburgh [131] and Sharma and Sohn [132], under oxidizing conditions, both $H_2AsO_4^-$ and $HAsO_4^{2-}$ inorganic As species

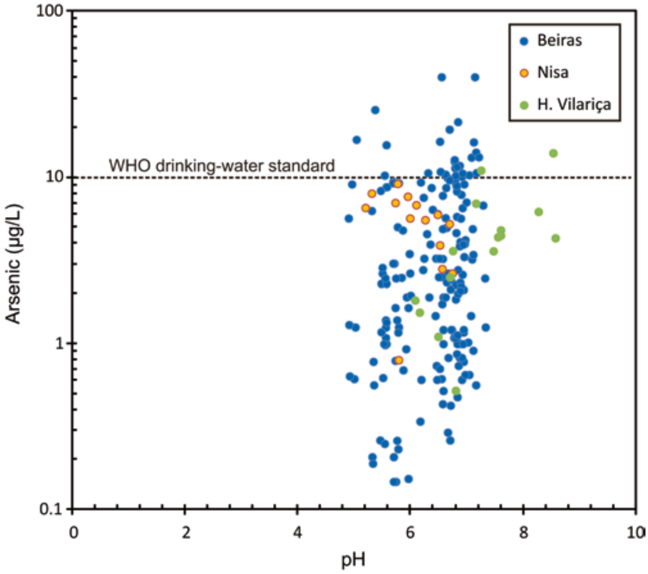


Fig. 11.5 Arsenic concentration relative to pH for stream waters of the studied areas. The World Health Organization [156] drinking-water provisional guideline value of 10 µg/L is indicated for reference

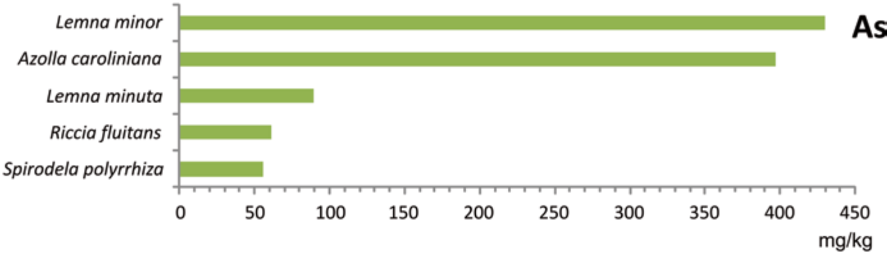


Fig. 11.6 Maximum As content in free-floating aquatic plants (mg/kg DW) of the studied areas

exist in the pH range found in the waters that were studied, although the form $H_2AsO_4^-$ may be predominant. At 28 of the sites sampled, the As concentration exceeded the limit (10 µg/L) established by the World Health Organization [156] for drinking water (Fig. 11.5).

The results of the chemical analysis of As on the most representative aquatic plant species in the studied areas revealed that As is highly accumulated by some species (Figs. 11.6, 11.7 and 11.8). High bioaccumulation levels were observed in several species at a magnitude much higher than the concentration in the surrounding water. The highest concentrations of As were found in the submerged species (Fig. 11.7) *Callitriche lusitanica* (2346 mg/kg, in dry weight, DW), *Ranunculus tripartitus* DC. (1463 mg/kg DW), *Callitriche brutia* Petagna (523 mg/kg DW),

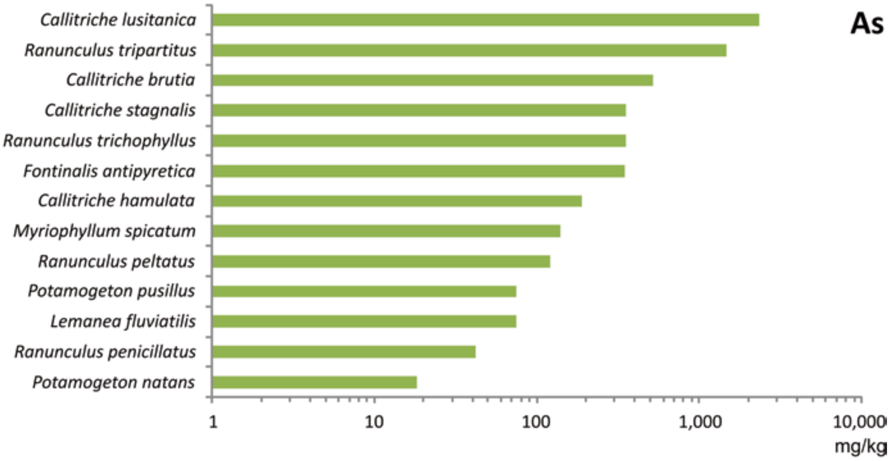


Fig. 11.7 Maximum As content in submerged aquatic plants (mg/kg DW) of the studied areas

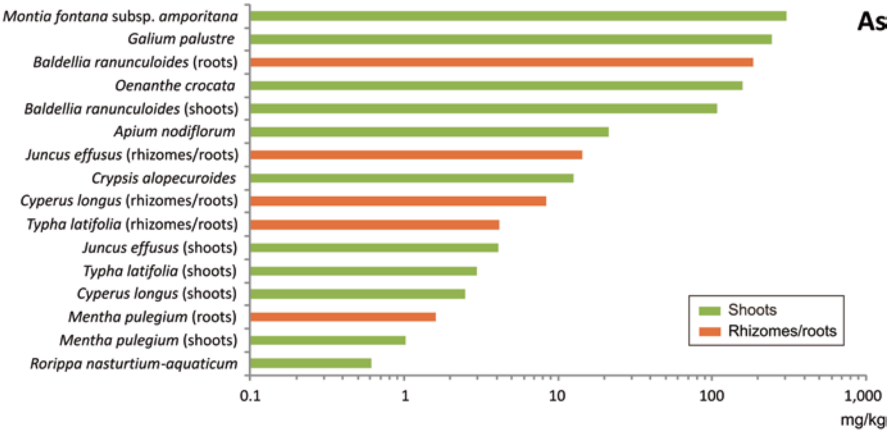


Fig. 11.8 Maximum As content in rooted emergent aquatic plants (mg/kg DW) of the studied areas

Callitriche stagnalis (354 mg/kg DW), *Ranunculus trichophyllus* Chaix ex Vill. (354 mg/kg DW), *Callitriche hamulata* Kütz. ex W.D.J. Koch (190 mg/kg DW), *Ranunculus peltatus* subsp. *saniculifolius* (Viv.) C.D.K. Cook (120 mg/kg DW), in the free-floating species (Fig. 11.6) *Lemna minor* (430 mg/kg DW), *Azolla caroliniana* Willd. (397 mg/kg DW), in the bryophyte *Fontinalis antipyretica* (346 mg/kg DW), and in the rooted emergent species (Fig. 11.8) *Montia fontana* L. (305 mg/kg DW), *Galium palustre* L. (247 mg/kg DW), and *Oenanthe crocata* L. (158 mg/kg DW). The measured concentrations in the remaining rooted emergent plants, such as *Apium nodiflorum* (L.) Lag., *Typha latifolia* L., and *Juncus effusus* L. were significantly lower when compared with the previous species, even in the rhizomes/roots.

The species *Callitriche stagnalis* and *Callitriche lusitanica* showed average BCFs of 1.1×10^4 and 1.8×10^4, respectively [42], revealing a great phytofiltration ability. The highest concentrations of As were therefore found in the plants from the Callitrichaceae family. Similar behavior was reported by Robinson et al. [147] in the Taupo Volcanic Zone of New Zealand, where As concentrations of 4215 mg/kg (DW) in *Callitriche stagnalis* and 422 mg/kg (DW) in *Callitriche petriei* R. Mason was found in waters with high As concentration (mean of 90 μg/L). The submerged species *Ranunculus trichophyllus* and *Ranunculus peltatus* subsp. *saniculifolius* also showed a high BCF, with averages of 7.5×10^3 and 1.1×10^4, respectively [42], and showed a very highly significant ($P < 0.001$) positive correlation with the As present in the water [42]. Therefore, this species may serve as an indicator of As pollution.

The free-floating species *Lemna minor* and *Azolla caroliniana* showed good ability to accumulate As in a similar way with average BCFs of 6.1×10^3 and 5.5×10^3 [42]. They therefore have a great potential for As accumulation in fresh waters. *Lemna minor* also showed a very significant ($P < 0.001$) positive correlation with the As present in the waters [42]. Therefore, this species may serve as a good indicator of As pollution. The bryophyte *Fontinalis antipyretica* showed a strong ability to accumulate large amounts of As, displaying a high BCF of approximately 1.2×10^4 [42]. In general, bryophytes have a great potential for rapid accumulation and exhibit seasonal fluctuations depending upon the environmental contaminants [84]. As this species does not have a root system, uptake occurs through the rhizoids as an ionic exchange between the environment and the basal portion of the plant.

The rooted emergent species *Montia fontana*, *Galium palustre,* and *Oenanthe crocata* also showed a significant As accumulation in their aerial organs and high BCF (with averages of 5.2×10^4, 1.4×10^4, and 4×10^4, respectively [42]). The remaining rooted emergent species *Apium nodiflorum*, *Typha latifolia*, and *Juncus effusus*, in spite of their high biomass and bioproductivity, did not show a significant As accumulation in their aerial organs. In these species, only the leaves of *Juncus effusus* showed a very highly significant ($P < 0.001$) positive correlation with the As found in the water [42]. Among the studied rooted emergent species, only in *Typha latifolia* and *Juncus effusus* the aerial parts (leaves) and the underground parts (rhizomes/roots) were separated. The As concentrations are significantly higher in the underground parts, and, in general, the TF values are below 1 [42]. Further studies on the rooted emergent species are therefore needed to investigate the mechanism of As uptake, translocation, and accumulation, considering both the water and the sediment and taking into account the relationship with Fe, S, and phosphate and determining the As species present.

11.4.1.4 Phytofiltration of Uranium-Contaminated Water

In the natural environment, U occurs almost entirely as ^{238}U in its hexavalent state (U^{6+}), and in minor quantities as ^{235}U, and in trace quantities as ^{234}U. In aqueous systems U reacts with oxygen to form uranyl ion UO_2^{2+} which is highly stable and soluble, which determines its toxicity [157–160]. Other soluble forms are UF_6, $UO_2(NO_3)_2$, UO_2Cl_2, UO_2F_2, uranyl acetates, sulfates, and carbonates [161]. Several physicochemical and biological variables may influence the U speciation, bioavailability, uptake, and toxicity in fresh surface waters, including pH, water hardness, natural organic matter, and microbial activity [94, 98, 102, 162–164]. The average global concentration of U in river water is ~0.3 µg/L [165], which is within the range 0.2–0.6 µg/L suggested by Palmer and Edmond [166].

The potential of some aquatic plants to accumulate U has been well demonstrated, supporting their possible use in phytofiltration of U-contaminated water. Among aquatic plants, algae are of considerable interest to ecological engineers due to their ability to sequester U as evidenced by the fact that many algae can survive in abundance under extreme environmental conditions (e.g., [119, 158]). Algae grow in a wide spectrum of contaminated waters from alkaline environments (*Chara*, *Nitella*) to acidic mine drainage wastewaters (*Mougeotia*, *Ulothrix*). Therefore, Kalin et al. [158] suggested that algae could provide a simple and long-term solution for removing U in waste streams through the combined processes of adsorption, reduction, and transformation.

Pettersson et al. [167] identified the water lily (*Nymphaea violacea* Lehm.) as an accumulator of several radionuclides when they observed high levels of U and Th series radionuclides in plant roots, rhizomes, and foliage in the vicinity of the Ranger Uranium Mine (Australia). At the same mine, an attempt to phytoremediate mine runoff water was tested using *Eleocharis dulcis* [81]. Members of Lemnacea are the most favored plants for phytoremediation and have been intensively described in literature as duck weeds including *Lemna*, *Spirodella*, *Wolffia*, and *Wolffiella* [133, 168]. *Lemna gibba* (612.36 ± 143.6 mg/kg DW) is an example of a U accumulator plant [169]. Other aquatic plants suggested as U accumulators are *Zostera japonica* Ascherson & Graebner and *Zostera marina* L. [170], *Phragmites australis* (Cav.) Trin ex. Steud. [171, 172], *Hydrilla verticillata* (L.f.) Royle [94], *Callitriche stagnalis* and *Fontinalis antipyretica* [41, 129], and *Spirodela punctata* [44].

Many microbial organisms, including bacteria, lichens, fungi, and algae, have been studied for their U-binding capacity, and the maximum U uptake was most frequently observed between pH 4 and 5 [98, 102, 173]. Similar results have been found in several other plant materials, such as dried roots [174] or coir pith [175]. Srivastava et al. [94] also observed in the aquatic plant *Hydrilla verticillata* that the maximum U uptake rates occurred at pH 5. Pratas et al. [41] found a very highly significant ($P < 0.001$) negative correlation between water pH and U concentration in *Callitriche stagnalis*.

The results of chemical analysis of the stream water samples at the studied sites show that U was detected in the surface waters at concentrations ranging from 0.23 to 1217 µg/L (Fig. 11.9). From a geographical interpretation of the sampling locations, it was clear that mine effluents contributed significantly to aquatic U contamination. Mean U concentration was higher in streams directly fed by mine drainage due to two locations with high concentrations, near Cunha Baixa and Urgeiriça mine sites [129].

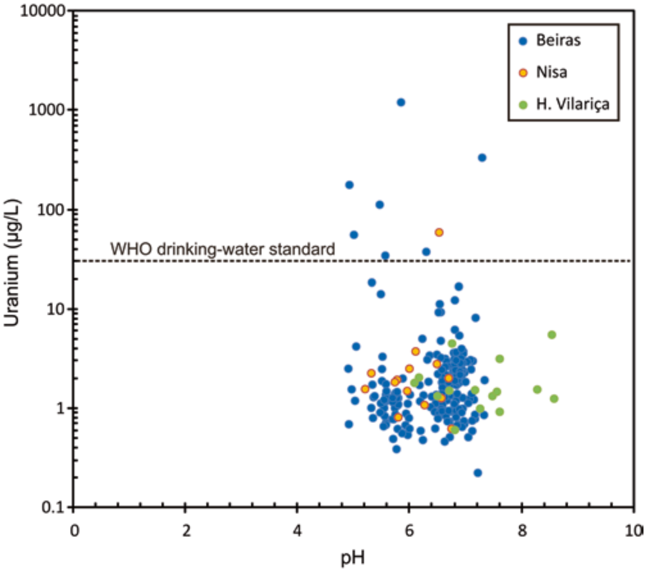


Fig. 11.9 Uranium concentration relative to pH for stream waters of the studied areas. The World Health Organization [156] drinking-water provisional guideline value of 30 µg/L is indicated for reference

According to Wang et al. [102], in an acidic environment ($pH < 4.0$), U(VI) occurs predominately as UO_2^{2+}, whereas at higher pH ranges ($4.0 < pH < 7.0$) composite hydrolyzed ionic species yield compounds such as UO_2OH^+, $(UO_2)_2(OH)_2^{2+}$ and $(UO_2)_3(OH)^{5+}$. When the pH is above 7.0, U(VI) precipitates easily. The pH of water samples ranged between 4.9 and 8.6 and reveals a complex behavior due to the wide hydrochemical variability. The mean U concentration (11.1 µg/L) is significantly higher than the range values estimated by Palmer and Edmond [166] as global riverine U flux. At 9 of the sites sampled, the U concentration exceeded the provisional guideline value (30 µg/L) indicated by the World Health Organization [156] for drinking water (Fig. 11.9). At these points, U concentrations could be directly linked to mining activities since these streams were directly fed by mine drainage.

The analysis of plants from the studied areas revealed high U bioaccumulation levels in several species and in magnitudes much higher than the ambient water concentrations (Figs. 11.10, 11.11 and 11.12). However, the water samples represent a "snapshot" while the U in the plant tissue may reflect more of an average of the U over time as the water U content could change after a rainfall or prolonged drought. In general, highest concentrations of U were observed in the submerged species and the lowest in the free-floating species. Among the submerged species highest concentrations of U were found in *Fontinalis antipyretica* (4979 mg/kg, DW), *Callitriche stagnalis* (1963 mg/kg DW) followed by *Callitriche hamulata* (379 mg/kg DW), *Ranunculus peltatus* subsp. *saniculifolius* (243 mg/kg DW), *Callitriche lusitanica* (218 mg/kg DW), and Ranunculus trichophyllus (65.8 mg/kgDW) (Fig. 11.11).

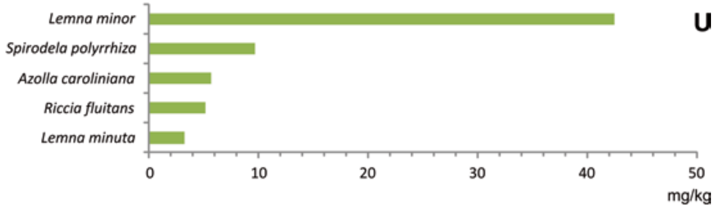


Fig. 11.10 Maximum U content in free-floating aquatic plants (mg/kg DW) of the studied areas

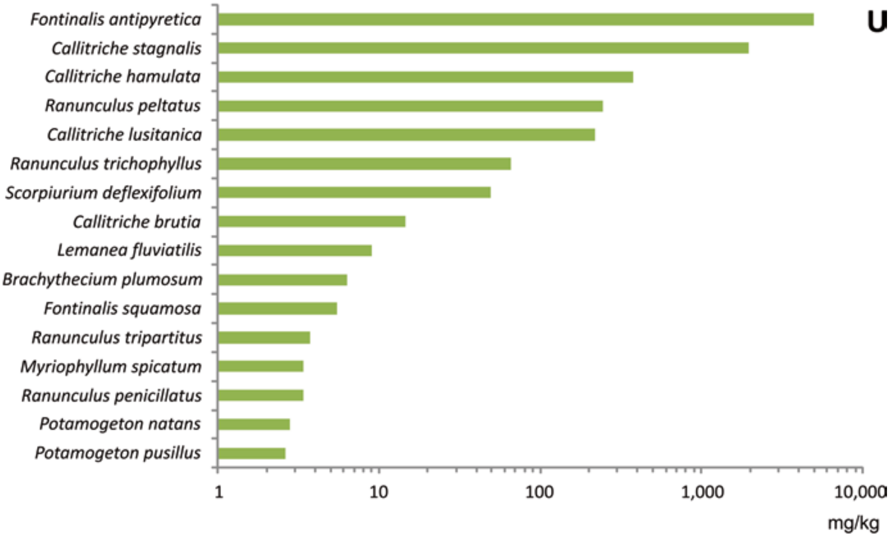


Fig. 11.11 Maximum U content in submerged aquatic plants (mg/kg DW) of the studied areas

The hydrophyte moss *Fontinalis antipyretica* showed a strong ability to accumulate large amounts of U, displaying a high mean BCF of approximately 1.0×10^4 [129] and a highly significant ($P < 0.001$) positive correlation with the U present in water [129]. Therefore, this species may serve as an indicator of U pollution. Accumulation of other metal(loid)s has also been demonstrated in *Fontinalis antipyretica*, such as As, Se, Cd, Cr, Cu, Pb, and Zn (e.g., [42, 176–185]). The species *Callitriche stagnalis*, *Callitriche hamulata*, and *Callitriche lusitanica* showed average BCFs of 3.0×10^3, 7.5×10^3, and 5.9×10^3, respectively [129], revealing a great phytofiltration ability. Furthermore, *Callitriche stagnalis* showed a highly significant ($P < 0.001$) positive correlation with the U present in the water [129]. Therefore, this species also serves as an excellent indicator of U pollution. High BCFs were also seen in *Ranunculus peltatus* subsp. *saniculifolius* and *Ranunculus trichophyllus*, with averages of 1.6×10^4 and 3.2×10^3, respectively [129].

Among the free-floating species (Fig. 11.10), *Lemna minor* showed good ability to accumulate U (42.5 mg/kg DW) as previously observed in *Lemna gibba* [169], with average BCF of 1.4×10^3 [129]. This plant belongs to the Lemnaceae family

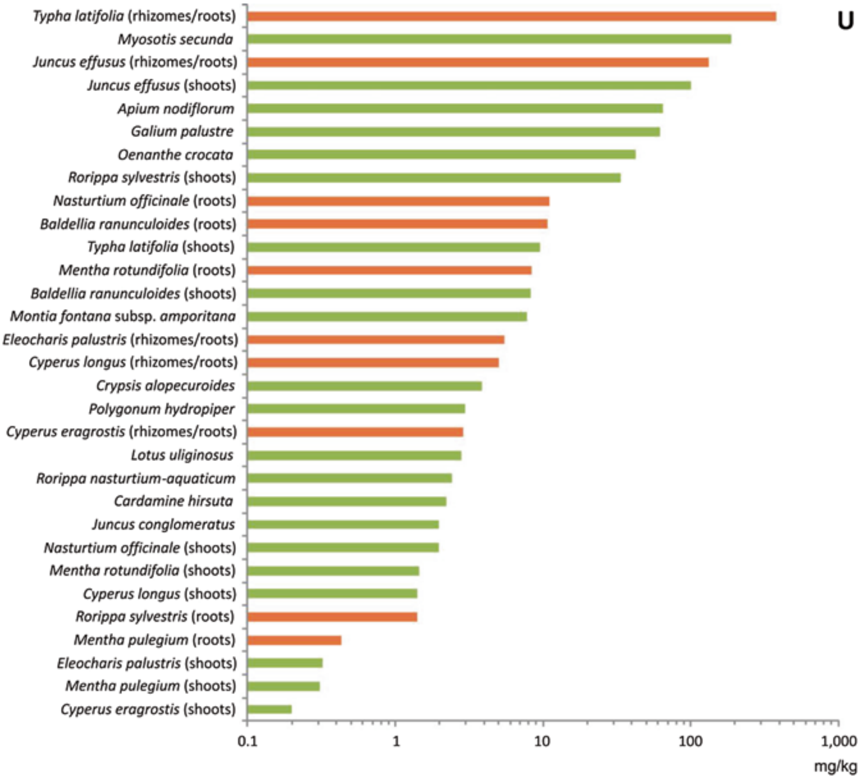


Fig. 11.12 Maximum U content in rooted emergent aquatic plants (mg/kg DW) of the studied areas

much studied in phytoremediation (e.g., [41, 128, 133, 149, 168, 186]). According to these studies, the fast growth rate, widespread distribution in natural wetlands, total independence from sediment, and adaptation to stress conditions like mine waters, makes such species a good option for water treatment technologies, in spite of the constant need for biomass removal. *Lemna minor* also showed a very significant ($P < 0.001$) positive correlation with the U present in the waters [129]. However, the U contents in free-floating plants probably cannot reflect their corresponding water U concentrations as they are moved with running river water. Nevertheless, *Lemna minor* also showed a very significant ($P < 0.001$) positive correlation with the U present in the standing water [41]. Therefore, this species may serve as a good indicator of U pollution.

Among the rooted emergent species high U concentrations were observed in rhizomes/roots of *Typha latifolia* (380 mg/kg DW), and *Juncus effusus* (132 mg/kg DW), and in the aerial parts of *Myosotis secunda* Al. Murray (188 mg/kg DW), *Juncus effusus* (99.9 mg/kg DW), *Apium nodiflorum* (64.5 mg/kg DW), *Galium palustre* (62.4 mg/kg DW), *Oenanthe crocata* (42.2 mg/kg DW), and *Rorippa sylvestris* (L.) Besser (33.8 mg/kg DW) (Fig. 11.12). *Myosotis secunda*, *Rorippa* *sylvestris*, *Juncus effusus*, *Apium nodiflorum*, *Galium palustre*, and *Oenanthe crocata* also showed high BCFs (mean BCF: 2.2×10^4, 4.8×10^3, 2.6×10^3, 3.6×10^3, 6.6×10^3, and 1.1×10^3, respectively) [129]. Only *Rorippa nasturtium-aquaticum* showed a significant ($P<0.05$) positive correlation with the U content of water. More studies are therefore needed on these emergent species taking into consideration the mechanism of U uptake and accumulation, partitioning of U between stems and roots, water column, and sediment.

Among the species studied, U concentrations are higher in the underground parts (significantly more in *Juncus effusus* and *Typha latifolia*), except for *Baldellia ranunculoides* (L.) Parl., *Cyperus eragrostis* Lam., *Mentha pulegium* L., and *Rorippa sylvestris*. In these species the mean TF values are above 1 suggesting better partitioning in the aerial parts. Pettersson et al. [167] identified the water lily, *Nymphaea violacea*, as an accumulator of several radionuclides when they observed high levels of ^{234}U, ^{238}U, ^{228}Th, ^{230}Th, ^{232}Th, ^{226}Ra, ^{210}Pb, and ^{210}Po in the plant, waters, and sediments in the vicinity of the Ranger Uranium mine (Australia). Higher levels of these contaminants were detected in the roots and rhizomes suggesting root uptake from sediment as the main uptake mechanism.

Preferential partitioning of U in roots may be attributed to the effect of U speciation in the mechanism of translocation and its complexation with phosphate. Studies have shown that among the physicochemical factors, pH is possibly the most important contributing factor [162, 163]. This can be attributed to the influence of pH on the speciation and bioavailability of metals and the activity of the functional groups of the biomass [102]. Other physicochemical variables which may influence the U speciation, bioavailability, and toxicity in fresh surface waters are water hardness, alkalinity, and natural organic matter [164]. In the studied areas, water pH varied from 4.9 to 8.6; this range favors the occurrence of U composite hydrolyzed ionic species. Variation in pH and the effect on U speciation may have affected the results. Only the species *Myosotis secunda* had a significant ($P<0.05$) negative correlation with the pH of the water. On the other hand, the species *Azolla caroliniana* showed a significant ($P<0.05$) positive correlation with the U present in the water [129]. Species with high U uptake, such as *Fontinalis antipyretica*, *Callitriche hamulata*, *Ranunculus peltatus* subsp. *saniculifolius*, *Callitriche lusitanica*, *Typha latifolia* (rhizomes/roots), *Juncus effusus*, and *Myosotis secunda*, may also be used in phytofiltration devices either in monoculture systems or in combined systems resembling the natural systems.

11.4.1.5 Phytofiltration of Lead-, Copper-, and Zinc-Contaminated Water

Among heavy metals, Pb is one of the most hazardous pollutants, due to its impact on human health and environment (e.g., [113]). The main sources of Pb pollution are mining and smelting, industrial effluents, fertilizers, pesticides, and municipal sewage sludge (e.g., [113, 187]). In plants, Pb toxicity leads to reduction in cell division and inhibition of photosynthesis [188], decreases in seed germination, as well as growth, dry biomass of roots and shoots, and disruption of mineral nutrition

[187]. Lead bioaccumulation potential and the effect of Pb stress have been studied recently in various aquatic plant species, including *Fontinalis antipyretica* [180], *Ceratophyllum demersum* L. [79, 189], *Potamogeton pectinatus* L. and *Potamogeton malaianus* Miq. [190], *Wolffia arrhiza* (L.) Horkel ex. Wimm. [191], *Lemna minor* [117, 186], *Lemna gibba* [117], *Najas indica* (Willd.) Cham. [192], *Typha latifolia* [193, 194], *Eichhornia crassipes* (Mart.) Solms [113, 195], and *Callitriche cophocarpa* Sendtn. [196].

In contrast to the Pb, certain heavy metals are required for the metabolic processes in plants. However, despite this, some of these metals, including Cu and Zn, become toxic at elevated levels (e.g., [197, 198]). Several aquatic plant species have been identified as accumulators of multi metals, including Cu and Zn, and as a result they might prove useful in phytofiltration technique. Some examples are as follows: *Ceratophyllum demersum* L. for Cu, Cr, Fe, and Mn [79]; *Fontinalis antipyretica* for Zn, Cu, and Cd [180, 181]; *Callitriche palustris* L. for Cu [199]; *Myriophyllum aquaticum* (Vell.) Verdc., *Ludwigina palustris* (L.) Ell., and *Mentha aquatica* L. for Cu, Fe, Hg, and Zn [200]; *Lemna minor* for Cu, Cd, Pb, and Zn [117, 201]; *Potamogeton pectinatus* L. and *Potamogeton malaianus* Miq. for Cd, Mn, Zn, and Cu [190]; *Lemna gibba* for Cu, Pb, and Zn [117, 202]; *Elodea canadensis* Michx. and *Elodea nuttallii* (Planch.) H. St. John for Cd, Cr, Cu, Mn, Ni, Pb, Zn, and Fe [203]; *Eichhornia crassipes* (Mart.) Solms for Cu, Zn, Cd, and Cr [195, 204–206]; and *Callitriche cophocarpa* Sendtn. for Tl, Cd, Zn, and Cr [196]. In the studied areas Pb, Cu, and Zn were detected in the surface waters at a concentration ranges of 0.1–13.4 µg/L, 0.45–125 µg/L, and 1.00–441 µg/L, respectively (Figs. 11.13, 11.14, and 11.15). Only at two of the sites sampled the Pb concentration exceeded the provisional guideline value (10 µg/L) indicated by the World Health Organization [156] for drinking water (Fig. 11.13).

The analytical results on the most representative aquatic plant species in the studied areas revealed the following significative uptake patterns: Pb from 90.5 to 1104 mg/kg in *Ranunculus trichophyllus*; rhizomes/roots of *Typha latifolia*, *Lemna minor*, *Spirodela polyrrhiza* (L.) Schleid.; and *Myriophyllum spicatum* (Figs. 11.16, 11.17, and 11.18); Cu from 81.8 to 161 mg/kg in *Callitriche lusitanica*, *Callitriche hamulata*, *Ranunculus trichophyllus*, and *Callitriche stagnalis* (Figs. 11.19, 11.20 and 11.21); and Zn from 900 to 34,162 mg/kg in *Lemna minor*, *Lemanea fluviatilis*, *Callitriche lusitanica*, *Callitriche brutia*, *Ranunculus trichophyllus*, *Fontinalis antipyretica*, and *Callitriche stagnalis* (Figs. 11.22, 11.23, and 11.24).

11.5 Conclusion

The studied aquatic plant species exhibited ability to accumulate several metal(loid)s, namely As, U, Pb, Cu, and Zn, in concentrations that are orders of magnitude higher than the surrounding water. This ability, reveled by several species, confirmed their high potential for the phytofiltration of contaminated waters. In general, submerged plants exhibited higher metal(loid) content. The highest U

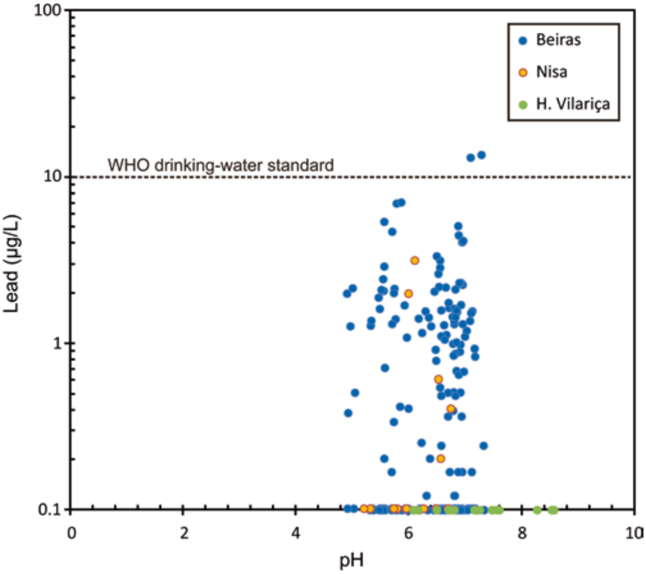


Fig. 11.13 Lead concentration relative to pH for stream waters of the studied areas. The World Health Organization [156] drinking-water provisional guideline value of 10 μg/L is indicated for reference

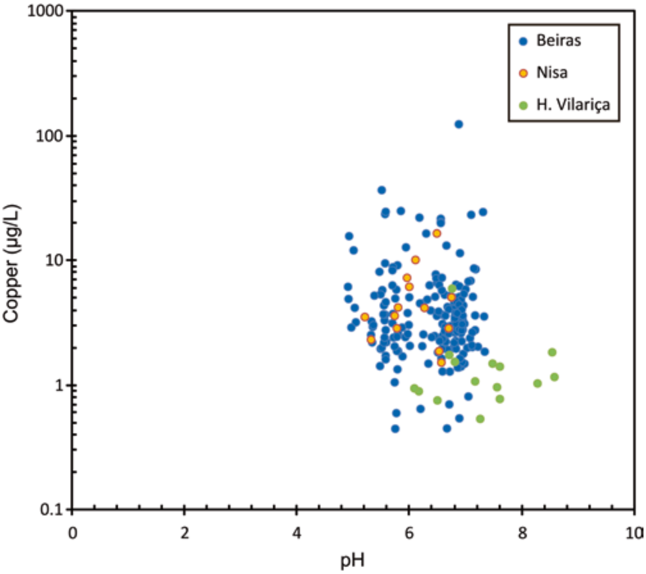


Fig. 11.14 Copper concentration relative to pH for stream waters of the studied areas

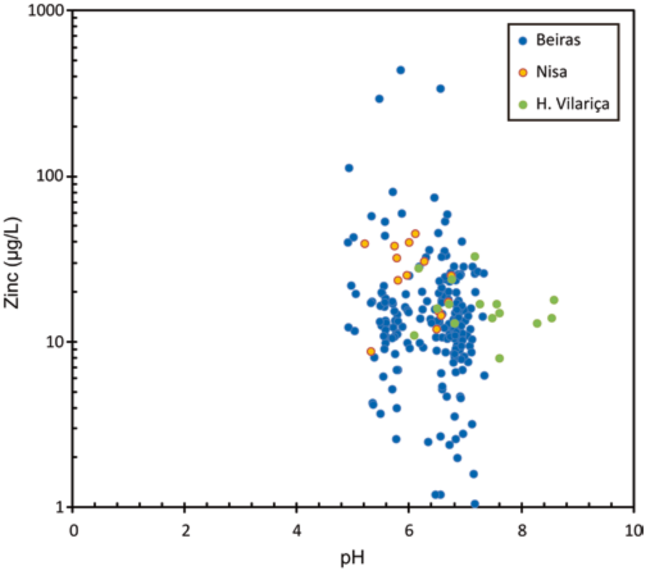


Fig. 11.15 Zinc concentration relative to pH for stream waters of the studied areas

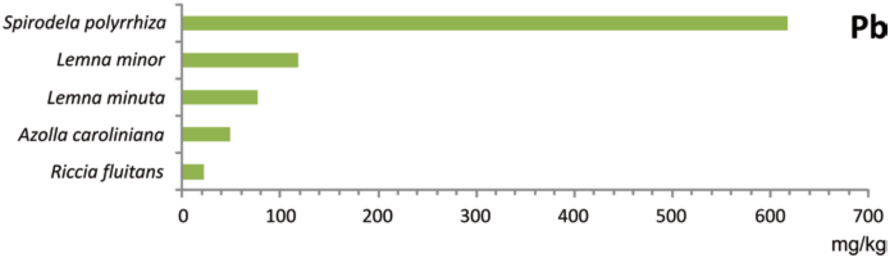


Fig. 11.16 Maximum Pb content in free-floating aquatic plants (mg/kg DW) of the studied areas

concentrations were observed in the bryophyte *Fontinalis antipyretica* and members of the monogeneric family Callitrichaceae. In the rooted emergent species, U seemed to be preferentially partitioned in rhizome/roots; maximum U content was observed in *Typha latifolia* rhizomes. The highest concentrations of As were found in the representatives of Callitrichaceae family. Other species with high As concentrations were *Lemna minor*, *Azolla caroliniana*, *Ranunculus trichophyllus*, *Fontinalis antipyretica*, *Montia fontana*, and *Galium palustre*.

The accumulation patterns of U and As of some of the aforementioned plants may also make them potential tools as bioindicators for trace elements in limnetic environment. Any adverse ecological impact in the aquatic food chain of these metal(loid)s by studied aquatic plants would be considered for future research. The abundance of *Fontinalis antipyretica* and Callitrichaceae family members, their biomass, relatively high bioproductivity, and ability to accumu-

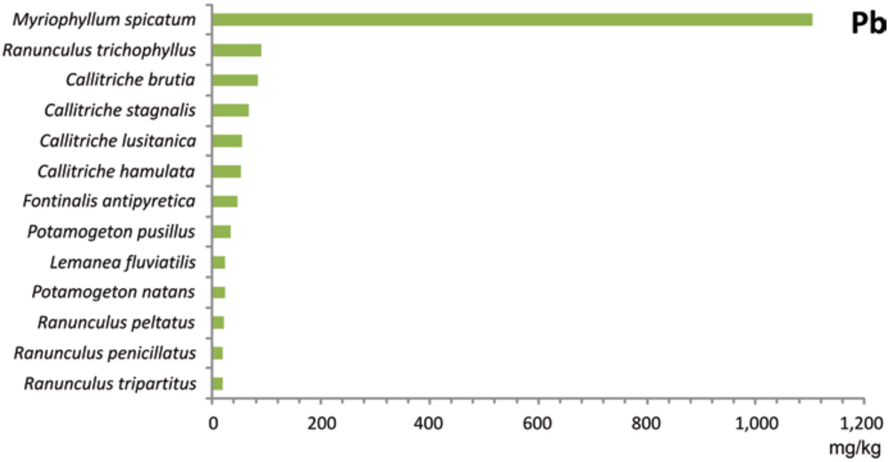


Fig. 11.17 Maximum Pb content in submerged aquatic plants (mg/kg DW) of the studied areas

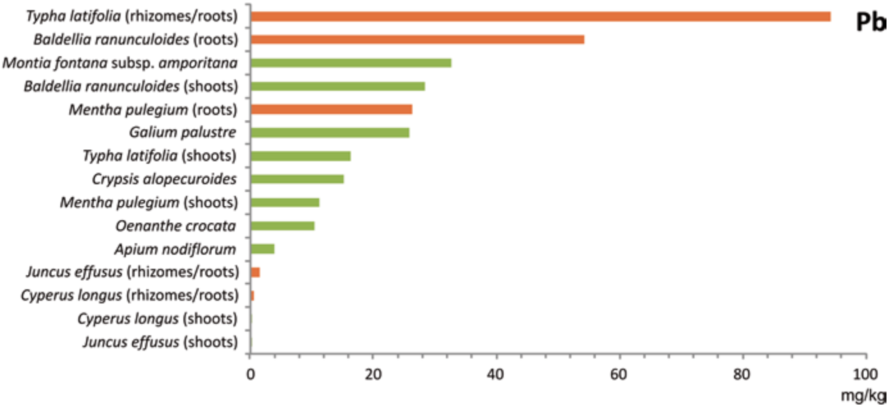


Fig. 11.18 Maximum Pb content in rooted emergent aquatic plants (mg/kg DW) of the studied areas

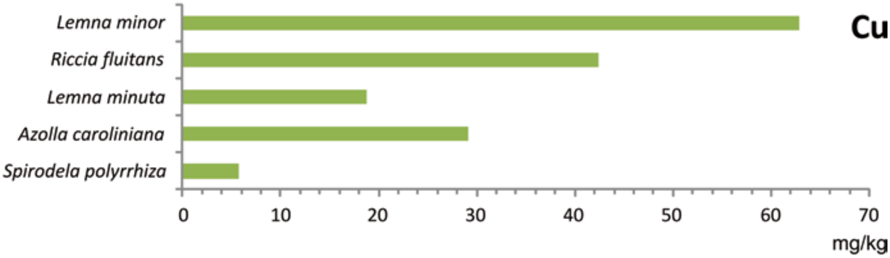


Fig. 11.19 Maximum Cu content in free-floating aquatic plants (mg/kg DW) of the studied areas

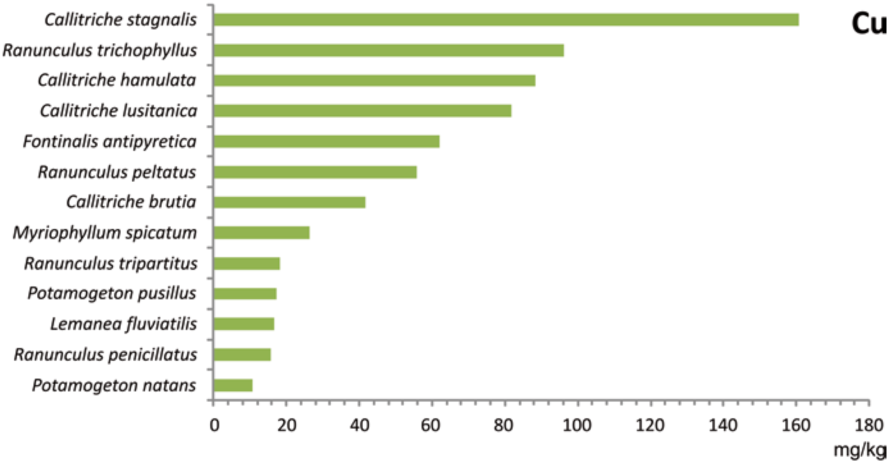


Fig. 11.20 Maximum Cu content in submerged aquatic plants (mg/kg DW) of the studied areas

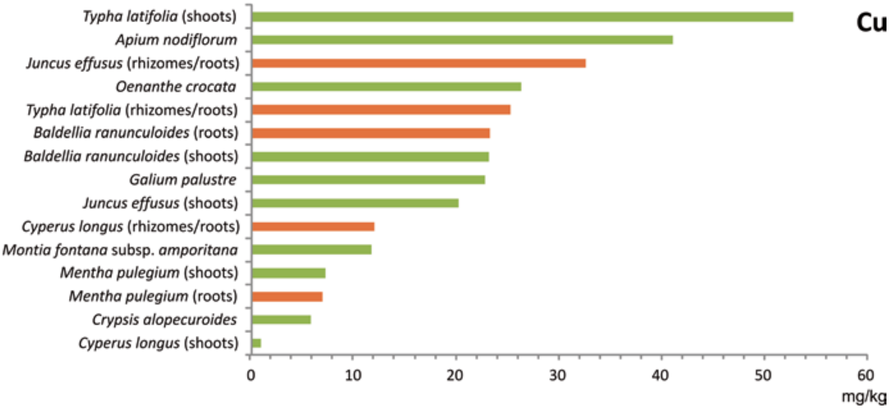


Fig. 11.21 Maximum Cu content in rooted emergent aquatic plants (mg/kg DW) of the studied areas

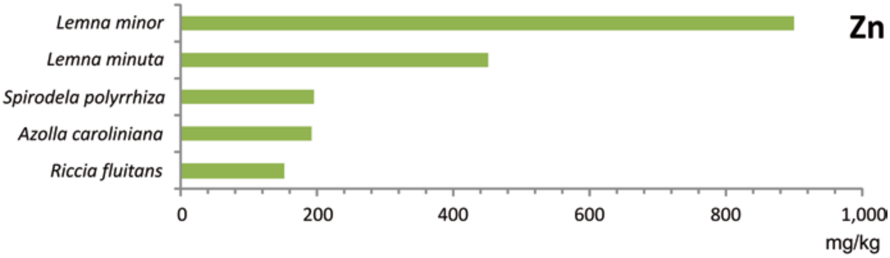


Fig. 11.22 Maximum Zn content in free-floating aquatic plants (mg/kg DW) of the studied areas

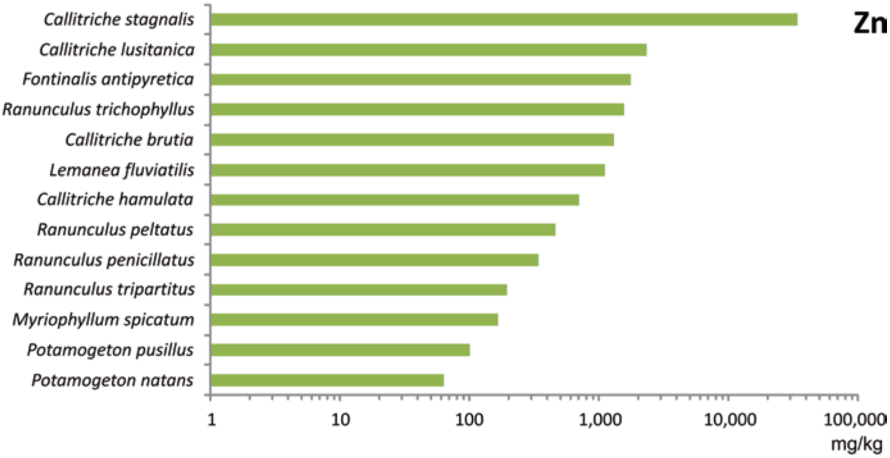


Fig. 11.23 Maximum Zn content in submerged aquatic plants (mg/kg DW) of the studied areas

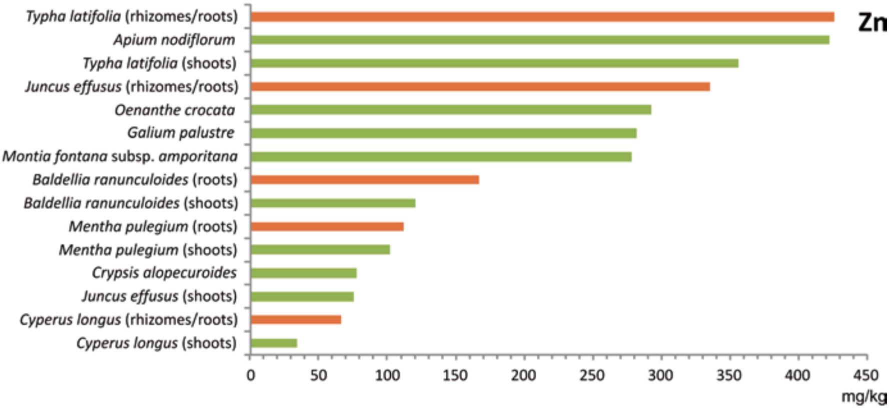


Fig. 11.24 Maximum Zn content in rooted emergent aquatic plants (mg/kg DW) of the studied areas

late several toxic elements at the same time make them promising candidates for the development of phytofiltration methodologies. Other species with high metal(loid) uptake such as *Lemna minor*, *Azolla caroliniana*, *Ranunculus trichophyllus*, *Montia fontana*, and *Galium palustre* can also be used in phytofiltration applications either in monoculture systems or in a combined systems representing the natural systems.

References

1. Lottermoser BG (2003) Mine wastes: Characterization, treatment and environmental impacts. Springer, Berlin, p 400
2. Chaney RL, Malik M, Li YM, Brown SL, Angle JS, Baker AJM (1997) Phytoremediation of soil metals. Curr Opin Biotechnol 8:279–284
3. Ensley BD (2000) Rationale for use of phytoremediation. In: Raskin I, Ensley BD (eds) Phytoremediation of toxic metals: Using plants to clean up the environment. Wiley, New York, pp 3–11
4. ITRC (2001) Phytotechnology technical and regulatory guidance document. Interstate Technology & Regulatory Council, Phytotechnologies Work Team, Washington, DC, p 124
5. Prasad MNV (2004) Phytoremediation of metals and radionuclides in the environment: The case for natural hyperaccumulators, metal transporters, soil-amending chelators and transgenic plants. In: Prasad MNV (ed) Heavy metal stress in plants: from biomolecules to ecosystems, 2nd edn. Springer, Berlin, pp 345–391
6. Mendez MO, Maier RM (2008) Phytoremediation of mine tailings in temperate and arid environments. Rev Environ Sci Biotechnol 7:47–59
7. Dickinson NM, Baker AJM, Doronila A, Laidlaw S, Reeves RD (2009) Phytoremediation of inorganics: realism and synergies. Int J Phytoremediation 11:97–114
8. Favas PJC, Pratas J, Varun M, D'Souza R, Paul MS (2014) Phytoremediation of soils contaminated with metals and metalloids at mining areas: Potential of native flora. In: Hernández-Soriano MC (ed) Environmental risk assessment of soil contamination. InTech, Rijeka, pp 485–517
9. El-Ramady HR, Abdalla N, Alshaal T, Elhenawy AS, Shams MS, Faizy SE-DA et al (2015) Giant reed for selenium phytoremediation under changing climate. Environ Chem Lett 13:359–380
10. Varun M, D'Souza R, Favas PJC, Pratas J, Paul MS (2015) Utilization and supplementation of phytoextraction potential of some terrestrial plants in metal-contaminated soils. In: Ansari AA, Gill SS, Gill R, Lanza GR, Newman L (eds) Phytoremediation: Management of environmental contaminants, vol 1, Springer. Cham, Heidelberg, pp 177–200
11. Roy M, Giri AK, Dutta S, Mukherjee P (2015) Integrated phytobial remediation for sustainable management of arsenic in soil and water. Environ Int 75:180–198
12. Favas PJC, Pratas J, Chaturvedi R, Paul MS, Prasad MNV (2016) Tree crops on abandoned mines for environmental remediation and industrial feedstock. In: Prasad MNV (ed) Bioremediation and Bioeconomy. Elsevier, Amsterdam, pp 219–249
13. ITRC (2009) Phytotechnology technical and regulatory guidance and decision trees, revised. PHYTO-3. Interstate Technology & Regulatory Council, Phytotechnologies Team, Washington, DC, p 204
14. Nwoko CO (2010) Trends in phytoremediation of toxic elemental and organic pollutants. Afr J Biotechnol 9(37):6010–6016
15. Bose S, Rai V, Bhattacharya S, Chaudhuri P, Bhattacharyya AK (2011) Phytoremediation: A promising technology of bioremediation for the removal of heavy metal and organic pollutants from the soil. In: Golubev IA (ed) Handbook of phytoremediation. Nova Science, New York, pp 263–296
16. Gaur N, Flora G, Yadav M, Tiwari A (2014) A review with recent advancements on bioremediation-based abolition of heavy metals. Environ Sci Processes Impacts 16(2): 180–193
17. Yavari S, Malakahmad A, Sapari NB (2015) A review on phytoremediation of crude oil spills. Water Air Soil Pollut 226(8):279
18. Zhang X, Wang J, Liu X, Gu L, Hou Y, He C et al (2015) Potential of *Sagittaria trifolia* for phytoremediation of diesel. Int J Phytoremediat 17:1220–1226
19. He Y, Chi J (2016) Phytoremediation of sediments polluted with phenanthrene and pyrene by four submerged aquatic plants. J Soils Sediments 16:309–317

20. Schnoor JL, Licht LA, McCutcheon SC, Wolfe NL, Carreira LH (1995) Phytoremediation of organic and nutrient contaminants. Environ Sci Technol 29:318A–323A
21. Rylott EL, Bruce NC (2008) Plants disarm soil: engineering plants for the phytoremediation of explosives. Trends Biotechnol 27(2):73–81
22. Berti WR, Cunningham SD (2000) Phytostabilization of metals. In: Ensley BD (ed) Raskin I. Wiley, Phytoremediation of toxic metals. Using plants to clean up the environment. New York, pp 71–88
23. Domínguez MT, Madrid F, Marañón T, Murillo JM (2009) Cadmium availability in soil and retention in oak roots: potential for phytostabilization. Chemosphere 76:480–486
24. Ali H, Khan E, Sajad MA (2013) Phytoremediation of heavy metals: Concepts and applications. Chemosphere 91:869–881
25. Brooks RR (1998) Phytoremediation by volatilisation. In: Brooks RR (ed) Plants that hyperaccumulate heavy metals: their role in phytoremediation, microbiology, archaeology, mineral exploration and phytomining. CAB International, New York, pp 289–312
26. Pilon-Smits E, Pilon M (2000) Breeding mercury-breathing plants for environmental cleanup. Trends Plant Sci 5(6):235–236
27. Ruiz ON, Daniell H (2009) Genetic engineering to enhance mercury phytoremediation. Curr Opin Biotechnol 20:213–219
28. Pilon-Smits EAH, LeDuc DL (2009) Phytoremediation of selenium using transgenic plants. Curr Opin Biotechnol 20:207–212
29. McGrath SP (1998) Phytoextraction for soil remediation. In: Brooks RR (ed) Plants that hyperaccumulate heavy metals: their role in phytoremediation, microbiology, archaeology, mineral exploration and phytomining. CAB International, New York, pp 261–287
30. Blaylock MJ, Huang JW (2000) Phytoextraction of metals. In: Raskin I, Ensley BD (eds) Phytoremediation of toxic metals: Using plants to clean up the environment. Wiley, New York, pp 53–70
31. Ma LQ, Komar KM, Tu C, Zhang W, Cai Y, Kennelley ED (2001) A fern that hyperaccumulates arsenic. Nature 409:579
32. McGrath SP, Zhao FJ (2003) Phytoextraction of metals and metalloids from contaminated soils. Curr Opin Biotechnol 14:277–282
33. Hernández-Allica J, Becerril JM, Garbisu C (2008) Assessment of the phytoextraction potential of high biomass crop plants. Environ Pollut 152:32–40
34. Pedron F, Petruzzelli G, Barbafieri M, Tassi E (2009) Strategies to use phytoextraction in very acidic soil contaminated by heavy metals. Chemosphere 75:808–814
35. Xie QE, Yan XL, Liao XY, Li X (2009) The arsenic hyperaccumulator fern *Pteris vittata* L. Environ Sci Technol 43(22):8488–8495
36. Van der Ent A, Baker AJM, Reeves RD, Pollard AJ, Schat H (2013) Hyperaccumulators of metal and metalloid trace elements: Facts and fiction. Plant Soil 362:319–334
37. Frers C (2009) El uso de plantas acuáticas en el tratamiento de aguas residuales. El Planeta Azul, Carmen de Areco
38. Dhote S, Dixit S (2009) Water quality improvement through macrophytes—a review. Environ Monit Assess 152:149–153
39. Krishna R, Fulekar MH, Pathank B (2012) Rhizofiltration: A green technology for remediation of heavy metals. Int J Innov BioSci 2(4):193–199
40. Dushenkov S, Kapulnik Y (2000) Phytofiltration of metals. In: Raskin I, Ensley BD (eds) Phytoremediation of toxic metals: Using plants to clean up the environment. Wiley, New York, pp 89–106
41. Pratas J, Favas PJC, Paulo C, Rodrigues N, Prasad MNV (2012) Uranium accumulation by aquatic plants from uranium-contaminated water in Central Portugal. Int J Phytoremediat 14:221–234
42. Favas PJC, Pratas J, Prasad MNV (2012) Accumulation of arsenic by aquatic plants in large-scale field conditions: Opportunities for phytoremediation and bioindication. Sci Total Environ 433:390–397

43. Rezania S, Ponraj M, Talaiekhozani A, Mohamad SE, Din MFM, Taib SM et al (2015) Perspectives of phytoremediation using water hyacinth for removal of heavy metals, organic and inorganic pollutants in wastewater. J Environ Manage 163:125–133
44. Nie X, Dong F, Liu N, Liu M, Zhang D, Kang W et al (2015) Subcellular distribution of uranium in the roots of *Spirodela punctata* and surface interactions. Appl Surf Sci 347:122–130
45. Crowley DE, Alvey S, Gilbert ES (1997) Rhizosphere ecology of xenobiotic-degrading microorganisms. In: Kruger EL, Anderson TA, Coats JR (eds) Phytoremediation of soil and water contaminants. ACS Symposium Series, Washington, pp 20–36
46. Khan MS, Zaidi A, Wani PA, Oves M (2009) Role of plant growth promoting rhizobacteria in the remediation of metal contaminated soils. Environ Chem Lett 7:1–19
47. Schnoor JL (2000) Phytostabilization of metals using hybrid poplar trees. In: Raskin I, Ensley BD (eds) Phytoremediation of toxic metals: Using plants to clean up the Environment. Wiley, New York, pp 133–150
48. Brooks RR, Chambers MF, Nicks LJ, Robinson BH (1998) Phytomining. Trends Plant Sci 3:359–362
49. Brooks RR, Chiarucci A, Jaffré T (1998) Revegetation and stabilization of mine dumps and other degraded terrain. In: Brooks RR (ed) Plants that hyperaccumulate heavy metals: Their role in phytoremediation, microbiology, archaeology, mineral exploration and phytomining. CAB International, New York, pp 227–247
50. Prasad MNV (2015) Engineered phyto-covers as natural caps for containment of hazardous mine and municipal solid waste dump sites – possible energy sources. In: Öztürk M, Ashraf M, Aksoy A, Ahmad MSA (eds) Phytoremediation for green energy. Springer, Dordrecht, pp 55–68
51. ITRC (2003) Technical and regulatory guidance document for constructed treatment wetlands. Interstate Technology & Regulatory Council, Wetlands Team, p 199
52. Vymazal J (2009) The use constructed wetlands with horizontal sub-surface flow for various types of wastewater. Ecol Eng 35:1–17
53. Olguín EJ, Sánchez-Galván G (2010) Aquatic phytoremediation: Novel insights in tropical and subtropical regions. Pure Appl Chem 82(1):27–38
54. Fonder N, Headley T (2013) The taxonomy of treatment wetlands: A proposed classification and nomenclature system. Ecol Eng 51:203–211
55. Horne AJ (2000) Phytoremediation by constructed wetlands. In: Terry N, Bañuelos G (eds) Phytoremediation of contaminated soil and water. Lewis Publishers, New York, pp 13–39
56. Kadlec RH, Wallace SD (2008) Treatment wetlands, 2nd edn. CRC Press, Boca Raton, FL, p 366
57. Vymazal J, Kröpfelová L (2008) Wastewater treatment in constructed wetlands with horizontal sub-surface flow. Springer, Dordrecht, p 566
58. Sobolewski A (1999) A review of processes responsible for metal removal in wetlands treating contaminated mine drainage. Int J Phytoremediat 1(1):19–51
59. Nyquist J, Greger M (2009) A field study of constructed wetlands for preventing and treating acid mine drainage. Ecol Eng 35:630–642
60. Adams A, Raman A, Hodgkins D (2013) How do the plants used in phytoremediation in constructed wetlands, a sustainable remediation strategy, perform in heavy-metal contaminated mine sites? Water Environ J 27(3):373–386
61. Zorrig W, Rabhi M, Ferchichi S, Smaoui A, Abdelly C (2012) Phytodesalination: a solution for salt-affected soils in arid and semi-arid regions. J Arid Land Stud 22:299–302
62. Ravindran KC, Venkatesan K, Balakrishnan V, Chellappan KP, Balasubramanian T (2007) Restoration of saline land by halophytes for Indian soils. Soil Biol Biochem 39:2661–2664
63. Brooks RR, Robinson BH (1998) Aquatic phytoremediation by accumulator plants. In: Brooks RR (ed) Plants that hyperaccumulate heavy metals: their role in phytoremediation, microbiology, archaeology, mineral exploration and phytomining. CAB International, New York, pp 203–226

64. Kikuchi T, Tanaka S (2012) Biological removal and recovery of toxic heavy metals in water environment. Crit Rev Environ Sci Technol 42:1007–1057
65. Bracamonte SC, Domingo LM (2002) Plantas aquáticas de las lagunas y humedales de Castilla-La Mancha. Real Jardín Botánico, Junta de Comunidades de Castilla-La Mancha, Madrid, p 340
66. Dhir B (2013) Phytoremediation: Role of aquatic plants in environmental clean-up. Springer, New Delhi, p 111
67. Arber A (1920) A study of aquatic angiosperms. Cambridge Univ. Press, Cambridge, p 436
68. Sculthorpe CD (1967) The biology of aquatic vascular plants. St. Martin's Press, New York, p 610
69. Outridge PM, Noller BN (1991) Accumulation of toxic trace elements by freshwater vascular plants. Rev Environ Contam Toxicol 121:1–63
70. Rai PK (2009) Heavy metal phytoremediation from aquatic ecosystems with special reference to macrophytes. Crit Rev Environ Sci Technol 39:697–753
71. Hutchinson GE (1975) A treatise on limnology, Vol 3: Limnological Botany. John Wiley & Sons, New York, p 672
72. Gray BR, Hill WR, Stewart AJ (2001) Effects of development time, biomass and ferromanganese oxides on nickel sorption by stream periphyton. Environ Pollut 112:61–71
73. Bradac P, Wagner B, Kistler D, Traber J, Behra R, Sigg L (2010) Cadmium speciation and accumulation in periphyton in a small stream with dynamic concentration variations. Environ Pollut 158:641–648
74. Stout L, Nüsslein K (2010) Biotechnological potential of aquatic plant-microbe interactions. Curr Opin Biotechnol 21:339–345
75. Krawczyk-Bärsch E, Lünsdorf H, Arnold T, Brendler V, Eisbein E, Jenk U, Zimmermann U (2011) The influence of biofilms on the migration of uranium in acid mine drainage (AMD) waters. Sci Total Environ 409:3059–3065
76. Castro MCR, Ureea G, Guasch H (2015) Influence of the interaction between phosphate and arsenate on periphyton's growth and its nutrient uptake capacity. Sci Total Environ 503–504:122–132
77. Srivastava S, Bhargava A (2016) Biofilms and human health. Biotechnol Lett 38:1–22
78. Hedin RS, Nairn RW, Kleinmann RLP (1994) The passive treatment of coal mine drainage. U.S. Bureau of Mines Information, Circular 9389, p 35
79. Rai UN, Sinha S, Tripathi RD, Chandra P (1995) Wastewater treatability potential of some aquatic macrophytes: Removal of heavy metals. Ecol Eng 5(1):5–12
80. Nyquist J, Greger M (2007) Uptake of Zn, Cu, and Cd in metal loaded *Elodea canadensis*. Environ Exp Bot 60:219–226
81. Overall RA, Parry DL (2004) The uptake of uranium by *Eleocharis dulcis* (Chinese water chestnut) in the Ranger Uranium Mine constructed wetland filter. Environ Pollut 132: 307–320
82. Entry JA, Vance NC, Hamilton MA, Zabowski D, Watrud LS, Adriano DC (1996) Phytoremediation of soil contaminated with low concentrations of radionuclides. Water Air Soil Pollut 88:167–176
83. Markert B (1993) Plants as biomonitors: indicators for heavy metals in the terrestrial environment. VCH, Weinheim, p 645
84. Cenci RM (2000) The use of aquatic moss (*Fontinalis antipyretica*) as monitor of contamination in standing and running waters: limits and advantages. J Limnol 60(Suppl 1):53–61
85. Fritioff Å, Greger M (2006) Uptake and distribution of Zn, Cu, Cd, and Pb in an aquatic plant *Potamogeton natans*. Chemosphere 63:220–227
86. Rahman MA, Hasegawa H (2011) Aquatic arsenic: Phytoremediation using floating macrophytes. Chemosphere 83:633–646
87. Tsezos M, Volesky B (1981) Biosorption of uranium and thorium. Biotechnol Bioeng 23:583–604
88. Volesky B, Holan ZR (1995) Biosorption of heavy metals. Biotechnol Prog 11:235–250

89. Abia AA, Horsfall M Jr, Didi O (2003) The use of chemically modified and unmodified cassava waste for the removal of Cd, Cu and Zn ions from aqueous solution. Bioresource Technol 90(3):345–348
90. Gamez G, Gardea-Torresdey JL, Tiemann KJ, Parsons J, Dokken K, Yacaman MJ (2003) Recovery of gold(III) from multi-elemental solutions by alfalfa biomass. Adv Environ Res 7(2):563–571
91. Raize O, Argaman Y, Yannai S (2004) Mechanisms of biosorption of different heavy metals by brown marine macroalgae. Biotechnol Bioeng 87:451–458
92. Gardea-Torresdey JL, De La Rosa G, Peralta-Videa JR (2004) Use of phytofiltration technologies in the removal of heavy metals: A review. Pure Appl Chem 76(4):801–813
93. Gadd GM (2009) Biosorption: critical review of scientific rationale, environmental importance and significance for pollution treatment. J Chem Technol Biotechnol 84:13–28
94. Srivastava S, Bhainsa KC, D'Souza SF (2010) Investigation of uranium accumulation potential and biochemical responses of an aquatic weed *Hydrilla verticillata* (L.f.) Royle. Bioresource Technol 101:2573–2579
95. Olguín EJ, Sánchez-Galván G (2012) Heavy metal removal in phytofiltration and phycoremediation: The need to differentiate between bioadsorption and bioaccumulation. New Biotechnol 30(1):3–8
96. Ungureanu G, Santos S, Boaventura R, Botelho C (2015) Arsenic and antimony in water and wastewater: Overview of removal techniques with special reference to latest advances in adsorption. J Environ Manage 151:326–342
97. Ebbs SD, Brady DJ, Kochian LV (1998) Role of uranium speciation in the uptake and translocation of uranium by plants. J Exp Bot 49:1183–1190
98. Li PF, Mao ZY, Rao XJ, Wang XM, Min MZ, Qiu LW, Liu ZL (2004) Biosorption of uranium by lake-harvested biomass from a cyanobacterium bloom. Bioresource Technol 94:193–195
99. Sawalha MF, Peralta-Videa JR, Duarte-Gardea M, Gardea-Torresdey JL (2008) Removal of copper, lead, and zinc from contaminated water by saltbush biomass. Analysis of the optimum binding, stripping, and binding mechanism. Bioresour Technol 99(10):4438–4444
100. Marchand L, Mench M, Jacob DL, Otte ML (2010) Metal and metalloid removal in constructed wetlands, with emphasis on the importance of plants and standardized measurements: A review. Environ Pollut 158:3447–3461
101. Kumar D, Gaur JP (2011) Metal biosorption by two cyanobacterial mats in relation to pH, biomass concentration, pretreatment and reuse. Bioresource Technol 102:2529–2535
102. Wang J, Hu X, Wang J, Bao Z, Xie S, Yang J (2010) The tolerance of *Rhizopus arrihizus* to U(VI) and biosorption beaviour of U(VI) onto *R. arrihizus*. Biochem Eng J 51:19–23
103. Ha NTH, Sakakibara M, Sano S (2011) Accumulation of indium and other heavy metals by *Eleocharis acicularis*: An option for phytoremediation and phytomining. Bioresource Technol 102:2228–2234
104. Mirza N, Pervez A, Mahmood Q, Shah MM, Shafqat MN (2011) Ecological restoration of arsenic contaminated soil by *Arundo donax* L. Ecol Eng 37:1949–1956
105. Xue PY, Yan CZ (2011) Arsenic accumulation and translocation in the submerged macrophyte *Hydrilla verticillata* (L.f.) Royle. Chemosphere 85:1176–1181
106. Tu S, Ma LQ (2004) Comparison of arsenic and phosphate uptake and distribution in arsenic hyperaccumulating and nonhyperaccumulating fern. J Plant Nutr 27(7):1227–1242
107. Sheoran V, Sheoran AS, Poonia P (2009) Phytomining: a review. Miner Eng 22:1007–1019
108. Morais I, Campos JS, Favas PJC, Pratas J, Pita F, Prasad MNV (2015) Nickel accumulation by *Alyssum serpyllifolium* subsp. *lusitanicum* (Brassicaceae) from serpentine soils of Bragança and Morais (Portugal) ultramafic massifs: plant-soil relationships and prospects for phytomining. Aust J Bot 63(2):17–30
109. OECD (2002) Environmental remediation of uranium production facilities. OECD Nuclear Energy Agency, International Atomic Energy Agency, Paris, p 323
110. Cardwell AJ, Hawker DW, Greenway M (2002) Metal accumulation in aquatic macrophytes from Southeast Queensland, Australia. Chemosphere 48:653–663

111. Abhilash PC, Pandey VC, Srivastava P, Rakesh PS, Chandran S, Singh N, Thomas AP (2009) Phytofiltration of cadmium from water by *Limnocharis flava* (L.) Buchenau grown in free-floating culture system. J Hazard Mater 170(2-3):791–797
112. Pratas J, Paulo C, Favas PJC, Venkatachalam P (2014) Potential of aquatic plants for phytofiltration of uranium-contaminated waters in laboratory conditions. Ecol Eng 69:170–176
113. Malar S, Vikram SS, Favas PJC, Perumal V (2014) Lead heavy metal toxicity induced changes on growth and antioxidative enzymes level in water hyacinths [*Eichhornia crassipes* (Mart.)]. Bot Stud 55:54
114. Malar S, Sahi SV, Favas PJC, Venkatachalam P (2015) Mercury heavy-metal-induced physiochemical changes and genotoxic alterations in water hyacinths [*Eichhornia crassipes* (Mart.)]. Environ Sci Pollut Res 22:4597–4608
115. Chowdhury R, Favas PJC, Pratas J, Jonathan MP, Ganesh PS, Sarkar SK (2015) Accumulation of trace metals by mangrove plants in Indian Sundarban Wetland: Prospects for phytoremediation. Int J Phytoremediat 17:885–894
116. Rodriguez-Hernandez MC, Bonifas I, Alfaro-De la Torre MC, Flores-Flores JL, Bañuelos-Hernández B, Patiño-Rodríguez O (2015) Increased accumulation of cadmium and lead under Ca and Fe deficiency in *Typha latifolia*: A study of two pore channel (TPC1) gene responses. Environ Exp Bot 115:38–48
117. Sasmaz M, Topal EIA, Obek E, Sasmaz A (2015) The potential of *Lemna gibba* L. and *Lemna minor* L. to remove Cu, Pb, Zn, and As in gallery water in a mining area in Keban, Turkey. J Environ Manage 163:246–253
118. González CI, Maine MA, Cazenave J, Hadad HR, Benavides MP (2015) Ni accumulation and its effects on physiological and biochemical parameters of *Eichhornia crassipes*. Environ Exp Bot 117:20–27
119. Jha VN, Tripathi RM, Sethy NK, Sahoo SK (2016) Uptake of uranium by aquatic plants growing in fresh water ecosystem around uranium mill tailings pond at Jaduguda, India. Sci Total Environ 539:175–184
120. Paulo C (2006) Selecção de plantas aquáticas e perspectivas na fitorremediação de escorrências uraníferas (Aquatic plant selection and perspective in uranium contaminated water phytoremediation), Master thesis. Instituto Superior Técnico, Lisboa
121. Favas PJC, Pratas JS (2007) Uptake of heavy metals, and arsenic by an aquatic plant in the vicinity of the abandoned Ervedosa tin mine (NE Portugal). Geochim Cosmochim Acta 71(15):A270–A270
122. Paulo C, Pratas J (2008) Environmental contamination control of water drainage from uranium mines by aquatic plants. In: Prasad MNV (ed) Trace elements as contaminants and nutrients: Consequences in ecosystems and human health. Wiley, Chichester, pp 623–651
123. Pratas J, Favas P, Rodrigues N, Prasad M (2010) Arsenic accumulation in aquatic plants (Central Portugal). In: Kallel A, Hassairi A, Bulucea CA, Mastorakis N (eds) Advances in waste management. WSEAS Press, Kantaoui, pp 73–76
124. Pratas J, Favas P, Rodrigues N, Prasad M, Freitas H (2010) Phytofiltration of uranium by aquatic plants of Central Portugal. In: Kallel A, Hassairi A, Bulucea CA, Mastorakis N (eds) Advances in waste management. WSEAS Press, Kantaoui, pp 77–80
125. Rodrigues N, Pratas J, Tavares L, Branches A (2010) Natural immobilization of uranium in streams. WSEAS Trans Environ Dev 6(7):539–548
126. Carvalho FP, Oliveira JM, Malta M (2011) Radionuclides in plants growing on sludge and water from uranium mine water treatment. Ecol Eng 37:1058–1063
127. Favas PJC, Pratas J, Prasad MNV, D'Souza R, Varun M, Paul M (2013) Potential for phytoremediation of multi-element contaminated water using aquatic plants. Curr Opin Biotech 24S:S128
128. Teixeira S, Vieira MN, Marques JE, Pereira R (2014) Bioremediation of an iron-rich mine effluent by *Lemna minor*. Int J Phytoremediat 16(12):1228–1240
129. Favas PJC, Pratas J, Varun M, D'Souza R, Paul MS (2014) Accumulation of uranium by aquatic plants in field conditions: Prospects for phytoremediation. Sci Total Environ 470–471:993–1002

130. Adriano D (2001) Trace elements in terrestrial environments: Biogeochemistry, bioavailability and risks of metals. Springer, New York, p 867
131. Smedley PL, Kinniburgh DG (2002) A review of the source, behaviour and distribution of arsenic in natural waters. Appl Geochem 17:517–568
132. Sharma VK, Sohn M (2009) Aquatic arsenic: Toxicity, speciation, transformations, and remediation. Environ Int 35:743–759
133. Rahman MA, Hasegawa H, Ueda K, Maki T, Okumura C, Rahman MM (2007) Arsenic accumulation in duckweed (*Spirodela polyrhiza* L.): A good option for phytoremediation. Chemosphere 69:493–499
134. García J, Rousseau DPL, Morató J, Lesage E, Matamoros V, Bayona JM (2010) Contaminant removal processes in subsurface-flow constructed wetlands: a review. Crit Rev Environ Sci Technol 40:561–661
135. Singhakant C, Koottatep T, Satayavivad J (2009) Enhanced arsenic removals through plant interactions in subsurface-flow constructed wetlands. J Environ Sci Health 44:163–169
136. Sasmaza A, Obek E (2009) The accumulation of arsenic, uranium, and boron in *Lemna gibba* L. exposed to secondary effluents. Ecol Eng 35:1564–1567
137. Rahman KZ, Wiessner A, Kuschk P, Afferden M, Mattusch J, Müller RA (2011) Fate and distribution of arsenic in laboratory-scale subsurface horizontal-flow constructed wetlands treating an artificial wastewater. Ecol Eng 37:1214–1224
138. Hozhina EI, Khramov AA, Gerasimov PA, Kumarkov AA (2001) Uptake of heavy metals, arsenic, and antimony by aquatic plants in the vicinity of ore mining and processing industries. J Geochem Explor 74:153–162
139. Adhikari AR, Acharya K, Shanahan SA, Zhou X (2011) Removal of nutrients and metals by constructed and naturally created wetlands in the Las Vegas Valley, Nevada. Environ Monit Assess 180:97–113
140. Wang J, Zhao FJ, Meharg AA, Raab A, Feldmann J, McGrath SP (2002) Mechanisms of arsenic hyperaccumulation in *Pteris vittata*. Uptake kinetics, interactions with phosphate, and arsenic speciation. Plant Physiol 130:1552–1561
141. Zhao FJ, McGrath SP, Meharg AA (2010) Arsenic as a food chain contaminant: mechanisms of plant uptake and metabolism and mitigation strategies. Annu Rev Plant Biol 61:535–559
142. Dhankher OP (2005) Arsenic metabolism in plants: an inside story. New Phytol 168:503–505
143. Lizama AK, Fletcher TD, Sun G (2011) Removal processes for arsenic in constructed wetlands. Chemosphere 84:1032–1043
144. Reay PF (1972) Accumulation of arsenic from arsenic-rich natural waters by aquatic plants. J Appl Ecol 9:557–565
145. Robinson BH, Brooks RR, Outred HA, Kirkman JH (1995) The distribution and fate of arsenic in the Waikato river system, North Island, New Zealand. Chem Spec Bioavailab 7:89–96
146. Mkandawire M, Taubert B, Dudel EG (2004) Capacity of *Lemna gibba* L. (duckweed) for uranium and arsenic phytoremediation in mine tailing waters. Int J Phytoremediat 6:347–362
147. Robinson B, Kim N, Marchetti M, Moni C, Schroeter L, Dijssel C, Milne G, Clothier B (2006) Arsenic hyperaccumulation by aquatic macrophytes in the Taupo Volcanic Zone, New Zealand. Environ Exp Bot 58:206–215
148. Robinson BH, Duwig C, Bolan NS, Kannathasan M, Saravanan A (2003) Uptake of arsenic by New Zealand watercress (*Lepidium sativum*). Sci Total Environ 301:67–73
149. Alvarado S, Guédez M, Lué-Merú MP, Nelson G, Alvaro A, Jesús AC, Gyula Z (2008) Arsenic removal from waters by bioremediation with the aquatic plants Water Hyacinth (*Eichhornia crassipes*) and Lesser Duckweed (*Lemna minor*). Bioresource Technol 99:8436–8440
150. Islam MS, Ueno Y, Sikder MT, Kurasaki M (2013) Phytofiltration of arsenic and cadmium from the water environment using *Micranthemum umbrosum* (J.F. Gmel) S.F. Blake as a hyperaccumulator. Int J Phytoremediat 15(10):1010–1021

151. Farnese FS, Oliveira JA, Lima FS, Leão GA, Gusman GS, Silva LC (2014) Evaluation of the potential of *Pistia stratiotes* L. (water lettuce) for bioindication and phytoremediation of aquatic environments contaminated with arsenic. Braz J Biol 74:S103–S112
152. Chen G, Liu X, Brookes PC, Xu J (2015) Opportunities for phytoremediation and bioindication of arsenic contaminated water using a submerged aquatic plant: *Vallisneria natans* (Lour.) H.Hara. Int J Phytoremediat 17:249–255
153. Wells JM, Richardson DHS (1985) Anion accumulation by the moss *Hylocomium splendens*: uptake and competition studies involving arsenate, selenate, selenite, phosphate, sulphate and sulphite. New Phytol 101:571–583
154. Mukherjee S, Kumar S (2005) Arsenic uptake potential of water lettuce (*Pistia stratiotes* L.). Int J Environ Stud 62:249–258
155. Srivastava S, Sounderajan S, Udas A, Suprasanna P (2014) Effect of combinations of aquatic plants (Hydrilla, Ceratophyllum, Eichhornia, Lemna and Wolffia) on arsenic removal in field conditions. Ecol Eng 73:297–301
156. WHO (2011) Guidelines for drinking-water quality, 4th edn. World Health Organization, Geneva, p 564
157. Malczewska B, Myers O, Shuey C, Lewis J (2003) Recommendations for a uranium health-based ground water standard. Ground Water Quality Bureau, Environment Department, New Mexico, p 71
158. Kalin M, Wheeler WN, Meinrath G (2005) The removal of uranium from mining waste water using algal/microbial biomass. J Environ Radioact 78:151–177
159. Schöner A, Noubactep C, Büchel G, Sauter M (2009) Geochemistry of natural wetlands in former uranium milling sites (eastern Germany) and implications for uranium retention. Chem Erde-Geochem 69:91–107
160. Bhalara PD, Punetha D, Balasubramanian K (2014) A review of potential remediation techniques for uranium(VI) ion retrieval from contaminated aqueous environment. J Environ Chem Eng 2:1621–1634
161. Durakoviae A (1999) Medical effects of internal contamination with uranium. Croatian Med J 40(1):49–66
162. Laurette J, Larue C, Llorens I, Jaillard D, Jouneau P-H, Bourguignon J, Carrière M (2012) Speciation of uranium in plants upon root accumulation and root-to-shoot translocation: A XAS and TEM study. Environ Exp Bot 77:87–95
163. Laurette J, Larue C, Mariet C, Brisset F, Khodja H, Bourguignon J, Carrière M (2012) Influence of uranium speciation on its accumulation and translocation in three plant species: Oilseed rape, sunflower and wheat. Environ Exp Bot 77:96–107
164. Markich SJ (2013) Water hardness reduces the accumulation and toxicity of uranium in a freshwater macrophyte (*Ceratophyllum demersum*). Sci Total Environ 443:582–589
165. Mangini A, Sonntag C, Bertsch G, Mueller E (1979) Evidence for a higher natural uranium content in world rivers. Nature 278:337–339
166. Palmer MR, Edmond JM (1993) Uranium in river water. Geochim Cosmochim Acta 57:4947–4955
167. Pettersson HBL, Johnston HA, Murray AS (1993) Uptake of uranium and thorium series radionuclides by the water lily, *Nymphaea violacea*. J Environ Radioact 19:85–108
168. Miretzky P, Saralegui A, Cirelli AF (2004) Aquatic macrophytes potential for the simultaneous removal of heavy metals Buenos Aires, Argentina. Chemosphere 57(8):997–1005
169. Mkandawire M, Dudel EG (2005) Accumulation of arsenic in *Lemna gibba* L. (duckweed) in tailing waters of two abandoned uranium mining sites in Saxony, Germany. Sci Total Environ 336:81–89
170. Kondo K, Kawabata H, Ueda S, Hasegawa H, Inaba J, Mitamura O, Seike Y, Ohmomo Y (2003) Distribution of aquatic plants and absorption of radionuclides by plants through the leaf surface in brackish Lake Obuchi, Japan, bordered by nuclear fuel cycle facilities. J Radioanal Nucl Chem 257:305–312
171. Gerth A, Hebner A, Kiessig G, Zellmer A (2005) Passive treatment of minewater at the Schlema-Alberoda site. In: Merkel B, Hasche-Berge A (eds) Uranium in the environment: Mining impact and consequences. Springer, Berlin, pp 409–414

172. Černe M, Smodiš B, Štrok M (2011) Uptake of radionuclides by a common reed (*Phragmites australis* (Cav.) Trin. Ex Steud.) grown in the vicinity of the former uranium mine at Žirovski vrh. Nucl Eng Des 241:1282–1286
173. Bhat SV, Melo JS, Chaugule BB, D'Souza SF (2008) Biosorption characteristics of uranium(VI) from aqueous medium onto *Catenella repens*, a red alga. J Hazard Mater 158:628–635
174. Bhainsa KC, D'Souza SF (2001) Uranium (VI) biosorption by dried roots of *Eichhornia crassipes* (water hyacinth). J Environ Sci Health 36:1621–1631
175. Parab H, Joshi S, Shenoy N, Verma R, Lali A, Sudersanan M (2005) Uranium removal from aqueous solution by coir pith: equilibrium and kinetic studies. Bioresource Technol 96:1241–1248
176. Burton MAS, Peterson PJ (1979) Metal accumulation by aquatic bryophytes from polluted mine streams. Environ Pollut 19:39–46
177. Say PJ, Harding JPC, Whitton BA (1981) Aquatic mosses as monitors of heavy metal contamination in the river Etherow. Great Britain Environ Pollut Ser B 2:295–307
178. Mouvet C (1984) Accumulation of chromium and copper by the aquatic moss *Fontinalis antipyretica* L. ex Hedw. transplanted in a metal-contaminated river. Environ Technol Lett 5:541–548
179. Goncalves EPR, Boaventura RAR (1998) Uptake and release kinetics of copper by the aquatic moss *Fontinalis antipyretica*. Water Res 32(4):1305–13
180. Siebert A, Bruns I, Krauss G-J, Miersch J, Markert B (1996) The use of the aquatic moss *Fontinalis antipyretica* L. ex Hedw. as a bioindicator for heavy metals: 1. Fundamental investigations into heavy metal accumulation in *Fontinalis antipyretica* L. ex Hedw. Sci Total Environ 177:137–144
181. Bruns I, Friese K, Markert B, Krauss G-J (1997) The use of *Fontinalis antipyretica* L. ex Hedw. as a bioindicator for heavy metals: 2. Heavy metal accumulation and physiological reaction of *Fontinalis antipyretica* L. ex Hedw. in active biomonitoring in the River Elbe. Sci Total Environ 204:161–176
182. Vázquez MD, Wappelhorst O, Markert B (2004) Determination of 28 elements in aquatic moss *Fontinalis antipyretica* Hedw. and water from the upper reaches of the river Nysa (CZ, D), by ICPMS, ICP-OES and AAS. Water Air Soil Pollut 152:153–172
183. Gapeeva MV, Dolotov AV, Chemeris EV (2010) Prospects of using mosses (*Fontinalis antipyretica* Hedw. and *Pylaisia polyantha* (Hedw.) Bruch et al.) as indicators of environmental contamination with heavy metals. Russ J Ecol 41(1):28–31
184. Díaz S, Villares R, Carballeira A (2012) Uptake kinetics of As, Hg, Sb, and Se in the aquatic moss *Fontinalis antipyretica* Hedw. Water Air Soil Poll 223:3409–3423
185. Mechora Š, Germ M, Stibilj V (2012) Selenium and its species in the aquatic moss *Fontinalis antipyretica*. Sci Total Environ 438:122–126
186. Uysal Y, Taner F (2009) Effect of pH, temperature, and lead concentration on the bioremoval of lead from water using *Lemna minor*. Int J Phytoremediat 11:591–608
187. Sharma P, Dubey RS (2005) Lead toxicity in plants. Braz J Plant Physiol 17:35–52
188. Ekmekci Y, Tanyolac D, Ayhan B (2009) A crop tolerating oxidative stress induced by excess lead: maize. Acta Physiol Plant 31:319–330
189. Mishra S, Srivastava S, Tripathi R, Kumar R, Seth C, Gupta D (2006) Lead detoxification by coontail (*Ceratophyllum demersum* L.) involves induction of phytochelatins and antioxidant system in response to its accumulation. Chemosphere 65:1027–1039
190. Peng K, Luo C, Lou L, Li X, Shen Z (2008) Bioaccumulation of heavy metals by the aquatic plants *Potamogeton pectinatus* L. and *Potamogeton malaianus* Miq. And their potential use for contamination indicators and in wastewater treatment. Sci Total Environ 392:22–29
191. Piotrowska A, Bajguz A, Godlewska B, Czerpak R, Kaminska M (2009) Jasmonic acid as modulator of lead toxicity in aquatic plant *Wolffia arrhiza* (Lamnaceae). Environ Exp Bot 66:507–513
192. Singh R, Tripathi RD, Dwivedi S, Kumar A, Trivedi PK, Chakrabarty D (2010) Lead bioaccumulation potential of an aquatic macropyte *Najas indica* are related to antioxidant system. Bioresour Technol 101:3025–3032

193. Alonso-Castro AJ, Carranza-Álvarez C, Alfaro-De la Torre MC, Chávez-Guerrero L, García-De la Cruz RF (2009) Removal and accumulation of cadmium and lead by *Typha latifolia* exposed to single and mixed metal solutions. Arch Environ Contam Toxicol 57:688–696
194. Lyubenova L, Pongrac P, Vogel-Mikuš K, Mezek GK, Vavpetič P, Grlj N, Regvar M, Pelicon P, Schröder P (2013) The fate of arsenic, cadmium and lead in *Typha latifolia*: A case study on the applicability of micro-PIXE in plant ionomics. J Hazard Mater 248–249:371–378
195. Smolyakov BS (2012) Uptake of Zn, Cu, Pb, and Cd by water hyacinth in the initial stage of water system remediation. Appl Geochem 27:1214–1219
196. Augustynowicz J, Tokarz K, Baran A, Płachno BJ (2014) Phytoremediation of water polluted by thallium, cadmium, zinc, and lead with the use of macrophyte *Callitriche cophocarpa*. Arch Environ Contam Toxicol 66:572–581
197. Alloway BJ (1995) Soil processes and the behavior of metals. In: Alloway BJ (ed) Heavy metals in soils. Blackie, Glasgow, pp 38–57
198. Succuro JS, McDonald SS, Lu CR (2009) Phytoremediation: The wave of the future. In: Kirakosyan A, Kaufman PB (eds) Recent advances in plant biotechnology. Springer, Dordrecht, pp 119–135
199. Samecka-Cymerman A, Kempers AJ (2001) Bioindication of heavy metals with aquatic macrophytes: The case of a stream polluted with power plant sewages in Poland. J Toxicol Environ Health 62(1):57–67
200. Kamal M, Ghaly AE, Mahmoud N, Côté R (2004) Phytoaccumulation of heavy metals by aquatic plants. Environ Int 29:1029–1039
201. Hou W, Chen X, Song G, Wang Q, Chang CC (2007) Effects of copper and cadmium on heavy metal polluted waterbody restoration by duckweed (*Lemna minor*). Plant Physiol Biochem 45:62–69
202. Khellaf N, Zerdaoui M (2009) Phytoaccumulation of zinc by the aquatic plant, *Lemna gibba* L. Bioresource Technol 100:6137–6140
203. Thiébaut G, Gross Y, Gierlinski P, Boiché A (2010) Accumulation of metals in *Elodea canadensis* and *Elodea nuttallii*: Implications for plant–macroinvertebrate interactions. Sci Total Environ 408:5499–5505
204. Zhu YL, Zayed AM, Quian JH, De Souza M, Terry N (1999) Phytoaccumulation of trace metals by wetland plants: II. Water hyacinth. J Environ Qual 28:339–344
205. Hu C, Zhang L, Hamilton D, Zhou W, Yang T, Zhu D (2007) Physiological responses induced by copper bioaccumulation in *Eichhornia crassipes* (Mart.). Hydrobiologia 579:211–218
206. Mishra VK, Tripathi BD (2009) Accumulation of chromium and zinc from aqueous solutions using water hyacinth (*Eichhornia crassipes*). J Hazard Mater 164:1059–1063

Chapter 12
Phytoremediation of Heavy Metals Contaminated Soils Through Transgenic Plants

Neerja Srivastava

12.1 Introduction

Environmental pollution by heavy metals has become a serious problem in the world. The mobilization of heavy metals through extraction from ores and subsequent processing for different applications has led to the release of these elements into the environment. The problem of heavy metals' pollution is becoming more and more serious with increasing industrialization and disturbance of natural biogeochemical cycles. Unlike organic substances, heavy metals are essentially nonbiodegradable and therefore accumulate in the environment. The accumulation of heavy metals in soils and waters poses a risk to the environmental and human health. These elements accumulate in the body tissues of living organisms (bioaccumulation) and their concentrations increase as they pass from lower trophic levels to higher trophic levels (a phenomenon known as biomagnification). In soil, heavy metals cause toxicological effects on soil microbes, which may lead to a decrease in their numbers and activities [1, 2].

The concept of phytoremediation has evoked considerable interest in plant metal accumulation [3]. Using hyperaccumulation as a means of cleaning up metal-contaminated soil and water has been proposed [4] based on well-documented observations that several plant species not only tolerate otherwise toxic levels of certain metals in the soil but even hyperaccumulate them in their shoots [5]. Plants ideal for phytoremediation should posses multiple traits. They must be fast growing, have high biomass, deep roots, be easy to harvest, and should tolerate and accumulate a range of heavy metals in their aerial and harvestable parts. To date, no plant has been described that fulfils all these criteria. However, a rapidly growing nonaccumulator could be engineered so that it achieves some of the properties of hyperaccumulators. Recent progress in determining the molecular basis for metal accumulation and

---

N. Srivastava (✉)
Department of Biochemistry, IBSBT, CSJM University, Kalyanpur,
Kanpur, UP 208024, India
e-mail: neerjas70@gmail.com

A.A. Ansari et al. (eds.), *Phytoremediation*, DOI 10.1007/978-3-319-40148-5_12

tolerance by hyperaccumulators has been significant and provides us with a strong scientific basis to outline some strategies for achieving this goal [6].

12.1.1 Heavy Metals

From a chemical point of view, the term heavy metal is strictly ascribed to transition metals with atomic mass over 20 and specific gravity above 5. In biology, "heavy" refers to a series of metals and also metalloids that can be toxic to both plants and animals even at very low concentrations. Some of these heavy metals, such as As, Cd, Hg, Pb, or Se, are not essential, since they do not perform any known physiological function in plants. Others, such as Co, Cu, Fe, Mn, Mo, Ni, and Zn, are essential elements required for normal growth and metabolism of plants. These latter elements can easily lead to poisoning when their concentration rises to supra-optimal values [7].

Mining, manufacturing, and the use of synthetic products (e.g., pesticides, paints, batteries, industrial waste, and land application of industrial or domestic sludge) can result in heavy metal contamination of urban and agricultural soils. Heavy metals also occur naturally but rarely at toxic levels. Potentially contaminated soils may occur at old landfill sites (particularly those that accepted industrial wastes), old orchards that used insecticides containing arsenic as an active ingredient, fields that had past applications of waste water or municipal sludge, areas in or around mining waste piles and tailings, industrial areas where chemicals may have been dumped on the ground, or in areas downwind from industrial sites [8]. Excess heavy metal accumulation in soils is toxic to humans and other animals. Exposure to heavy metals is normally chronic (exposure over a longer period of time), due to food chain transfer. Acute (immediate) poisoning from heavy metals is rare through ingestion or dermal contact, but is possible. Chronic problems associated with long-term heavy metal exposures are as follows:

Lead—mental lapse.
Cadmium—affects kidney, liver, and GI tract.
Arsenic—skin poisoning, affects kidneys and central nervous system.

The most common problems causing *cationic* metals (metallic elements whose forms in soil are positively charged cations, e.g., Pb^{2+}) are mercury, cadmium, lead, nickel, copper, zinc, chromium, and manganese. The most common anionic compounds (elements whose forms in soil are combined with oxygen and are negatively charged, e.g., MoO_4^{2-}) are arsenic, molybdenum, selenium, and boron [8]. Metal pollution has harmful effect on biological systems and does not undergo biodegradation. Toxic heavy metals such as Pb, Co, Cd can be differentiated from other pollutants, since they cannot be biodegraded but can be accumulated in living organisms, thus causing various diseases and disorders even in relatively lower concentrations [9]. Heavy metals, with soil residence times of thousands of years, pose numerous health dangers to higher organisms. They are also known to have effect on plant growth, ground cover, and have a negative impact on soil microflora [10]. It is well known that heavy metals cannot be chemically degraded and need to be physically removed or be transformed into nontoxic compounds [11, 12].

12.1.2 Basic Soil Chemistry and Potential Risks of Heavy Metals

The most common heavy metals found at contaminated sites, in order of abundance are Pb, Cr, As, Zn, Cd, Cu, and Hg [13]. Those metals are important since they are capable of decreasing crop production due to the risk of bioaccumulation and biomagnification in the food chain. There is also the risk of superficial and groundwater contamination. Knowledge of the basic chemistry, environmental, and associated health effects of these heavy metals is necessary in understanding their speciation, bioavailability, and remedial options. The fate and transport of a heavy metal in soil depends significantly on the chemical form and speciation of the metal. Once in the soil, heavy metals are adsorbed by initial fast reactions (minutes, hours), followed by slow adsorption reactions (days, years) and are, therefore, redistributed into different chemical forms with varying bioavailability, mobility, and toxicity [14, 15]. This distribution is believed to be controlled by reactions of heavy metals in soils such as (1) mineral precipitation and dissolution; (2) ion exchange, adsorption, and desorption; (3) aqueous complexation; (4) biological immobilization and mobilization; and (5) plant uptake [16, 17].

12.2 Lead

Lead is a metal belonging to group IV and period 6 of the periodic table with atomic number 82, atomic mass 207.2, density 11.4 g cm^{-3}, melting point 327.4 °C, and boiling point 1725 °C. It is a naturally occurring, bluish gray metal usually found as a mineral combined with other elements, such as sculpture (i.e., Pubs, $PbSO_4$), or oxygen ($PbCO_3$), and ranges from 10 to 30 mg kg^{-1} in the earth's crust [18]. Typical mean BP concentration for surface soils worldwide averages 32 mg kg^{-1} and ranges from 10 to67 mg kg^{-1} [19]. Lead ranks fifth behind Fe, Cu, Al, and Zn in industrial production of metals [17]. Ionic lead, Pb(II), lead oxides and hydroxides, and lead metaloxyanion complexes are the general forms of Pb that are released into the soil, groundwater, and surface waters. The most stable forms of lead are Pb(II) and lead-hydroxy complexes. Lead(II) is the most common and reactive form of Pb, forming mononuclear and polynuclear oxides and hydroxides [20]. The predominant insoluble Pb compounds are lead phosphates, lead carbonates (form when the pH is above 6), and lead (hydr)oxides [21]. Lead sulfide (PbS) is the most stable solid form within the soil matrix and forms under reducing conditions, when increased concentrations of sulfide are present. Under anaerobic conditions a volatile organolead (tetramethyl lead) can be formed due to microbialalkylation [20].

Lead(II) compounds are predominantly ionic (e.g., $Pb^{2+}SO_4^{2-}$), whereas Pb(IV) compounds tend to be covalent (e.g., tetraethyl lead, $Pb(C_2H_5)_4$). Some Pb (IV) compounds, such as PbO_2, are strong oxidants. Lead forms several basic salts, such as $Pb(OH)_2 \bullet 2PbCO_3$, which was once the most widely used white paint pigment and the source of considerable chronic lead poisoning to children who ate peeling white paint. Many compounds of Pb(II) and a few Pb(IV) compounds are useful. The two most common of these are lead dioxide and lead sulfate, which are participants in the reversible reaction that occurs during the charge and discharge of lead storage battery. In addition to the inorganic compounds of lead, there are a number of organolead compounds such as tetraethyllead. The toxicities and environmental effects of organolead compounds are particularly noteworthy because of the former widespread use and distribution of tetraethyllead as a gasoline additive. Although more than 1000 organolead compounds have been synthesized, those of commercial and toxicological importance are largely limited to the alkyl(methyl and ethyl) lead compounds and their salts (e.g., dimethyldiethyl lead, trimethyllead chloride, and diethyl lead dichloride).

Inhalation and ingestion are the two routes of exposure, and the effects from both are the same. Pb accumulates in the body organs (i.e., brain), which may lead to poisoning (plumbism) or even death. The gastrointestinal tract, kidneys, and central nervous system are also affected by the presence of lead. Children exposed to lead are at risk for impaired development, lower IQ, shortened attention span, hyperactivity, and mental deterioration, with children under the age of six being at a more substantial risk. Adults usually experience decreased reaction time, loss of memory, nausea, insomnia, anorexia, and weakness of the joints when exposed to lead [22]. Lead is not an essential element. It is well known to be toxic and its effects have been more extensively reviewed than the effects of other trace metals. Lead can cause serious injury to the brain, nervous system, red blood cells, and kidneys [23]. Exposure to lead can result in a wide range of biological effects depending on the level and duration of exposure. Various effects occur over a broad range of doses, with the developing young and infants being more sensitive than adults. Lead poisoning, which is so severe as to cause evident illness, is now very rare. Lead performs no known essential function in the human body, it can merely do harm after uptake from food, air, or water. Lead is a particularly dangerous chemical, as it can accumulate in individual organisms, but also in entire food chains.

The most serious source of exposure to soil lead is through direct ingestion (eating) of contaminated soil or dust. In general, plants do not absorb or accumulate lead. However, in soils testing high in lead, it is possible for some lead to be taken up. Studies have shown that lead does not readily accumulate in the fruiting parts of vegetable and fruit crops (e.g., corn, beans, squash, tomatoes, strawberries, and apples). Higher concentrations are more likely to be found in leafy vegetables (e.g., lettuce) and on the surface of root crops (e.g., carrots). Since plants do not take up large quantities of soil lead, the lead levels in soil considered safe for plants will be much higher than soil lead levels where eating of soil is a concern (pica). Generally, it has been considered safe to use garden produce grown in soils with total lead levels less than 300 ppm. The risk of lead poisoning through the food chain increases as the soil lead level rises above this concentration. Even at soil levels above 300 ppm, most of the risk is from lead-contaminated soil or dust deposits on the plants rather than from uptake of lead by the plant [17, 24].

12.3 Arsenic

Arsenic is a metalloid in group VA and period 4 of the periodic table that occurs in a wide variety of minerals, mainly as As_2O_3, and can be recovered from processing of ores containing mostly Cu, Pb, Zn, Ag, and Au. It is also present in ashes from coal combustion. Arsenic has the following properties: atomic number 33, atomic mass 75,density 5.72 g cm^{-3}, melting point 817 °C, and boiling point613°C, and exhibits fairly complex chemistry and can be present in several oxidation states (−III, 0, III, V) [25]. In aerobic environments, As (V) is dominant, usually in the form of arsenate (AsO_4^{3-}) in various protonation states: H_3AsO_4, H_2AsO^{4-}, $HAsO_4^{2-}$, and AsO_4^{3-}. Arsenate and other anionic forms of arsenic behave as chelates and can precipitate when metal cations are present [26]. Metal arsenate complexes are stable only under certain conditions.

Arsenic (V) can also coprecipitate with or adsorb onto iron oxyhydroxides under acidic and moderately reducing conditions. Coprecipitates are immobile under these conditions, but arsenic mobility increases as pH increases [25]. Under reducing conditions As (III) dominates, existing as arsenite (AsO_3^{3-}), and its protonated forms H_3AsO_3, H_2AsO^{3-}, and$HAsO_3^{2-}$. Arsenite can adsorb or coprecipitate with metal sulfides and has a high affinity for other sulfur compounds. Elemental arsenic and arsine, AsH_3, may be present under extreme reducing conditions. Biotransformation (via methylation) of arsenic creates methylated derivatives of arsine, such as dimethyl arsine $HAs(CH_3)_2$ and trimethylarsine $As(CH_3)_3$ which are highly volatile. Since arsenic is often present in anionic form, it does not form complexes with simple anions such as $Cl^-$and SO_4^{2-}. Arsenic speciation also includes organometallic forms such as methylarsinic acid $(CH_3)AsO_2H_2$ and dimethylarsinic acid $(CH_3)_2AsO_2H$. Many As compounds adsorb strongly to soils and are therefore transported only over short distances in groundwater and surface water. Arsenic is associated with skin damage, increased risk of cancer, and problems with circulatory system [27].

12.4 Zinc

Zinc is a transition metal with the following characteristics: period 4, group IIB, atomic number 30, atomic mass 65.4, density 7.14 g cm^{-3}, melting point 419.5 °C, and boiling point 906 °C. Zinc occurs naturally in soil (about 70mg kg^{-1} in crustal rocks) [28], but Zn concentrations are rising unnaturally, due to anthropogenic additions. Most Zn is added during industrial activities, such as mining, coal, and waste combustion and steel processing. Many foodstuffs contain certain concentrations of Zn. Drinking water also contains certain amounts of Zn, which may be higher when it is stored in metal tanks. Industrial sources or toxic waste sites may cause the concentrations of Zn in drinking water to reach levels that can cause health problems. Zinc is a trace element that is essential for human health. Zinc shortages can cause birth defects. The world's Zn production is still on the rise which means that more and more Zn ends up in the environment. Water is polluted with Zn, due to the presence of large quantities present in the wastewater of industrial plants. A consequence is that Zn polluted sludge is continually being deposited by rivers on their banks. Zinc may also increase the acidity of waters. Some fish can accumulate Zn in their bodies, when they live in Zn-contaminated waterways. When Zn enters the bodies of these fish, it is able to biomagnify up the food chain. Water-soluble zinc that is located in soils can contaminate groundwater. Plants often have a Zn uptake that their systems cannot handle, due to the accumulation of Zn in soils. Finally, Zn can interrupt the activity in soils, as it negatively influences the activity of microorganisms and earthworms, thus retarding the breakdown of organic matter [29].

12.5 Cadmium

Cadmium is located at the end of the second row of transition elements with atomic number 48, atomic weight 112.4, density 8.65 g cm^{-3}, melting point 320.9 °C, and boiling point 765 °C. Together with Hg and Pb, Cd is one of the big three heavy metal poisons and is not known for any essential biological function. In its compounds, Cd occurs as the divalent Cd(II) ion. Cadmium is directly below Zn in the periodic table and has a chemical similarity to that of Zn, an essential micronutrient for plants and animals. This may account in part for Cd's toxicity; because Zn being an essential trace element, its substitution by Cd may cause the malfunctioning of metabolic processes [30].

Cadmium is present as an impurity in several products, including phosphate fertilizers, detergents, and refined petroleum products. In addition, acid rain and the resulting acidification of soils and surface waters have increased the geochemical mobility of Cd, and as a result its surface-water concentrations tend to increase as lake water pH decreases [30]. Cadmium is produced as an inevitable by-product of Zn and occasionally lead refining. The application of agricultural inputs such as fertilizers, pesticides, and biosolids (sewage sludge); the disposal of industrial wastes or the deposition of atmospheric contaminants increases the total concentration of Cd in soils; and the bioavailability of this Cd determines whether plant Cd uptake occurs to a significant degree [31]. Cadmium is very biopersistent but has few toxicological properties and, once absorbed by an organism, remains resident for many years.

Since the 1970s, there has been sustained interest in possible exposure of humans to Cd through their food chain, for example, through the consumption of certain species of shellfish or vegetables. Concern regarding this latter route (agricultural crops) led to research on the possible consequences of applying sewage sludge (Cd-rich biosolids) to soils used for crops meant for human consumption, or of using cadmium-enriched phosphate fertilizer [30]. This research has led to the stipulation of highest permissible concentrations for a number of food crops [32].

Cadmium in the body is known to affect several enzymes. It is believed that the renal damage that results in proteinuria is the result of Cd adversely affecting enzymes responsible for reabsorption of proteins in kidney tubules. Cadmium also reduces the activity of delta-aminolevulinic acid synthetase, arylsulfatase, alcohol dehydrogenase, and lipoamide dehydrogenase, whereas it enhances the activity of deltaaminolevulinic acid dehydratase, pyruvate dehydrogenase, and pyruvate decarboxylase [33]. The most spectacular and publicized occurrence of cadmium poisoning resulted from dietary intake of cadmium by people in the Jintsu River Valley, near Fuchu, Japan. The victims were afflicted by *itaiitai* disease, which means *ouch, ouch* in Japanese. The symptoms are the result of painful osteomalacia (bone disease) combined with kidney malfunction. Cadmium poisoning in the Jintsu River Valley was attributed to irrigated rice contaminated from an upstream mine producing Pb, Zn, and Cd. The major threat to human health is chronic accumulation in the kidneys leading to kidney dysfunction. Food intake and tobacco smoking are the main routes by which Cd enters the body [33].

12.6 Copper

Copper is a transition metal which belongs to period 4 and group IB of the periodic table with atomic number 29, atomic weight 63.5, density 8.96 g cm^{-3}, melting point 1083 °C, and boiling point 2595 °C. The metal's average density and concentrations in crustal rocks are 8.1 ×103 kgm^{-3} and 55 mg kg^{-1}, respectively [28]. Copper is the third most used metal in the world [34]. Copper is an essential micronutrient required in the growth of both plants and animals. In humans, it helps in the production of blood hemoglobin. In plants, Cu is especially important in seed production, disease resistance, and regulation of water. Copper is indeed essential, but in high doses it can cause anemia, liver and kidney damage, and stomach and intestinal irritation. Copper normally occurs in drinking water from Cu pipes, as well as from additives designed to control algal growth. While Cu's interaction with the environment is complex, research shows that most Cu introduced into the environment is, or rapidly becomes, stable and results in a form which does not pose a risk to the environment. In fact, unlike some man-made materials, Cu is not magnified in the body or bioaccumulated in the food chain. In the soil, Cu strongly complexes to the organic implying that only a small fraction of copper will be found in solution as ionic copper, Cu(II).

The solubility of Cu is drastically increased at pH 5.5 [35] which is rather close to the ideal farmland pH of 6.0–6.5 [36]. Copper and Zn are two important essential elements for plants, microorganisms, animals, and humans. The connection between soil and water contamination and metal uptake by plants is determined by many chemical and physical soil factors as well as the physiological properties of the crops. Soils contaminated with trace metals may pose both direct and indirect threats: direct, through negative effects of metals on crop growth and yield, and indirect, by entering the human food chain with a potentially negative impact on human health. Even a reduction of crop yield by a few percent could lead to a significant long-term loss in production and income. Some food importers are now specifying acceptable maximum contents of metals in food, which might limit the possibility for the farmers to export their contaminated crops [37].

12.7 Mercury

Mercury belongs to same group of the periodic table with Zn and Cd. It is the only liquid metal at STP. It has atomic number 80, atomic weight 200.6, density 13.6 g cm^{-3}, melting point −13.6 °C, and boiling point 357 °C and is usually recovered as a by-product of ore processing [25]. Release of Hg from coal combustion is a major source of Hg contamination. Releases from manometers at pressure measuring stations along gas/oil pipelines also contribute to Hg contamination. After release to the environment, Hg usually exists in mercuric (Hg^{2+}), mercurous (Hg_2^{2+}), elemental (HgO), or alkylated form (methyl/ethyl mercury). The redox potential and pH of the system determine the stable forms of Hg that will be present. Mercurous and mercuric mercury are more stable under oxidizing conditions. When mildly reducing conditions exist, organic or inorganic Hg may be reduced to elemental Hg, which may then be converted to alkylated forms by biotic or abiotic processes. Mercury is most toxic in its alkylated forms which are soluble in water and volatile in air [25]. Mercury(II) forms strong complexes with a variety of both inorganic and organic ligands, making it very soluble in oxidized aquatic systems [26]. Sorption to soils, sediments, and humic materials is an important mechanism for the removal of Hg from solution. Sorption is pH dependent and increases as pH increases. Mercury may also be removed from solution by coprecipitation with sulfides. Under anaerobic conditions, both organic and inorganic forms of Hg may be converted to alkylated forms by microbial activity, such as by sulfur reducing bacteria. Elemental mercury may also be formed under anaerobic conditions by demethylation of methyl mercury, or by reduction of Hg(II). Acidic conditions ($pH<4$) also favor the formation of methyl mercury, whereas higher pH values favor precipitation of HgS(s) [25]. Mercury is associated with kidney damage [27].

12.8 Nickel

Nickel is a transition element with atomic number 28 and atomic weight 58.69. In low pH regions, the metal exists in the form of the nickelous ion, Ni(II). In neutral to slightly alkaline solutions, it precipitates as nickelous hydroxide, $Ni(OH)_2$, which is a stable compound. This precipitate readily dissolves in acid solutions forming

Ni(III) and in very alkaline conditions; it forms nickelite ion, $HNiO_2$, that is soluble in water. In very oxidizing and alkaline conditions, nickel exists in form of the stable nickelo-nickelic oxide, Ni_3O_4, that is soluble in acid solutions. Other nickel oxides such as nickelic oxide, Ni_2O_3, and nickel peroxide, NiO_2, are unstable in alkaline solutions and decompose by giving of oxygen. In acidic regions, however, these solids dissolve producing Ni^{2+} [38].

Nickel is an element that occurs in the environment only at very low levels and is essential in small doses, but it can be dangerous when the maximum tolerable amounts are exceeded. This can cause various kinds of cancer on different sites within the bodies of animals, mainly of those that live near refineries. The most common application of Ni is an ingredient of steel and other metal products. The major sources of nickel contamination in the soil are metal plating industries, combustion of fossil fuels, and nickel mining and electroplating [39]. It is released into the air by power plants and trash incinerators and settles to the ground after undergoing precipitation reactions. It usually takes a longtime for nickel to be removed from air. Nickel can also end up in surface water when it is a part of wastewater streams. The larger part of all Ni compounds that are released to the environment will adsorb to sediment or soil particles and become immobile as a result. In acidic soils, however, Ni becomes more mobile and often leaches down to the adjacent groundwater. Microorganisms can also suffer from growth decline due to the presence of Ni, but they usually develop resistance to Ni after a while. Nickel is not known to accumulate in plants or animals and as a result Ni has not been found to biomagnify up the food chain. For animals Ni is an essential foodstuff in small amounts. The primary source of mercury is the sulfide ore cinnabar [17].

12.9 Heavy Metals in Plants

Although some metals are essential for plant and animal life, many are toxic at high concentrations and awareness of the extent and severity of soil and water contamination they cause is growing. Besides their natural availability in soils, specific sources of heavy metals are mine tailings, leaded gasoline and lead-based paints [40, 41], fertilizers, animal manure, biosolids, compost, pesticides, coal combustion residues, and atmospheric deposition [42, 43]. Metal(loid)s of environmental concern are As, Cd, Cr, Cu, Pb, Hg, Ni, Se, Mo, Zn, Tl, Sb, and others [44]. Their anthropogenic application to soils is often related to the use of residuals, like biosolids, livestock manure, and compost, adversely affecting human, crop, and wildlife health [42]. In plants, some metals play an important role as micronutrients, being essential for growth at low concentrations. Most of them are cofactors of enzymes and are involved in important processes such as photosynthesis (Mn, Cu), DNA transcription (Zn), hydrolysis of urea into carbon dioxide and ammonia (Ni), and legume nodulation and nitrogen fixation (Co, Zn, Co). Some are involved in flowering and seed production and in plant growth (Cu, Zn), especially when their availability is very low (Table 12.1).

Table 12.1 Role and toxicity of metals in plants and humans (Vamerali et al., 2010 [43])

Metal	Essential for plants	Functions in plants	Toxicity threshold in plant tissues (mg kg^{-1} dw)	Some hyperaccumulators	Threshold for Hyperaccumulation in aboveground Biomass (mg kg-1dw)	Essential for human	Toxicity symptoms in humans
As	No	-	≳20(a)	*Pteris vittata* L. (f)	>1,000 (f)	No	Cancer (e.g., lung and skin); cardiovascular, gastrointestinal, hepatic, and renal diseases
Cd	No	-	5–10 (b)	*Thlaspi caerulescens* J. & C. Presl. (e)	>100 (e)	No	Stomach irritations (vomiting and diarrhea), lung damage, kidney diseases, cancer(probably)
Co	Yes	Cofactor of biosynthetic enzymatic activities; essential for Rhizobium	60–170 (c)	*Haumaniastrum robertii* (Robyns) P A. Duvign. & Plancke (e)	>1,000 (e)	Yes	Contact dermatitis; mutagenic and carcinogenic effects
Cr	No	-	1–2 (b)	*Brassica juncea* (L.) *Czern.;B.napus* (L.); *Vallisneria americana* (d)	>20,000 (g)	Yes	Genotoxic carcinogens (Cr6?); lung cancer
Cu	Yes	Constituent of enzymes; role in photo synthesis; involved in reproductive and in determining yield and quality in crops	15–20 (b)	*Brassica juncea (L.) Czern.; Vallisneria americana* Michx. (d)	>1,000 (e)	Yes	Inhibition of dihydrophil hydratase (in hemopoiesis); accumulation in liver and kidney
Mn	Yes	Constituent of enzymes; activation of enzymes; photosynthesis; reproductive phase; resistance against biotic and abiotic stress	170–2,000 (h)	*Agrostis castellana* Boiss. & Reuter (d)	>10,000 (e)	Yes	Neurological symptoms; affection of liver function

Ni	Yes	Constituent of enzymes; activation of urease	20–30 (b)	*Alyssum* spp.; *Thlaspi* spp. (e)	>1,000 (e)	Yes	Accumulation in lungs Irreversible neurological damage; renal disease; cardiovascular effects; reproductive toxicity
Pb	No	–	10–20 (b)	*Thlaspi rotundifolium* (L.) Gaudin ssp. *Cepaefolium* (Wulfen) Rouy & Fouc; *T. Caerulescens* J. & C. Presl.; *Alyssum Wulfenienum* Bernh.; *Arrhenatherumelatius* (L) Beauv.; *Festuca ovina* L. (e)	>1,000 (e)	No	Inhibition of copper absorption; nausea, vomiting, loss of appetite, abdominal cramps, diarrhea, headaches
Zn	Yes	Constituent of cell membranes; activation of enzymes; DNA transcription; involved in reproductive phase and in determining yield and quality of crops; resistance against biotic and abiotic stress; legume nodulation and nitrogen fixation	150–200 (b)	*Thlaspi* spp.; *Cardaminopsis* spp. (e)	>10,000 (e)	Yes	

Hyperaccumulator plant species refer to temperate climates. Variations in phytotoxicity thresholds take into account interspecific and environmental variability
(a) Wallace et al. [223]; (b) Fo¨rstner [224]; (c) Li et al. [225]; (d) McCutcheon and Schnoor [226]; (e) Terry and Ban˜uelos [227]; (f) Visoottiviseth et al. [228]; (g) Zayed and Terry [229]; (h) Adriano [80]

Interactions for uptake and transport may occur between metals or with macronutrients, depending on their relative concentrations. For instance, Cu reduces the uptake of Cd and Ni in soybean seedlings [45], whereas its uptake is inhibited by Cr, Cd, Co, and Ni in barley. Nickel can compete with Cu, Zn, and Co and, to a greater extent, with iron uptake [46]. Lead is also an antagonist in the uptake of Fe, more than Mn and Co [47] and can inhibit enzymes such as ureases. In rice, the competition in uptake between arsenate and phosphate, which may markedly reduce plant growth, is well known [48]. Interactions between metals for uptake across cellular membranes and vacuoles and transport depend on the expression and functionality of specific transporter families shared by various metals [43, 49]. Phytotoxicity is mainly associated with nonessential metals like As, Cd, Cr, and Pb, which generally have very low toxicity thresholds [50] and lower values for hyperaccumulation (especially for Cd) than the other metals. The above-mentioned metals, except Cr, are also not essential for humans, and may enter the food chain through ingestion of contaminated edible products at various levels, depending on the metal in question. Arsenic, Cr, and Pb are not easily transferred to aboveground plant biomass, mainly being stored in root cells [51–53], whereas Zn is easily accumulated in green tissues like leaves [43, 54].

12.10 Traditional Remediation of Metal-Contaminated Soil

Various physical, chemical, and biological processes are already being used in soil remediation [55] such as: (1) soil washing, (2) solidification/stabilization by either physical inclusion or chemical interactions between the stabilizing agent and the pollutant, (3) vitrification, (4) electrokinetic treatment, (5) chemical oxidation or reduction, (6) excavation and off-site treatment or storage at a more appropriate site ("dig and dump"), and (7) incineration. In contrast to these traditional remediation approaches, a number of researchers and organizations have proposed the adoption of less invasive, alternative remediation options ("gentle" remediation technologies), the so-called green remediation, based on life cycle analysis (LCA) in order to conserve resources and minimize environmental impacts [56]. Phytoremediation is widely viewed as an ecologically responsible alternative to the environmentally destructive physical remediation methods currently practiced, given that it is based on the use of green plants to extract, sequester, and/or detoxify pollutants. This is not a new concept since constructed wetlands, reed beds, and floating-plant systems are common for the treatment of different wastewaters for many years [57, 58].

12.11 Phytoremediation

The term phytoremediation was coined from the Greek phyto, meaning "plant," and the Latin suffix remedium, "able to cure" or "restore," by Ilya Raskin in 1994, and is used to refer to plants which can remediate a contaminated medium [43].

Phytoremediation, also called green remediation, botanoremediation, agroremediation, or vegetative remediation, can be defined as an in situ remediation strategy that uses vegetation and associated microbiota, soil amendments, and agronomic techniques to remove, contain, or render environmental contaminants harmless [59, 60]. The idea of using metal-accumulating plants to remove heavy metals and other compounds was first introduced in 1983, but the concept has actually been implemented for the past 300 years on wastewater discharges [61, 62]. Plants may breakdown or degrade organic pollutants or remove and stabilize metal contaminants. The methods used to phytoremediate metal contaminants are slightly different from those used to remediate sites polluted with organic contaminants. As it is a relatively new technology, phytoremediation is still mostly in its testing stages and as such has not been used in many places as a full-scale application. However, it has been tested successfully in many places around the world for many different contaminants. Phytoremediation is energy efficient, esthetically pleasing method of remediating sites with low to- moderate levels of contamination, and it can be used in conjunction with other more traditional remedial methods as a finishing step to the remedial process.

The advantages of phytoremediation compared with classical remediation are that (1) it is more economically viable using the same tools and supplies as agriculture, (2) it is less disruptive to the environment and does not involve waiting for new plant communities to recolonize the site, (3) disposal sites are not needed, (4) it is more likely to be accepted by the public as it is more esthetically pleasing then traditional methods, (5) it avoids excavation and transport of polluted media thus reducing the risk of spreading the contamination, and (6) it has the potential to treat sites polluted with more than one type of pollutant. The disadvantages are as follows: (1) it is dependent on the growing conditions required by the plant (i.e., climate, geology, altitude, and temperature), (2) large-scale operations require access to agricultural equipment and knowledge, (3) success is dependent on the tolerance of the plant to the pollutant, (4) contaminants collected in senescing tissues may be released back into the environment in autumn, (5) contaminants may be collected in woody tissues used as fuel, (6) time taken to remediate sites far exceeds that of other technologies, (7) contaminant solubility may be increased leading to greater environmental damage and the possibility of leaching. Potentially useful phytoremediation technologies for remediation of heavy metal-contaminated soils include phytoextraction (phytoaccumulation), phytostabilization, and phytofiltration [17, 63]. Phytoremediation takes advantage of the plant's ability to remove pollutants from the environment or to make them harmless or less dangerous [64]. It can be applied to a wide range of organic [65, 66] and inorganic contaminants. Phytoremediation is a general term including several processes, among which phytoextraction and phytostabilization are the most reliable for heavy metals [43].

12.12 Different Strategies of Phytoremediation

12.12.1 Phytostabilization

Phytostabilization, also referred to as in-place inactivation, is primarily concerned with the use of certain plants to immobilize soil sediment and sludges [67]. Contaminant are absorbed and accumulated by roots, adsorbed onto the roots, or precipitated in the *rhizosphere*. This reduces or even prevents the mobility of the contaminants preventing migration into the groundwater or air and also reduces the bioavailability of the contaminant thus preventing spread through the food chain. Plants for use in phytostabilization should be able to (1) decrease the amount of water percolating through the soil matrix, which may result in the formation of a hazardous leachate; (2) act as barrier to prevent direct contact with the contaminated soil; and (3) prevent soil erosion and the distribution of the toxic metal to other areas [21]. Phytostabilization can occur through the process of sorption, precipitation, complexation, or metal valence reduction. This technique is useful for the cleanup of Pb, As, Cd, Cr, Cu, and Zn [68]. It can also be used to reestablish a plant community on sites that have been denuded due to the high levels of metal contamination. Once a community of tolerant species has been established, the potential for wind erosion (and thus spread of the pollutant) is reduced, and leaching of the soil contaminants is also reduced. Phytostabilization is advantageous because disposal of hazardous material/biomass is not required, and it is very effective when rapid immobilization is needed to preserve ground and surface waters [17, 67, 68] (Fig. 12.1).

12.12.2 Phytotransformation

It is also known as phytodegradation. It is the breakdown of contaminants taken up by plants through metabolic processes within the plant or the breakdown of contaminants external to the plant through the effect of compounds (such as enzymes) produced by the plants. The main mechanism is plant uptake and metabolism causing degradation in plants. Additionally, degradation may occur outside the plant, due to the release of compounds that cause the transformation [69, 70].

12.12.3 Phytovolatilization

Toxic metals such as Se, As, and Hg can be biomethylated to form volatile molecules that can be lost to the atmosphere. Although it was known for a long time that microorganisms play an important role in the volatilization of Se from soils [71], a plant's ability to perform the same function was only recently discovered. Again,

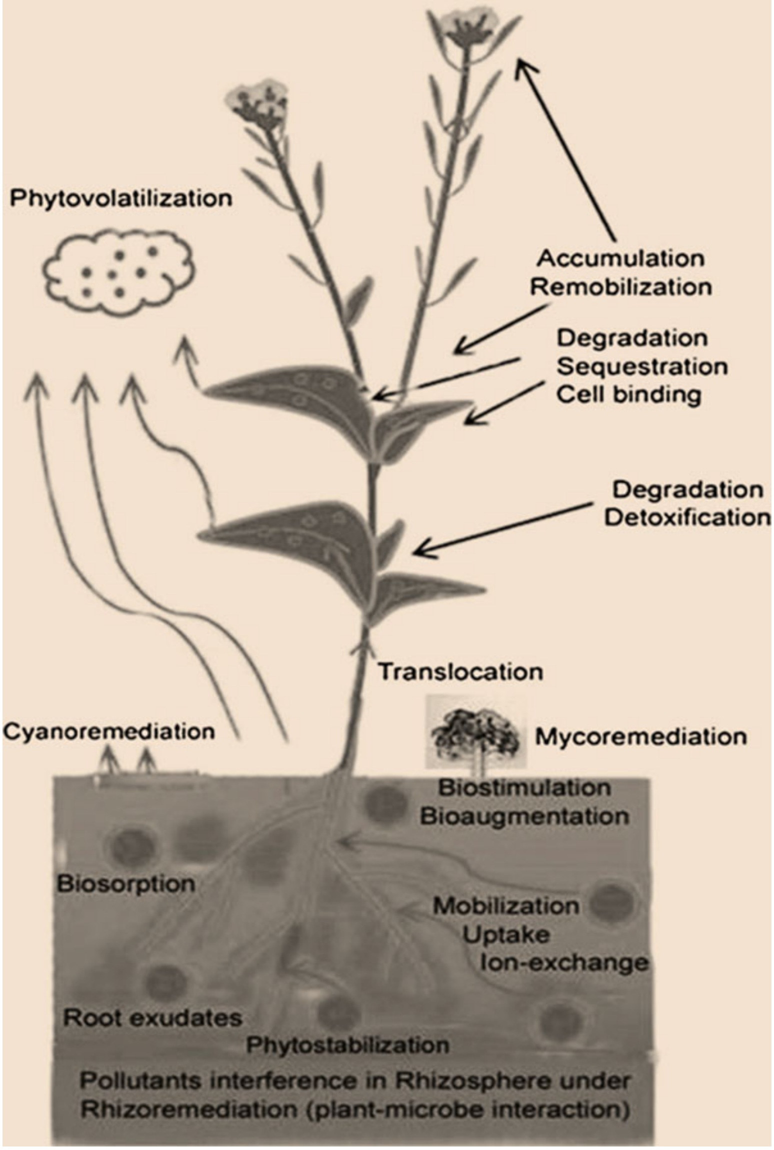


Fig. 12.1 An overview of biotechnological approaches for phytoremediation. Reproduced from Mani and Kumar Int. J. Environ. Sci. Technol. 11:843–872 [70], © 2014 Springer (modified from Dhankher et al. [267])

B. juncea was identified as a valuable plant for removing Se from soils [72, 73]. Se volatilization in the form of methyl selenate was proposed as a major mechanism of Se removal by plants [74, 75]. Some plants can also remove Se from soil by accumulating nonvolatile methyl selenate derivatives in the foliage. An enzyme responsible for the formation of methyl selenocystine in the Se accumulator *Astragalus bisculatus* was recently purified and characterized [76]. The unique property of elemental mercury is that it is a liquid at room temperature and thus is easily volatilized; however, because of its high reactivity, mercury in the environment exists mainly as a divalent cation Hg^{2+}. Bacteria can catalyze the reduction of the mercuric ion to elemental mercury using mercury reductase, a soluble NADPH-dependent FAD-containing disulfide oxidoreductase (NADPH, reduced nicotinamide adenine dinucleotide phosphate; FAD, flavin adenine dinucleotide) [77]. A modified bacterial gene encoding a functional mercuric ion reductase was recently introduced into *Arabidopsis thaliana* [78]. Transformants showed greater resistance to HgCl2 and produced large amounts of Hg vapor compared to control plants. Although the practicality of using mercury-volatilizing plants for environmental remediation is questionable, this elegant work points to a new environmental use of plant molecular biology [79].

12.12.4 Phytofiltration

Phytofiltration is the use of plant roots (rhizofiltration) or seedlings (blastofiltration), is similar in concept to phytoextraction, but is used to absorb or adsorb pollutants, mainly metals, from groundwater and aqueous waste streams rather than the remediation of polluted soils [20, 63]. Rhizosphere is the soil area immediately surrounding the plant root surface, typically up to a few millimeters from the root surface. The contaminants are either adsorbed onto the root surface or are absorbed by the plant roots. Plants used for rhizofiltration are not planted directly in situ but are acclimated to the pollutant first. Plants are hydroponically grown in clean water rather than soil, until a large root system has developed. Once a large root system is in place, the water supply is substituted for a polluted water supply to acclimatize the plant. After the plants become acclimatized, they are planted in the polluted area where the roots uptake the polluted water and the contaminants along with it. As the roots become saturated, they are harvested and disposed of safely. Repeated treatments of the site can reduce pollution to suitable levels as was exemplified in Chernobyl where sunflowers were grown in radioactively contaminated pools [17, 27]).

12.12.5 Metal Phytoextraction

As heavy metals are the main inorganic contaminants, among existing phytotechnologies much interest is devoted to phytoextraction and its improvement [80–83]. Phytoextraction is a green technology, born 15 years ago from the studies of Raskin et al. [84] and later of Brooks et al. [85], which exploits the ability of plants to translocate a great fraction of metals taken up to harvestable biomass.

Contaminated biomass may be used for energy production, whereas remaining ashes are dumped, included in construction materials, or subjected to metal extraction (phytomining; [85]). Although promising, phytoextraction has many limitations, deriving from scarce metal availability in soils, difficulties in root uptake, symplastic mobility and xylem loading, as well as the great energy cost required for detoxification and storage within shoots [6, 86]. Plants show differing morpho-physiological responses to soil metal contamination. Most are sensitive to very low concentrations; others have developed tolerance, and a reduced number show hyperaccumulation [5, 85, 87]. The latter capacity has practically opened up the way to phytoextraction [88, 89]. Metal accumulation is expressed by the metal biological absorption coefficient (BAC), i.e., the plant (harvestable)-to-soil metal concentration ratio [90].

Besides convenient BAC, both the high bioconcentration factor (BCF, root-to-soil metal concentration ratio) and the translocation factor (TF, shoot-to-root metal concentration ratio) can positively affect phytoextraction. Tolerant plant species tend to restrict soil–root and root–shoot transfers, and therefore have much less accumulation in biomass, whereas hyperaccumulators actively take up and translocate metals into aboveground tissues. Plants with high BAC (greater than 1) are suitable for phytoextraction; those with high BCF (higher than 1) and low TF (lower than 1) have potential for phytostabilization [91]. Desirable characteristics for a plant species in phytoextraction are (1) fast growth and high biomass, (2) extended root system for exploring large soil volumes, (3) good tolerance to high concentrations of metals in plant tissues, (4) high translocation factor, (5) adaptability to specific environments/sites; and (6) easy agricultural management. All these traits are difficult to combine, and there are basically two available phytoextraction strategies, which make use of hyperaccumulators or biomass plant species, respectively. Hyperaccumulators, such as the well studied *Thlaspi caerulescens* J. & C. Presl. and *Alyssum bertolonii* Desv. [92, 93] are able to take up specifically one or a few metals, generally producing a small shoot biomass with high metal concentrations [5, 94]. Instead, high-yielding biomass plant species can absorb a wide range of heavy metals at generally low concentration [43].

12.13 Hyperaccumulator Plants

The discovery of hyperaccumulator plant species has revolutionized phytoremediation technology since these plants have an innate capacity to absorb metal at levels 50-500 times greater than average plants [95]. Hyperaccumulators are a subgroup of accumulator species often endemic to naturally mineralized soils, which accumulate high concentrations of metals in their foliage [5, 79]. Metal hyperaccumulators are naturally capable of accumulating heavy metals in their aboveground tissues, without developing any toxicity symptoms. A metal hyperaccumulator is a plant that can concentrate the metals to a level of 0.1 % (of the leaf dry weight) for Ni, Co, Cr, Cu, Al, and Pb; 1 % for Zn and Mn; and 0.01 % for Cd and Se [5, 96]. The time taken by plants to reduce the amount of heavy metals in contaminated soils depends on biomass production as well as on their bioconcentration factor (BCF), which is the ratio of metal concentration in the shoot tissue to the soil [97]. It is determined by the capacity of the roots to take up metals and their ability to accumulate, store, and detoxify metals while maintaining metabolism, growth, and biomass production [6, 98, 99]. With the exception of hyperaccumulators, most plants have metal bioconcentration factors of less than 1, which means that it takes longer than a human lifespan to reduce soil contamination by 50 % [100].

Hyperaccumulators have a bioconcentration factor greater than 1, sometimes reaching as high as 50-100. The relationship between metal hyperaccumulation and tolerance is still a subject of debate. Views range from no correlation between hyperaccumulators and the degree of tolerance to metals [101] to strong association between these traits [61]. It is increasingly being realized that to cope with high concentrations of metals in their tissue, plants must also tolerate the metals that they accumulate [102]. There has long been a general agreement that metal hyperaccumulation is an evolutionary adaptation by specialized plants to live in habitats that are naturally rich in specific minerals that confers on them the qualities of increased metal tolerance, protection against herbivores or pathogens, drought tolerance, and allelopathy [103, 104]. The hypothesis of protection against pathogens and herbivores is considered the most accepted one [105–109]. However, the mechanisms of metal uptake, tolerance to high metal concentrations, and the exact roles that high level of elements play in the survival of hyperaccumulators have continued to be debated [102].

Hyperaccumulation of heavy metal ions is a striking phenomenon exhibited by approximately <0.2 % of angiosperms [7, 110]. Metal hyperaccumulators have been reported to occur in over 450 species of vascular plants from 45 angiosperm families (Table 12.2) including members of the Asteraceae, Brassicaceae, Caryophyllaceae, Cyperaceae, Cunoniaceae, Fabaceae, Flacourtiaceae, Lamiaceae, Poaceae, Violaceae, and Euphorbiaceae [111], but are well represented in Brassicaceae especially in the genera *Alyssum* and *Thlaspi*, wherein accumulation of more than one metal has been reported [43, 94, 112, 113] (Table 12.2). *Pteris vittata* (Chinese brake fern) is known to accumulate up to 95 % of the arsenic taken up from soil in its fronds [114, 115]. The best known angiosperm hyperaccumulator of metals is *Thlaspi* (now: *Noccaea*) *caerulescens* (*pennycress*), which can accumulate large amounts of Zn (39,600 mg/kg) and Cd (1800 mg/kg) without apparent damage [7, 116, 117]. This small, self pollinating diploid plant can easily grow under lab conditions and therefore represents an excellent experimental system for studying the mechanisms of metal uptake, accumulation, and tolerance in relation to metal phytoextraction. Apart from *T. caerulescens*, *Brassica juncea* has also been used as a model system to investigate the physiology and biochemistry of metal accumulation in plants [102].

Table 12.2 Important plant species that are metal hyperaccumulators

Metals	Number of hyperaccumulator species	Plant species that accumulate specific metals	Family	Reference
Ni	320	*Berkheya coddii*	Asteraceae	Robinson et al. [230]; Moradi et al. [231]
		Alyssum serpyllifolium, A. bertolonii	Brassicaceae	Becerra-Castro et al. [232]; Barzanti et al. [233]
		Sebertia acuminata	Sapotaceae	Jaffre et al. [234]; Perrier [235]
		Phidiasia lindavii	Acanthaceae	Reeves et al. [236]
		Bornmuellera kiyakii	Brassicaceae	Reeves et al. [237]
Cu	34	*Ipomea alpina*	Convolvulaceae	Cunningham and Ow [59]
		Crassula helmsii	Crassulaceae	Küpper et al. [238]
		Commelina communis	Commelinaceae	Wang and Zhong [239]
Co	34	*Haumaniastrum robertii*	Lamiaceae	Brooks [85]
		Crotalaria cobalticola	Fabaceae	Oven et al. [240]
Se	20	*Astragalus bisulcatus*	Fabaceae	Galeas et al. [241]
		Stanleya pinnata	Brassicaceae	Freeman et al. [242]; Hladun et al. [243]
Zn	18	*Thlaspi caerulescens*	Brassicaceae	Kupper and Kochian [244]
		Arabis gemmifera, A. paniculata)	Brassicaceae	Kubota and Takenaka [245]; Tang et al. [246]
		Sedum alfredii	Crassulaceae	Sun et al. [247]
		Arabidopsis halleri	Brassicaceae	Zhao et al. [248]
		Picris divaricata	Asteraceae	Du et al. [249]
Pb	14	*Sesbania drummondii*	Fabaceae	Sahi et al. [250]; Sharma et al. [251]
		Hemidesmus indicus	Apocynaceae	Chandra Sekhar et al. [252]
		Arabis paniculata	Brassicaceae	Tang et al. [246]
		Plantago orbignyana	Plantaginaceae	Bech et al. [253]
Mn	9	*Austromyrtus bidwillii*	Myrtaceae	Bidwell et al. [254]
		Phytolacca americana	Phytolaccaceae	Pollard et al. [255]
		Virotia neurophylla	Proteaceae	Fernando et al. [256]
		Gossia bidwillii	Myrtaceae	Fernando et al. [257]
		Maytenus founieri	Celastraceae	Fernando et al. [258]

(continued)

Table 12.2 (continued)

Metals	Number of hyperaccumulator species	Plant species that accumulate specific metals	Family	Reference
Cd	4	*Thlaspi caerulescens*	Brassicaceae	Basic et al. [117]
		Arabidopsis halleri	Brassicaceae	Dahmani-Muller et al. [259]; Bert et al. [260]
		Bidens pilosa	Asteraceae	Sun et al. [261]
Cr	na	*Salsola kali*	Amaranthaceae	Gardea-Torresday et al. [262]
		Leersia hexandra	Poaceae	Zhang et al. [263]
		Gynura pseudochina	Asteraceae	Mongkhonsin et al. [264]
Ti	na	*Iberis intermedia*	Brassicaceae	Leblanc et al. [265]
		Brassica oleracea	Brassicaceae	Al-Najar et al. [266]

Reproduced from Bhargava A, Carmona FF, Bhargava M, Srivastava S. Approaches for enhanced phytoextraction of heavy metals. Journal of Environmental Management. 2012; 105:103-120 [102], with permission of Elsevier

12.14 High Biomass Crops

For successful and economically feasible phytoextraction, it is necessary to use plants having a metal bioconcentration factor of 20 and a biomass production of 10 tonnes per hectare (t/ha); or plants with a metal bioconcentration factor of 10 and a biomass production of 20 t/ha [100]. The rate of phytoextraction is directly proportional to plant growth rate and the total amount of metal phytoextracted is correlated with the plant biomass, which makes the process of phytoextraction very slow [118]. This necessitates the identification of fast growing (largest potential biomass and greatest nutrient responses) and strongly metal-accumulating genotypes. *B. juncea*, while having one-third the concentration of Zn in its tissue, is considered to be more effective at Zn removal from soil than *T. caerulescens*, a known hyperaccumulator of Zn [119]. This advantage is primarily due to the fact that *B. juncea* produces ten times more biomass than *T. caerulescens*.

Recently, interest has arisen in the use of high-biomass crops for phytoextraction of metals [120, 121]. Fast growing trees are ideal low cost candidates for phytoextraction due to their extensive root systems, high rates of water uptake and transpiration, rapid growth, large biomass production, and easy harvesting with subsequent resprouting without disturbance of the site [100]. Several tree species have evoked interest in the phytoremediation of metal-contaminated soils and show great prospects for heavy metal remediation [122–127]. Poplar and willow, though not hyperaccumulators, are effective because of their greater biomass and deep root systems, which makes them effective remediators of metal contamination. Poplars can be grown in a wide range of climatic conditions and are used with increasing frequency in "short-rotation forestry" systems for pulp and paper production [100]. This raises the possibility of using plantations of poplars across several multiyear cycles to remove heavy metals from contaminated soils. Importantly, it is unlikely that poplars will enter the human food chain or end up as feedstock for animals. Likewise, several species of willow (*Salix dasyclados, Salix smithiana,* and *Salix caprea*) display good accumulation capabilities and remediation effectiveness, similar to herbaceous hyperaccumulators like *Arabidopsis halleri* and *T. caerulescens*, compensating lower metal content in shoots with higher biomass production [124, 128, 129]. However, the use of perennial tree species having extensive root systems with elevated metal content would require excavation and disposal, especially short rotation coppice (SRC) after several harvests and at the process end [102, 130].

12.15 Plant Limitations

When the concept of phytoextraction was reintroduced (approximately two decades ago),engineering calculations suggested that a successful plant-based decontamination of even moderately contaminated soils would require crops able to concentrate metals in excess of 1–2 %. Accumulation of such high levels of heavy metals is highly toxic and would certainly kill the common nonaccumulator plant. However, in hyperaccumulator species such concentrations are attainable. Nevertheless, the extent of metal removal is ultimately limited by plant ability to extract and tolerate only a finite amount of metals. On a dry weight basis, this threshold is around 3 % for Zn and Ni, and considerably less for more toxic metals, such as Cd and Pb. The other biological parameter which limits the potential for metal phytoextraction is biomass production. With highly productive species, the potential for biomass production is about 100 t fresh weight/ha. The values of these parameters limit the annual removal potential to a maximum of 400 kg metal/ha/year. It should be mentioned, however, that most metal hyperaccumulators are slow growing and produce little biomass. These characteristics severely limit the use of hyperaccumulator plants for environment cleanup.

It has been suggested that phytoremediation would rapidly become commercially available if metal removal properties of hyperaccumulator plants, such as *T. caentiescens* could be transferred to high-biomass producing species, such as Indian mustard (*Brassica juncea*) or maize (*Zea mays*) [131]. Biotechnology has already been successfully employed to manipulate metal uptake and tolerance properties in several species. For example, in tobacco (*Nicotiana tabacum*) increased metal tolerance has been obtained by expressing the mammalian metallothionein, metal-binding proteins, genes [95, 132, 133].

12.16 Improving Phytoextraction

The goal of remediating metal-contaminated soil is generally to extract the metal from the large soil volume and transfer it to a smaller volume of plant tissue for harvest and disposal. This is due to the fact that metals cannot be metabolized or broken down to less toxic forms. The amount of pollutant a plant can remove from the soil is a function of its tissue concentration multiplied by the quantity of biomass formed [134]. Low yield and slow growth rates have been cited as limiting factors for the development of effective metal phytoremediator plants [116]. Most of the known metal accumulating plants are metal selective, show slow growth rate, produce relatively little biomass, and can be used for phytoextraction in their natural habitats only [135]. Thus, while the amounts of metal concentration per unit of plant biomass can be high, the total amounts of metal removed at a site during a given period can be quite low. For example, although *T. caerulescens* can take up sufficient levels of metals to make harvesting and metal recovery economical, they are often limited by their small biomass [136, 137]. Moreover, the use of hyperaccumulator plants can be limited because of less information about their agronomic characteristics, pest management, breeding potential, and physiological processes [138]. However, a rapidly growing nonaccumulator could be modified to enable it to achieve some of the properties of hyperaccumulators. Two approaches are currently being explored to develop and/or improve the metal accumulating plants: (1) conventional breeding, and (2) genetic engineering [102].

12.17 Genetically Engineered Plants for Phytoremediation

The genetic and biochemical basis is becoming an interesting target for genetic engineering, because the knowledge of molecular genetics model organisms can enhance the understanding of the essential metal metabolism components in plants. A fundamental understanding of both uptake and translocation processes in normal plants and metal hyperaccumulators, the regulatory control of these activities, and the use of tissue specific promoters offer great promise that the use of molecular biology tools can give scientists the ability to develop effective and economic phytoremediation plants for soil metals [61, 139]. Plants such as *Populus angustifolia*, *Nicotiana tabacum*, or *Silene cucubalus* have been genetically engineered to overexpress glutamylcysteine synthetase, and thereby provide enhanced heavy metal accumulation as compared with a corresponding wild-type plant [139, 140].

Candidate plants for genetic engineering for phytoremediation should be a high biomass plant with either short or long duration (trees), which should have inherent capability for phytoremediation. The candidate plants should be amicable for genetic transformation. Some of high biomass hyperaccumulators for which regeneration protocols are already developed include Indian mustard (*Brassica juncea*), sunflower (*Helianthus annuus*), tomato (*Lycopersicon esculentum*), and yellow poplar (*Liriodendron tulipifera*) [141, 142]. The application of powerful genetic and molecular techniques may surely identify a range of gene families that are likely to be involved in transition metal transport. Considerable progress has been made recently in identifying plant genes encoding metal ion transporters and their homologous in hyperaccumulator plants. Therefore, it is hoped that genetic engineering may offer a powerful new means by which to improve the capacity of plants to remediate environmental pollutants [142, 143].

Genetic engineering applied to crops aims at manipulating the plant's capacity to tolerate, accumulate and metabolize pollutants. Many genes involved in the acquisition, allocation and detoxification of metals have been identified and characterized from a variety of organisms, especially bacteria and yeasts [144]. Transgenic plants have been engineered to overproduce recombinant proteins playing possible roles in chelation, assimilation, and membrane transport of metals. Enhanced tolerance and accumulation have been achieved through overproduction of metal chelating molecules such as citrate [145], phytochelatins [146, 147], metallothioneins [148, 149], phytosiderophores and ferritin [150], or overexpression of metal transporter proteins [43, 151–156].

As many genes are involved in metal uptake, translocation, sequestration, chemical modification, and tolerance, the overexpression of any (combination) of these genes is a possible strategy for genetic engineering. Depending on which phytoremediation application is to be used, the genetic engineering strategy may strive to create plants that accumulate more metals in harvestable plant parts (phytoextraction), or adsorb more metals at their root surface (rhizofiltration, phytostabilization). A plant property essential for all phytoremediation applications is plant tolerance (Table 12.3), so enhancing plant metal tolerance is an obvious avenue for genetic engineering approaches. Enhanced tolerance to metals may be achieved by reducing metal uptake, by more efficient sequestration of metals in plant storage compartments, overproduction of metal chelating molecules, or increasing activity of enzymes involved in general (oxidative) stress resistance [157].

The overexpression of metal transporter genes may lead to enhanced metal uptake, translocation, and/or sequestration, depending on the tissues where the gene is expressed (root, shoot, vascular tissue, or all), and on the intracellular targeting (e.g., cell membrane, vacuolar membrane). The overexpression of genes involved in synthesis of metal chelators may lead to enhanced or reduced metal uptake, as well as enhanced metal translocation and/or sequestration, depending on the type of chelator and its location [157]. Unless regulatory genes are identified that simultaneously induce many metal-related genes, it is feasible that more than one gene will need to be upregulated in order to substantially enhance metal phytoremediation capacity. Encouraging for transgenic approaches, classic genetic studies indicate that there are usually very few genes (1–3) responsible for metal tolerance [158]. Also, metal accumulation, tolerance, and plant productivity are not necessarily correlated [158, 159]. Therefore, it should be possible to breed or genetically engineer a plant with high metal tolerance and metal accumulation as well as high productivity. This would be the ideal plant for metal phytoextraction [157]. According to Eapen and D'Souza [141], the possible targets for genetic manipulations are given [160].

Table 12.3 Important factors for different metal phytoremediation applications

	Tolerance	Root uptake	Translocation	Shoot accumulation	Biotransformation	Rhizosphere—microbes
Phytostabilization	x				(x)	
Phytoextraction	x	x	x	x		(x)
Phytovolatilization	x	x	(x)		x	(x)
Rhizofiltration	x	x				

Note: the importance of biomass is incorporated in shoot accumulation (=shoot conc. × biomass) and in tolerance (=growth)

12.18 Metallothioneins

Overproduction of various metal chelator molecules has been shown to affect plant metal tolerance and accumulation. Several research groups have overexpressed the metal-chelating proteins metallothioneins (MTs). The expression of the human MT2 gene in tobacco or oil seed rape resulted in higher Cd tolerance at the seedling level [161]. Similarly, the expression of the mouse MT1 gene in tobacco led to enhanced Cd tolerance at the seedling level [162]. The overexpression of a pea MT in *A. thaliana* resulted in a severalfold higher Cu accumulation [148, 157]. An attempt to improve tolerance to Cd, Zn and Ni was made by introducing a metallothionein gene in tobacco [163, 164]. Macek et al. [165] also showed that Cd accumulation significantly increased in tobacco plants bearing the transgene coding for the polyhistidine cluster combined with yeast metallothionein [43].

In some instances an increased Cd tolerance has been reported (up to 200 μM Cd^{2+}; [166]) and in others, an altered distribution of Cd has been observed in transgenic plants that express MTs. For example, the human MT-II gene and MT-II fused to the β-glucuronidase gene were expressed in tobacco under control of the CaMV 35S promoter with a double enhancer ($35S^2$). In vitro grown transgenic seedlings expressing the fusion protein accumulated 60–70 % less Cd in their shoots than the control plants did [167]. The best transgenic lines expressing the *35S2-hMT-II* gene were grown in the greenhouse and field. Little or no effect on the amount of Cd accumulated was observed, however there was a significant modification in the Cd distribution48. In the control plants, 70–80 % of the Cd was translocated to the leaves whereas in the MT expressing plants only 40–50 % was translocated. Reduced translocation to the leaves was accompanied with increased Cd levels in both roots and stem. Moreover, there was an altered distribution of Cd within the leaves of MT-expressing plants, a 73 % decrease of Cd in the leaf lamina with a concomitant increase in Cd levels in the midrib.

A modified distribution is of particular interest for crops in the objective of translocating Cd to the nonconsumed segments of the plant or to the harvestable parts of plants for phytoremediation. The choice of promoter is of great importance and several different promoters have been evaluated for the expression of MTs. The mouse MT promoter was found to be inactive in tobacco49; the CaMV 35S promoter was not affected by Cd exposure in tobacco, whereas the ribulose bisphosphate carboxylase (*rbcS*) promoter is repressed at high Cd concentrations and the mannopine synthase (*mas*) promoter is induced by Cd [168, 169].

The most pronounced effect of MT overexpression was observed by Hasegawa et al.[149], who overexpressed the yeast gene *CUP1* in cauliflower, leading to a 16-fold higher Cd tolerance, as well as higher Cd accumulation. Thus, it appears that the overexpression of MTs is a promising approach to enhance Cd/Cu tolerance and accumulation [157]. Recently, two metallothionein genes from *Prosopis juliflora*, *PjMT1* and *PjMT2* were cloned separately in pCAMBIA 1301 and transformed into *Nicotiana tabacum* by Balasundaram et al. [170]. When tested for cadmium accumulation and tolerance, *PjMT1* transformants showed better performance than *PjMT2* counterparts.

12.19 Phytochelatins

In a different approach to enhance metal tolerance and accumulation, the metal-binding peptides phytochelatins (PCs) were overproduced via expression of enzymes involved in their biosynthesis. Transgenic mustard (*Brassica juncea*) plants with higher levels of glutathione and phytochelatins were created through the overexpression of either of two glutathione synthesizing enzymes—γ--glutamylcysteine synthetase (γECS) or glutathione synthetase (GS). Both types of transgenics showed enhanced Cd tolerance and accumulation [146, 147], illustrating the importance of these metal-binding peptides for metal tolerance and accumulation. In a related study, γECS was overexpressed or knocked out (antisense approach) in *Arabidopsis*, leading to increased or decreased GSH levels [171]. Transgenics with decreased GSH levels showed reduced Cd tolerance, confirming the importance of GSH and PCs for Cd tolerance. However, plants with increased GSH levels did not show enhanced Cd tolerance, suggesting that GSH production is not limiting for PC production and Cd tolerance in this species. Harada et al. [172] also created transgenic plants with enhanced phytochelatin levels, through overexpression of cysteine synthase. The resulting transgenics displayed enhanced Cd tolerance but lower Cd concentrations. Overexpression of either gamma-glutamylcysteine synthetase or glutathione synthetase in transgenic *Brassica juncea* (L.) Czern. resulted in higher accumulation and tolerance of various metals such as Cd, Cr, and As, considered alone or mixed together [43, 173].

The study of Bennett et al. [156] is the first to demonstrate enhanced phytoextraction potential of transgenic plants using polluted environmental soil. The results confirm the importance of metal-binding peptides for plant metal accumulation and show that results from hydroponic systems have value as an indicator for phytoremediation potential. Of the six metals tested, the ECS and GS transgenics accumulated 1.5-fold more Cd, and 1.5- to 2-fold more Zn, compared with wild-type Indian mustard. Furthermore, the ECS transgenics accumulated 2.4–3-fold more Cr, Cu, and Pb, relative to WT. The grass mixture accumulated significantly less metal than Indian mustard: approximately twofold less Cd, Cu, Mn, and Zn, and 5.7-fold less Pb than WT Indian mustard. All transgenics removed significantly more metal from the soil compared with WT Indian mustard or an unplanted control. While WT did not remove more metal than the unplanted control for any of the metals tested, all three types of transgenics significantly reduced the soil metal concentration, and removed between 6 % (Zn) and 25 % (Cd) of the soil metal.

Guo et al. [174] have demonstrated that simultaneous expression of AsPCS1 and YCF1 in Arabidopsis led to elevate the tolerance to Cd and As and have higher amounts accumulation of these metals than corresponding single-gene transgenic lines and wild type. Such a stacking of modified genes involved in chelation of toxic metals by thiols and vacuolar compartmentalization represents a highly promising new tool for use in phytoremediation for multiple heavy metals cocontamination. The overexpression of a tobacco glutathione-*S*-transferase gene (parB) in *Arabidopsis* was reported to lead to enhanced Cu, Al, and Na tolerance [175]. Glutathione-*S*-transferases mediate glutathione conjugation, followed by transport of the resulting complex to the vacuole [157, 176]. Transgenic *Brassica juncea* overexpressing different enzymes involved in phytochelatin synthesis were shown to extract significant Cd, Cr, Cu, Pb, and Zn than wild plants [146].

12.20 Organic Acids

Enhanced production of the metal chelator citric acid was achieved by the overexpression of citrate synthase [145]. The resulting CS transgenics were shown to have enhanced Al tolerance, apparently via extracellular complexation of Al by citrate after excretion from root cells. The same CS transgenics take up more phosphorus [177] and are more resistant to iron deficiency [178], illustrating that citrate excretion can affect the uptake of different elements in different ways. As citrate amendment has been shown to enhance U uptake [179], it would be interesting to test these CS transgenics for U uptake [157]. The overexpression of citrate synthetase has shown to promote enhanced Al tolerance in plants [180]. Enhanced aluminum tolerance has been achieved by increasing organic acid synthase gene activity. Han et al. [181] isolated a full-length OsCS1 gene encoding for citrate synthase, which is highly induced by Al toxicity in rice (*Oryza sativa* L.). Insertion of OsCS1 in several independent transgenic tobacco lines and its expression increased citrate efflux and conferred great tolerance to aluminum [43].

12.21 Phytosiderophores

Another promising approach is the introduction of genes encoding for phytosiderophores. A first step in this direction was achieved by Higuchi et al. [182], who isolated genes encoding for nicotianamine synthase, a key enzyme in the phytosiderophore biosynthetic pathway in barley and rice. The increase of iron acquisition mediated by phytosiderophores was found to provide an advantage under Cd stress in maize [183]. Overproduction of ferritin through genetic modification also led to increased Fe uptake as well as Cd, Mn, and Zn, but only at alkaline pH [184]. This was due to high pH Fe deficiency, which stimulates metal uptake and translocation in shoots through an increase in root ferric reductase and H^+-ATPase activities [43]. The overproduction of the iron-chelator deoxymugineic acid (phytosiderophores) was achieved through the overexpression of nicotianamine aminotransferase (NAAT) in rice [185]. The resulting plants released more phytosiderophores and grew better on iron-deficient soils. Iron levels in the plants were not determined [157].

12.21.1 Ferritin

The overexpression of the iron-binding protein ferritin was shown to lead to a 1.3-fold higher iron level in tobacco leaves [186] and a threefold higher level in rice seeds [150, 157].

12.22 Metal Transporters

The genetic manipulation of several metal transporters has been shown to result in altered metal tolerance and/or accumulation. The overexpression of the Zn transporter ZAT (also known as AtMTP1) in *A. thaliana* gave rise to plants with enhanced Zn resistance and twofold higher root Zn accumulation [153]. ZAT is a putative vacuolar transporter and of the same gene family as the TgMTP1 isolated from the hyperaccumulator *T. goesingense* [187]. The overexpression of the calcium vacuolar transporter CAX2 from *A. thaliana* in tobacco resulted in enhanced accumulation of Ca, Cd, and Mn, and to higher Mn tolerance [155]. Another vacuolar transporter, AtMHX, was overexpressed in tobacco [188]. The resulting plants showed reduced tolerance to Mg and Zn, but it did not show altered accumulation of these elements. Another putative metal transporter gene from tobacco (NtCBP4), encoding a calmodulin-binding protein, when overexpressed resulted in enhanced Ni tolerance and reduced Ni accumulation, as well as reduced Pb tolerance and enhanced Pb accumulation [152]. When a truncated form of the protein was overexpressed, however, from which the calmodulin-binding part was removed, the resulting transgenics showed enhanced Pb tolerance and attenuated accumulation [189]. In order to enhance iron uptake by plants, two yeast genes encoding ferric reductase (FRE1 and FRE2, involved in iron uptake) were overexpressed in tobacco [151]. Iron content in the shoot of the transgenics was 1.5-fold higher compared with wild-type plants. Earlier, enhanced accumulation of various metals (Fe, Cu, Mn, Zn, Mg) was already observed in an *Arabidopsis* mutant with enhanced ferric-chelate reductase activity [190]. The affected gene in the *Arabidopsis* mutant meanwhile has been identified as FRO2 and isolated [191]; it will be interesting to see what effect its overexpression has on plant metal uptake. The overexpression of another metal transporter, AtNramp1, resulted in an increase in Fe tolerance [154], while the overexpression of AtNramp3 led to reduced Cd tolerance but no difference in Cd accumulation [192]. In addition to overexpressing metal transporters, it is also possible to alter their metal specificity. For instance, while IRT1, the *Arabidopsis* iron transporter, can transport Fe, Zn, Mn, and Cd, the substitution of one amino acid was shown to result in loss of either Fe and Mn transport capacity, or Zn transport capacity [193]. Expression of the bacterial heavy metal transporter MerC promoted the transport and accumulation of mercury in transgenic *Arabidopsis*, which may be a useful method for improving plants for the phytoremediation of mercury pollution [194].

Recently, transgenic *Sesbania grandiflora* (L.) pers (Fabaceae) and *Arabidopsis thaliana* (L.) (Brassicaceae) plants harboring the rabbit cytochrome p450 2E1 enzyme were evaluated by Mouhamad et al. [195] for their ability to accumulate heavy metals, potassium (K), calcium (Ca), manganese (Mn), zinc (Zn), copper (Cu), iron (Fe), lead (Pb), and bromine (Br), using X-ray Fluorescence analysis. When grown for 15 days on heavy metal-contaminated soils, transgenic cuttings of *S. grandiflora* and T3 *A. thaliana* plants recorded higher dry and fresh weight compared with their respective controls (*A. thaliana* and *S. grandiflora* plants transformed with an empty vector). Dry weight of transgenic *S. grandiflora* plants (0.321 g) was seven times higher than that of the wild type (0.049 g), and the fresh weight (4.421 g) was about 4.6 times higher. Likewise, the dry weight of CYP450 2E1 *A. thaliana* (0.198 g) was more than eight times higher than that seen in the control (0.024 g). Moreover, Fe, Mn, K, and Ca concentrations in transgenic plants were significantly higher than those in their corresponding controls. For instance, concentrations of accumulated K (~3000 and 2000 mg/kg dry weight in *S. grandiflora* and *A. thaliana*, respectively) were significantly higher than those recorded in their corresponding controls (2500 and 1500 mg/kg, respectively). In the same vein, translocation of all studied metals from soils cultured with transgenic plants was higher than in those cultured with the control plants. In conclusion, the obtained results show the potential in using transgenic *Sesbania* and *Arabidopsis* plants harboring the rabbit CYP450 2E1 for phytoremediation of mixed environmental contaminants. With the overexpression of such engineered transporters, it may be possible to tailor transgenic plants to accumulate specific metals [157].

12.23 Alteration of Metabolic Pathways

Rather than accelerating existing processes in plants, an alternative approach is to introduce an entirely new pathway from another organism. This approach was taken by Richard Meagher and coworkers, who introduced two bacterial genes in plants that together convert methylmercury to volatile elemental mercury. *MerB* encodes organomercuriallyase, which converts methylmercury to ionicmercury or Hg(II); *MerA* encodes mercuric reductase, which reduces ionic mercury to elemental mercury or Hg(O) [196]. Transgenic MerA *A. thaliana* plants showed significantly higher tolerance to Hg(II) and volatilized elemental mercury [78]. Transgenic MerB *A. thaliana* plants were significantly more tolerant to methylmercury and other organomercurials [197]. The MerB plants were shown to convert methylmercury to ionic mercury, a form that is ~100-fold less toxic to plants. MerA–MerB double-transgenics, obtained by crossing MerA and MerB transgenics, were compared with their MerA, MerB, and wild-type counterparts with respect to tolerance to organic mercury [197]. While MerB plants were tenfold more tolerant to organic mercury than wild-type plants, MerA–MerB plants were 50-fold more tolerant. When supplied with organic mercury, MerA–MerB double transgenics volatilized elemental mercury, whereas single transgenics and wild-type plants did not; thus, MerA–MerB plants were able to convert organic mercury all the way to elemental mercury, which was released in volatile form. The same *MerA/MerB* gene constructs were used to create mercury-volatilizing plants from other species. Transgenic MerA and MerB tobacco and yellow poplar also showed enhanced mercury tolerance [198]. In an initial experiment to analyze the potential of these plants for phytoremediation, MerA tobacco plants removed three- to fourfold more mercury from hydroponic medium than untransformed controls [136]. Transfer of *E. coli arsC* and *γ-ECS* genes to *Arabidopsis,* improved the efficiency of transgenics in transporting oxyanion arsenate to aboveground tissues, reducing it to arsenite, and sequestering it to thiol peptide complexes [199]. Chen et al. [200] simultaneously inserted 13 genes into rice using particle bombardment 62 [169].

No reports have been published at this point involving the expression of metal hyperaccumulator genes in nonaccumulator species. However, an alternative approach has been used to transfer hyperaccumulation capacity to a nonaccumulator high biomass species. Brewer et al. [201] used somatic hybridization (protoplast electrofusion) to create a hybrid between *Thlaspi caerulescens* and *Brassica napus*. Some of the hybrids showed high biomass combined with high metal tolerance and accumulation, making them attractive for metal phytoextraction. A different way of using genetic engineering to study metal metabolism is by creating hairy root cultures of plants using *Agrobacterium rhizogenes*. The resulting fast growing root culture can be grown in vitro indefinitely. Hairy root culture of *Thlaspi caerulescens* was shown to be more tolerant to Cd, and accumulated 1.5–1.7-fold more Cd than hairy roots of nonaccumulator species [137]. *Agrobacterium rhizogenes* infection may also be used to bring about root proliferation, and thus to increase the root surface area of a plant. The use of such plants may be attractive for rhizofiltration applications [157].

12.24 Alteration of Enzymes Relating to Oxidative Stress Management

Overexpression of enzymes involved in general stress resistance mechanisms present an alternative approach to bring about metal tolerance. Several studies using this approach have led to promising results. Ezaki et al. [175] reported that the overexpression of several genes involved in oxidative stress response (glutathione-*S*-transferase, peroxidase) resulted in enhanced Al tolerance. Oberschall et al. [202] overexpressed an aldose/aldehyde reductase responsible for detoxifying a lipid peroxide degradation product and found that the transgenics were more metal tolerant. The overexpression of glutathione reductase resulted in reduced Cd accumulation and enhanced Cd tolerance, as judged from chlorophyll content and chlorophyll fluorescence measurements [203]. Grichko et al. [204] found that the overexpression of 1-aminocyclopropane-1-carboxylicacid (ACC) deaminase led to an enhanced accumulation of a variety of metals, as well as higher metal tolerance. ACC is the precursor for ethylene, the plant hormone involved in senescence [157].

12.25 Alteration in Biomass Production

Phytohormones are generally targeted for increasing the biomass production. Eriksson et al. [205] reported that genetically modified trees with enhanced gibberellins biosynthesis provided higher growth and biomass as compared to the normal tree [160].

12.26 Development of Transgenic Plants for Remediation of Heavy Metals

Considering the above-mentioned targets there are many reports of transgenic plants with increased metal tolerance and accumulation. It is relevant that, most, if not all, transgenic plants created to date are based on the overexpressing genes involved in the biosynthesis pathways of metal-binding proteins and peptides [146, 156, 199, 206] and the genes that can convert a toxic ion into a less toxic form [207]. The effective use of biotechnology to design transgenic plants for efficient phytoremediation is only possible when a comprehensive knowledge of cellular mechanisms for metal tolerance and genetic basis for metal hyperaccumulation is well understood. In this case, particularly those genes should be undertaken which are helping in enhanced metal uptake, translocation, accumulation, sequestration in vacuole, and provided tolerance in natural metal hyperaccumulators. Modification or overexpression of the enzymes that are involved in synthesis of -GSH and PCs could be another good approach to enhance heavy metal accumulation and tolerance in plants. In one of the earlier study, it was found that overexpression of *E. coli* γ-ECS and -GSH synthetase in Indian mustard enhance the Cd accumulation than wild type [146]. The other reports carried out by Rugh et al. [207] and they modified yellow poplar trees with two bacterial genes, i.e., *merA* and *merB*, to detoxify methyl-Hg, which is then converted to Hg^o by *merA*. It is evident that the elemental Hg is less toxic and more volatile than the mercuric ion, and is released easily into the atmosphere. Pilon-Smits et al. [208] overexpressed the ATP-sulfurylase (APS) gene in Indian mustard and the transgenics had fourfold higher APS activity and accumulated three times more Se than wild type. Recently, Dhankher et al. [199] reported a genetics-based strategy to remediate arsenic (As) from contaminated soils by overexpressing two bacterial genes in *Arabidopsis*. One was the expression of *E. coli AsrC* gene, encoding arsenate reductase coupled with a light-induced soybean Rubisco promoter. However, the second gene was the *E. coli γ-ECS* coupled with a strong constitutive action promoter. Thereafter, the AsrC protein, expressed strongly in stem and leaves, catalyzes the reduction of arsenate to arsenite, whereas γ-ECS, which is the first enzyme in PC-biosynthetic pathway, increases the pool of PCs. The transgenic expressing both *AsrC* and *γ-ECS* proteins showed substantially higher As tolerance, when grown on As-contaminated soil. These plants accumulated a 4–17-fold greater fresh shoot weight and accumulated two- to threefold more As than wild-type plants. A summary of the most effective transgenes and the effects of their expression on tolerance, accumulation, and volatilization of metals in plants are described in Table 12.4 [160].

Though, the risks of escaping genes from transgenic plants have been found negligible [146], deployment of transgenic plants in field conditions requires certain precautions. Assessment of risk with use of transgenic plant should be accounted very carefully before any field testing or application [208]. One of the possible risks associated with transgenic application is biological transformation of metals into chemical species that are easily bioavailable. It will enhance exposure of various

Table 12.4 Genes introduced into plants and the effects of their expression on heavy metal tolerance, accumulation and volatilization

Gene	Product	Source	Target	Maximum observed effect[a]
me r A	Hg(II) reductase	Gram-negative bacteria	*Liriodendron tulipifera*	50 µmol l^{-1} $HgCl_2$; 500 mg $HgCl_2\,kg^{-1}$
			Nicotiana tabacum	V: Hg volatilization rate increase tenfold
mer A	Hg(II) reductase	Gram-negative bacteria	*Arabidopsis thaliana*	T: 10 µmol l^{-1} CH_3HgCl (>40-fold)
mer B	Organomercurial lyase	Gram-negative bacteria	*A. thaliana*	V: Up to 59 pg Hg(0) mg^{-1} fresh biomass min^{-1}
APS1	ATP sulfurylase	*A. thaliana*	*B. juncea*	A: Twofold increase in Se concentration
MT-I	MT	Mouse	*N. tabacum*	T: 200 µmol l^{-1} $CdCl_2$ (20-fold)
CUP1	MT	*Saccharomyces cerevisiae*	*B. oleracea*	T:400 µmol l^{-1} $CdCl_2$ (approximately 16-fold)
gsh2	GSH synthase	*E. coli*	*B. juncea*	A: Cd concentrations 125 %
gsh1	γ-Glu-Cys synthase	*E. coli*	*B. juncea*	A: Cd concentrations 190 %
NtCBP4	Cation channel	*N. tabacum*	*N. tabacum*	T: 250 mmol l^{-1} $NiCl_2$ (2.5-fold), Pb sensitive
				A: Pb concentrations 200 %
ZAT1	Zn transporter	*A. thaliana*	*A. thaliana*	T: Slight increase
TaPCS1	PCs	Wheat	*Nicotiana glauca* R. Graham	A: Pb concentrations 200 %

Reproduced from Yang X, Jin XF, Feng Y, Islam E. Molecular Mechanisms and Genetic bases of Heavy Metal Tolerance/Hyperaccumulation in Plants. *Journal of Integrative Plant Biology*, 2005;47(9):1025-1035 [143], with permission of John Wiley and Sons

[a] Relative values refer to control plants not expressing the transgene. *A* accumulation in the shoot; *GSH* glutathione, *MT* metallothionein; *T* tolerance; *V* volatilization; *PCs* Phytochelatins

wildlife and human beings to toxic heavy metals. Another aspect of concern could be uncontrolled distribution of transgenic plants owing to higher fitness of such plants in the particular climatic conditions and/or interbreeding with populations of wild relatives [199]. These risks have to be assessed and weighed not only against the benefits of the technique, but also against the nontargeted risks. Despite these limitations, the transgenic development offers potential role in environmental cleanup provided adequate prevention measures are adopted [160, 209, 210].

12.27 Risk Assessment Considerations

Transgenic plants with altered metal tolerance, accumulation, or transformation properties are valuable for various reasons. They shed new light on basic biological mechanisms involved in these processes: which pathways are involved and which enzymes are rate limiting. Plants with altered metal accumulation properties may also be applicable, not only for phytoremediation but also to enhance crop productivity in areas with suboptimal soil metal levels, or as “fortified foods” for humans or livestock [99]. When genetically engineered plants are used for any of these applications, a thorough risk assessment study should be performed in each case [211]. Some of the possible risks involved are biological transformation of metals into forms that are more bioavailable, enhanced exposure of wildlife and humans to metals (in the case of enhanced metal accumulation in palatable plant parts, or volatilization), uncontrolled spread of the transgenic plants due to higher fitness (e.g., metal tolerance) or general weedy nature, and/or uncontrolled spread of the transgene by interbreeding with populations of wild relatives (for a comprehensive report on this topic, see [212]). These risks will have to be assessed on a case-by-case basis and weighed against the benefits, and against the risks of doing nothing or using alternative technologies.

The actual risks involved with the use of transgenic plants for phytoremediation have never been tested. However, theoretical calculations of risks associated with the use of mercury volatilizating plants have been done by Meagher and coworkers [136, 198]. According to their calculations, the mercury emitted by these plants would pose no significant threat to the environment and would be negligible compared with other sources of mercury, such as burning of fossil fuels and medical waste. Even if the level of volatile mercury at the phytoremediation site is 400-fold higher than background levels, that would still be 25 times below regulatory limits. In addition, the retention time of elemental mercury in the atmosphere, before precipitation, is 1–2 years during which the mercury is diluted to nontoxic levels. Norman Terry and coworkers have done a similar theoretical analysis of the risk of volatile Se emitted by plants [213], and came to the conclusion that the volatile Se will likely be beneficial rather than toxic, as it is likely to precipitate in Se-deficient areas. Metal accumulation in plant shoots brings along the risk of wildlife ingestion, and any increase in metal accumulation via biotechnology will lead to a proportional increase of this risk. On the other hand, if a site can be cleaned in a shorter time, the duration of exposure may be reduced when using transgenics.

The risk of metal ingestion by wildlife may be minimized by fencing off the area, using deterrents such as periodic noise, and the use of less palatable plant species. The risk of transgenic plants or their genes “escaping” is not considered a significant problem by Meagher et al. [136], because they generally offer little or no advantage over untransformed plants, either in a pristine or a contaminated environment. However, before using specific transgenics for phytoremediation in the field, this could be verified by a greenhouse or pilot field experiment, analyzing transgenic gene frequency over a number of generations, on polluted and uncontaminated soil. To further minimize the risk of outcrossing to wild relatives, transgenic plant species may be chosen that have no compatible wild relatives, male-sterile transgenics may be bred, and/or the plants may be harvested before flowering.

12.28 Conclusions

It has been shown in multiple studies that plant trace element metabolism can be genetically manipulated, leading to plants with altered metal tolerance, accumulation, and/or biotransformation capacity. When natural plant processes were accelerated by genetic engineering, the typical increase in metal accumulation per plant was two- to threefold. This would potentially reduce the cost of phytoremediation to the same extent, if the same results hold true in the field. Furthermore, the introduction of a new pathway has led to plants that can detoxify Hg in ways that other plants cannot—this is potentially even more valuable. In the coming years some of these newly available transgenics will likely be put to the test in a more realistic phytoremediation setting. As more metal-related genes are discovered, facilitated by the genome sequencing projects, many new possibilities will open up for the creation of new transgenics with favorable properties for phytoremediation. In addition to constitutive overexpression of one gene, several genes may be overexpressed simultaneously, and the overexpression may be fine-tuned in specific tissues, under specific conditions, or in specific cellular compartments. Some promising strategies may be (1) the many newly discovered metal transporters, including the ones from hyperaccumulator plants (ZNT1, TgMTP1), may be overexpressed in high biomass plant species, targeted to different tissues and intracellular locations; (2) nicotianamine overproduction may be an interesting avenue to manipulate metal translocation and tolerance, as well as iron uptake in cereals, NA being the precursor of phytosiderophores.

Overproduction of NA is feasible via overexpression of enzymes from the NA biosynthesis pathway, the genes for which have been cloned [182, 214–216]; (3) overexpression of phytochelatin synthase (PS), the enzyme mediating PC synthesis from GSH, may further enhance metal tolerance and accumulation. The overexpression of PS is possible, because genes encoding PS have been cloned [217–219]. The overexpression of the vacuolar transporter responsible for shuttling the PC-metal complex into the vacuole may also enhance metal tolerance and accumulation; this too is possible because the *A. thaliana* gene encoding this transporter has been cloned [220]; (4) overproduction of histidine can be done because the genes involved in His biosynthesis have been cloned [221]. In fact, preliminary data suggest that histidine overproducing plants have enhanced Ni tolerance [222]; (5) are search area that may render a wealth of new information in the coming years is molecular biology of the rhizosphere. Manipulation of the quality and quantity of root-released compounds offer a promising alternative strategy to affect metal uptake or exclusion. Together, these new developments likely will give rise to much new information about metal metabolism in plants in the near future and may lead to the fruitful applications in environmental cleanup, nutrition, and crop productivity [157].

References

1. Khan S, Hesham AEL, Qiao M, Rehman S, He JZ (2010) Effects of Cd and Pb on soil microbial community structure and activities. Environ Sci Pollut Res 17:288–296
2. Ali H, Khan E, Sajad MA (2013) Phytoremediation of heavy metals—concepts and applications. Chemosphere 91:869–881
3. Salt DE, Smith RD, Raskin I (1998) Phytoremediation. Annu Rev Plant Physiol Plant Mol Biol 49:643–668
4. Chaney RL (1983) Plant uptake of inorganic waste. In: Parr JE et al (eds) Land treatment of hazardous wastes. Noyes Data, Park Ridge, pp 50–76
5. Baker AJM, Brooks RR (1989) Terrestrial higher plants which hyperaccumulate metallic elements. A review of their distribution, ecology and phytochemistry. Biorecovery 1:81–126
6. Clemens S, Palmgren MG, Krämer U (2002) A long way ahead: understanding and engineering plant metal accumulation. Trends Plant Sci 7(7):309–315
7. Rascioa N, Navari-Izzo F (2011) Heavy metal hyperaccumulating plants: How and why do they do it? And what makes them so interesting? Plant Sci 80:169–181
8. Auburn AL. Heavy metal soil contamination. soil quality—Urban Technical Note No. 3. 2000. United States Department of Agriculture Natural Resources Conservation Service.
9. Pehlivan E, Ozkan AM, Dinç S, Parlayici S (2009) Adsorption of Cu^{2+} and Pb^{2+} ion on dolomite powder. J Hazard Mater 167(1–3):1044–1049
10. Roy S, Labelle S, Mehta P et al (2005) Phytoremediation of heavy metal and PAH-contaminated Brownfield sites. Plant Soil 272(1-2):277–290
11. Gaur A, Adholeya A (2004) Prospects of arbuscular mycorrhizal fungi in phytoremediation of heavy metal contaminated soils. Curr Sci 86(4):528–534
12. Tangahu BV, Abdullah SRS, Basri H, Idris M, Anuar N, Mukhlisin M (2011) A review on heavy metals (As, Pb, and Hg) uptake by plants through phytoremediation. Int J Chem Eng 2011:939161. doi:10.1155/2011/939161, 31 p
13. USEPA (1996) Report: recent developments for in situ treatment of metals contaminated soils. Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency, Washington, DC
14. Shiowatana J, McLaren RG, Chanmekha N, Samphao A (2001) Fractionation of arsenic in soil by a continuous flow sequential extraction method. J Environ Qual 30(6):1940–1949
15. Buekers J (2007) Fixation of cadmium, copper, nickel and zinc in soil: kinetics, mechanisms and its effect on metal bioavailability, Ph.D. thesis, Katholieke Universiteit Lueven, DissertationesDe Agricultura, Doctoraatsprooefschrift nr
16. Levy DB, Barbarick KA, Siemer EG, Sommers LE (1992) Distribution and partitioning of trace metals in contaminated soils near Leadville, Colorado. J Environ Qual 21(2):185–195
17. Wuana RA, Okieimen FF (2011) Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation. ISRN Ecol 2011:402647. doi:10.5402/2011/402647, 20 p
18. USDHHS (1999) Toxicological profile for lead. United States Department of Health and Human Services, Atlanta
19. Kabata-Pendias A, Pendias H (2001) Trace metals in soils and plants, 2nd edn. CRC Press, Boca Raton
20. GWRTAC (1997) Remediation of metals-contaminated soils and groundwater. Rep. TE-97-01, GWRTAC-E Series. GWRTAC, Pittsburgh
21. Raskin I, Ensley BD (2000) Phytoremediation of toxic metals: using plants to clean up the environment. Wiley, New York
22. NSC, Lead Poisoning, National Safety Council (2009). http:// www.nsc.org/news resources/Resources/Documents/Lead Poisoning.pdf
23. Baldwin DR, Marshall W (1999) Heavy metal poisoning and its laboratory investigation. Ann Clin Biochem 36(3):267–300

24. Rosen CJ (2002) Lead in the home garden and urban soil environment. Communication and Educational Technology Services, University of Minnesota Extension, St Paul
25. Smith LA, Means JL, Chen A (1995) Remedial options for metals-contaminated sites. Lewis, Boca Raton
26. Bodek I, Lyman WJ, Reehl WF, Rosenblatt DH (eds) (1988) Environmental inorganic chemistry: properties, processes and estimation methods. Pergamon Press, New York
27. Scragg A (2006) Environmental biotechnology, 2nd edn. Oxford University Press, Oxford
28. Davies BE, Jones LHP (1988) Micronutrients and toxic elements. In: Wild A (ed) Russell's soil conditions and plant growth, 11th edn. Wiley, New York, pp 781–814
29. Greany KM (2005) An assessment of heavy metal contamination in the marine sediments of Las Perlas Archipelago, M.S. thesis. School of Life Sciences, Heriot-Watt University, Edinburgh
30. Campbell PGC (2006) Cadmium-A priority pollutant. Environ Chem 3(6):387–388
31. Weggler K, McLaughlin MJ, Graham RD (2004) Effect of chloride in soil solution on the plant vailability of biosolid-borne cadmium. J Environ Qual 33(2):496–504
32. McLaughlin MJ, Hamon RE, McLaren RG, Speir TW, Rogers SL (2000) Review: a bioavailability-based rationale for controlling metal and metalloid contamination of agricultural land in Australia and New Zealand. Aust J Soil Res 38(6):1037–1086
33. Manahan SE (2003) Toxicological chemistry and biochemistry, 3rd edn. CRC, Boca Raton
34. VCI (2011) Copper history/future. Van Commodities. http://trademetalfutures.com/copper-history.html
35. Martínez CE, Motto HL (2000) Solubility of lead, zinc and copper added to mineral soils. Environ Pollut 107(1):153–158
36. Eriksson J, Andersson A, Andersson R (1997) The state of Swedish farmlands. Tech. Rep. 4778. Swedish Environmental Protection Agency, Stockholm
37. Bjuhr J (2007) Trace metals in soils irrigated with waste water in a periurban area downstream Hanoi City, Vietnam, Seminar Paper. Institutionen för markvetenskap, Sveriges lantbruksuniversitet (SLU), Uppsala
38. Pourbaix M (1974) Atlas of electrochemical equilibria. Pergamon Press, New York, Translated from French by J.A. Franklin
39. Khodadoust AP, Reddy KR, Maturi K (2004) Removal of nickel and phenanthrene from kaolin soil using different extractants. Environ Eng Sci 21(6):691–704
40. The Conservation Foundation (1987) State of the environment: a view toward the nineties. The Conservation Foundation, Washington, DC
41. Pirbazari M, Badriyha BN, Ravindran V, Kim S (1989) Treatment of landfill leachate by biologically active carbon adsorbers. In: Bell JM (ed) Proceedings of 44th annual Purdue conference on industrial wastes. Lewis, Chelsea, pp 555–563
42. Adriano DC (2001) Trace elements in the terrestrial environments. Biogeochemistry, bioavailability, and risks of heavy metals, 2nd edn. Springer, New York
43. Vamerali T, Bandiera M, Mosca G (2010) Field crops for phytoremediation of metal-contaminated land. A review. Environ Chem Lett 8:1–17. doi:10.1007/s10311-009-0268-0
44. Basta NT, Ryan JA, Chaney RL (2005) Trace element chemistry in residual-treated soil. Key concepts and metal bioavailability. J Environ Qual 34:49–63
45. Cataldo DA, Wildung RE (1978) Soil and plant factors influencing the accumulation of heavy metals by plants. Environ Health Perspect 27:149–159
46. Cataldo DA, Garland TR, Wildung RE (1978) Nickel in plants: II. Distribution and chemical form in soybean plants. Plant Physiol 62:566–570
47. Gaweda M, Capecka E (1995) Effect of substrate pH on the accumulation of lead in radish (*Raphanus sativus L. subvar. radicula*) and spinach (*Spinacia oleracea L.*). Acta Physiol Plant 17:333–340
48. Meharg AA, Hartley-Whitaker J (2002) Arsenic uptake and metabolism in arsenic resistant and non-resistant plant species. New Phytol 154:29–43
49. Hall JL, Williams LE (2003) Transition metal transporters in plants. J Exp Bot 54:2601–2613

50. Clemens S (2006) Toxic metal accumulation, responses to exposure and mechanisms of tolerance in plants. Biochimie 88(11):1707–1719
51. Marin AR, Masscheleyn PH, Patrick WH Jr (1992) The influence of chemical form and concentration of arsenic on rice growth and tissue arsenic concentration. Plant Soil 139:175–183
52. Tiwari KK, Dwivedi S, Singh NK, Rai UN, Tripathi RD (2009) Chromium (VI) induced phytotoxicity and oxidative stress in pea (Pisum sativum L.): biochemical changes and translocation of essential nutrients. J Environ Biol 30:389–394
53. Mellem JJ, Baijnath H, Odhav B (2009) Translocation and accumulation of Cr, Hg, As, Pb, Cu and Ni by Amaranthus dubius (Amaranthaceae) from contaminated sites. J Environ Sci Heal A 44:568–575
54. Probst A, Liu H, Fanjul M, Liao B, Hollande E (2009) Response of Vicia faba L. to metal toxicity on mine tailing substrate: geochemical and morphological changes in leaf and root. Environ Exp Bot 66:297–308
55. Barcelo J, Poschenrieder C (2003) Phytoremediation: principles and perspectives. Contrib Science 2:333–344
56. Onwubuya K, Cundy A, Puschenreiter M, Kumpiene J, Bone B, Greaves J, Teasdale P, Mench M, Tlustos P, Mikhalovsky S, Waite S, Friesl-Hanl W, Marschner B, Muller I (2009) Developing decision support tools for the selection of ‘gentle’ remediation approaches. Sci Environ 407:6132–6142
57. Gomes HI (2012) Phytoremediation for bioenergy: challenges and opportunities. Environ Technol Rev 1:59–66
58. Rajeswari PM (2014) Phytoremediation-insights into plants as remedies. Malaya Journal of Biosciences 1(1):41–45, ISSN 2348-6236 print /2348-3075 online
59. Cunningham SD, Ow DW (1996) Promises and prospects of phytoremediation. Plant Physiol 110(3):715–719
60. Helmisaari HS, Salemaa M, Derome J, Kiikkilä O, Uhlig C, Nieminen TM (2007) Remediation of heavy metal contaminated forest soil using recycled organic matter and native woody plants. J Environ Qual 36(4):1145–1153
61. Chaney RL, Malik M, Li YM, Brown SL, Brewer EP, Angle JS, Baker AJM (1997) Phytoremediation of soil metals. Curr Opin Biotech 8:279–284
62. Henry RJ (2000) An overview of the phytoremediation of lead and mercury. Office of Solid Waste and Emergency Response Technology, Innovation office, United States Environmental Protection Agency, Washington, DC
63. Garbisu C, Alkorta I (2001) Phytoextraction: a cost-effective plant-based technology for the removal of metals from the environment. Bioresour Technol 77(3):229–236
64. Raskin I (1996) Plant genetic engineering may help with environmental cleanup. Proc Natl Acad Sci U S A 93:3164–3166
65. Anderson TA, Coats JR (1995) An overview of microbial degradation in the rhizosphere and its implications for bioremediation. In: Skipper HD, Turco RF (eds) Bioremediation, science and applications. SSSA/ASA/CSS, Madison, pp 135–143
66. Schnoor JL, Licht LA, McCutcheon SC, Wolfe NL, Carreira LH (1995) Phytoremediation of organic and nutrient contaminants. Environ Sci Technol 29:318–323
67. USEPA (2000) Introduction to phytoremediation. Tech. Rep. EPA 600/R-99/107. Office of Research and Development, United States Environmental Protection Agency, Cincinnati
68. Jadia CD, Fulekar MH (2009) Phytoremediation of heavy metals: recent techniques. Afr J Biotechnol 8(6):921–928
69. Mukhopadhyay S, Maiti SK (2010) Phytoremediation of metal mine waste. Appl Ecol Environ Res 8:207–222
70. Mani D, Kumar C (2014) Biotechnological advances in bioremediation of heavy metals contaminated ecosystems: an overview with special reference to phytoremediation. Int J Environ Sci Technol 11:843–872
71. Karlson U, Frankenberger WT Jr (1989) Accelerated rates of selenium volatilization from California soils. Soil Sci Soc Am J 53:749–753

72. Bauelos GS, Meek DW (1990) Accumulation of selenium in plants grown on selenium-treated soil. J Environ Qual 19:772
73. Bauelos GS, Cardon G, Mackey B, Ben-Asher J, Wu L, Beuselinck P, Akohoue S, Zambrzuski S (1993) Plant and environment interactions, boron and selenium removal in boron-laden soils by four sprinkler irrigated plant species. J Environ Qual 22:786–792
74. Zayed AM, Terry N (1994) Selenium volatilization in roots and shoots: effects of shoot removal and sulfate level. J Plant Physiol 143:8–14
75. Terry N, Carlson C, Raab TK, Zayed AM (1992) Rates of selenium volatilization among crop species. J Environ Qual 21:341–344
76. Neuhierl B, Böck A (1996) On the mechanism of selenium tolerance in selenium-accumulating plants: purification and characterization of a specific selenocysteine methyltransferase from cultured cells of *Astragalus bisculatus*. Eur J Biochem 239:235–238
77. Fox B, Walsh CT (1982) Mercuric reductase. Purification and characterization of a transposon-encoded flavoprotein containing an oxidation-reduction-active disulfide. J Biol Chem 253:4341–4348
78. Rugh CL, Wilde HD, Stack NM, Thompson DM, Summers AO, Meagher RB (1996) Mercuric ion reduction and resistance in transgenic *Arabidopsis thaliana* plants expressing a modified bacterial merA gene. Proc Natl Acad Sci U S A 93:3182–3187
79. Raskin I, Smith RD, Salt DE (1997) Phytoremediation of metals: using plants to remove pollutants from the environment. Curr Opin Biotechnol 8:221–226
80. Adriano DC (1986) Trace elements in the terrestrial environment. Springer, New York
81. Adriano DC (1992) Biogeochemistry of trace metals. Lewis, Boca Raton
82. Alloway BJ (1990) Soil processes and behaviour of metals. In: Alloway BJ (ed) Heavy metals in soils. Blackie, Glasgow, pp 7–28
83. Meeuseen JCL, Keizer MG, Reimsdijk WH, Haan FAM (1994) Solubility of cyanide in contaminated soil. J Environ Qual 23:785–792
84. Raskin I, Kumar PBAN, Dushenkov S, Salt D (1994) Bioconcentration of heavy metals by plants. Curr Opin Biotechnol 5:285–290
85. Brooks RR, Chambers MF, Nicks LJ, Robinson BH (1998) Phytomining. Trends Plant Sci 3:359–362
86. Meagher RB (2000) Phytoremediation of toxic elemental and organic pollutants. Curr Opin Plant Biol 3:153–162
87. Barceló J, Vázquez MD, Mádico J, Poschenrieder C (1994) Hyperaccumulation of zinc and cadmium in *Thlaspi caerulescens*. In: Varnavas SP (ed) Environmental contamination. CEP, Edinburgh, pp 132–134
88. Garbisu C, Alkorta I (2003) Basic concepts on heavy metal soil bioremediation. Min Proc Einviron Protect 3:229–236
89. van der Lelie D, Schwitzguébel JP, Glass DJ, Vangronsveld J, Baker A (2001) Assessing phytoremediation progress in the United States and Europe. Environ Sci Technol 35:446–452
90. Blaylock MJ, Salt DE, Dushenkov S, Zakharova O, Gussman C, Kapulnik Y, Ensley BD, Raskin I (1997) Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents. Environ Sci Technol 31:860–865
91. Yoon J, Cao X, Zhou O (2006) Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. Sci Total Environ 368:456–464
92. McGrath SP (1998) Phytoextraction for soil remediation. In: Brooks RR (ed) Plants that hyperaccumulate heavy metals. CAB International, Wallingford, pp 261–287
93. Robinson BH, Meblanc L, Petit D, Broks RR, Kirkman JH, Gregg PEH (1998) The potential of Thlaspi caerulescens for phytoremediation of contaminated soils. Plant Soil 203:47–56
94. Reeves RD, Baker AJM (2000) Metal accumulating plants. In: Raskin I, Ensley BD (eds) Phytoremediation of toxic metals: using plants to clean up the environment. Wiley, New York, pp p193–p229
95. Lasat MM (2000) Phytoextraction of metals from contaminated soil: a review of plant/soil/metal interaction and assessment of pertinent agronomic issues. J Hazard Subs Res 2:1–25

96. Baker AJM, McGrath SP, Reeves RD, Smith JAC (2000) Metal hyperaccumulator plants: a review of the ecology and physiology of a biological resource for phytoremediation of metal polluted soils. In: Terry N, Banuelos G (eds) Phytoremediation of contaminated soil and water. Lewis, Boca Raton, pp 85–107
97. McGrath SP, Zhao FJ (2003) Phytoextraction of metals and metalloids from contaminated soils. Curr Opin Biotechnol 14:277–282
98. Gleba D, Borisjuk NV, Borisjuk LG, Kneer R, Poulev A, Skarzhinskaya M, Dushenkov S, Logendra S, Gleba YY, Raskin I (1999) Use of plant roots for phytoremediation and molecular farming. Proc Natl Acad Sci U S A 96:5973–5977
99. Guerinot ML, Salt DE (2001) Fortified foods and phytoremediation: two sides of the same coin. Plant Physiol 125:164–167
100. Peuke AD, Rennenberg H (2005) Phytoremediation: molecular biology, requirements for application, environmental protection, public attention and feasibility. EMBO Rep 6:497–501
101. Baker AJM, Reeves RD, Hajar ASM (1994) Heavy metal accumulation and tolerance in British populations of the metallophyte Thlaspi caerulescens J. and C. Presl (Brassicaceae). New Phytol 127:61–68
102. Bhargava A, Carmona FF, Bhargava M, Srivastava S (2012) Approaches for enhanced phytoextraction of heavy metals. J Environ Manage 105:103–120
103. Boyd RS, Martens SN (1992) The raison d'être for metal hyperaccumulation by plants. In: Baker AJM, Proctor J, Reeves RD (eds) The vegetation of ultramafic (serpentine) soils. Intercept, Andover, UK, pp 279–289
104. Macnair MR (1993) The genetics of metal tolerance in vascular plants. New Phytol 124:541–559
105. Boyd RS, Marten SN (1994) SN Ni hyperaccumulated by Thlaspi montanum var. montanum is acutely toxic to an insect herbivore. Oikos 70:21–25
106. Huitson SB, Macnair MR (2003) Does Zn protect the Zn hyperaccumulator Arabidopsis halleri from herbivory by snails? New Phytol 159:453–459
107. Boyd RS (2007) The defense hypothesis of elemental hyperaccumulation: status, challenges and new directions. Plant Soil 293:153–176
108. Noret N, Meerts P, Vanhaelen M, Dos Santos A, Escarré J (2007) Do metal-rich plants deter herbivores? A field test of the defence hypothesis. Oecologia 152:92–100
109. Galeas ML, Klamper EM, Bennett LE, Freeman JL, Kondratieff BC, Quinn CF, Pilon-Smits EA (2008) Selenium hyperaccumulation reduces plant arthropod loads in the field. New Phytol 177:715–724
110. Baker AJM, Whiting SN (2002) In search of the Holy Grail—a further step in understanding metal hyperaccumulation? New Phytol 155:1–7
111. Padmavathiamma PK, Li LY (2007) Phytoremediation technology: hyperaccumulation metals in plants. Plant Soil 184:105–126
112. Prasad MNV, Freitas H (2003) Metal hyperaccumulation in plants - biodiversity prospecting for phytoremediation technology. Electronic J Biotechnol 6:275–321
113. Verbruggen N, Hermans C, Schat H (2009) Molecular mechanisms of metal hyperaccumulation in plants. New Phytol 181:759–776
114. Ma LQ, Komar KM, Tu C, Zhang W, Cai Y, Kenelley ED (2001) A fern that hyperaccumulates arsenic. Nature 409:579
115. Zhang W, Cai Y, Tu C, Ma LQ (2002) Arsenic speciation and distribution in an arsenic hyperaccumulating plant. Sci Total Environ 300:167–177
116. Brown SL, Chaney RF, Angle JS, Baker AJM (1995) Zn and Cd uptake by hyperaccumulator *Thlaspi caerulescens* and metal tolerant *Silene vulgaris*, grown on sludge-amended soils. Environ Sci Tech 29:1581–1585
117. Basic N, Keller C, Fontanillas P, Vittoz P, Besnard G, Galland N (2006) Cadmium hyperaccumulation and reproductive traits in natural *Thlaspi caerulescens* populations. Plant Biol 8:64–72
118. Shah K, Nongkynrih JM (2007) Metal hyperaccumulation and bioremediation. Biol Plant 51:618–634

119. Ebbs SD, Lasat MM, Brandy DJ, Cornish J, Gordon R, Kochian LV (1997) Heavy metals in the environment: phytoextraction of cadmium and zinc from a contaminated soil. J Environ Qual 26:1424–1430
120. Doty SL (2008) Enhancing phytoremediation through the use of transgenics and endophytes. New Phytol 179:318–333
121. Capuana M (2011) Heavy metals and woody plants-biotechnologies for phytoremediation. iForest 4:7–15
122. Pulford ID, Watson C (2003) Phytoremediation of heavy metal-contaminated land by trees—a review. Environ Int 29:529–540
123. Rosselli W, Keller C, Boschi K (2003) Phytoextraction capacity of trees growing on a metal contaminated soil. Plant Soil 256:265–272
124. Meers E, Vandecasteele B, Ruttens A, Vangronsveld J, Tack FMG (2007) Potential of five willow species (Salix spp.) for phytoextraction of heavy metals. Environ Exp Bot 60:57–68
125. Unterbrunner R, Puschenreiter M, Sommer P, Wieshammer G, Tlustos P, Zupan M (2007) Heavy metal accumulation in tree growing on contaminated sites in Central Europe. Environ Poll 148:107–114
126. Brunner I, Luster J, Günthardt-Goerg MS, Frey B (2008) Heavy metal accumulation and phytostabilisation potential of tree fine roots in a contaminated soil. Environ Poll 152:559–568
127. Domínguez MT, Marañón T, Murillo JM, Schulin R, Robinson BH (2008) Trace element accumulation in woody plants of the Guadiamar Valley, SW Spain: a large-scale phytomanagement case study. Environ Poll 152:50–59
128. Fischerová Z, Tlustos P, Száková J, Sichorova K (2006) A comparison of phytoremediation capability of selected plant species for given trace elements. Environ Poll 144:93–100
129. Keller C, Diallo S, Cosio C, Basic N, Galland N (2006) Cadmium tolerance and hyperaccumulation by Thlaspi caerulescens populations grown in hydroponics are related to plant uptake characteristics in the field. Func Plant Biol 33:673–684
130. Mench M, Lepp N, Bert V, Schwitzguébel JP, Gawronski SW, Schröder P, Vangronsveld J (2010) Successes and limitations of phytotechnologies at field scale: outcome, assessment and outlook from COST Action 859. J Soil Sedim 10:1039–1070
131. Brown SL, Chaney RL, Angle IS, Baker AJM (1995) Zinc and cadmium uptake by hyperaccumulator *Thlaspi caerulescens* grown in nutrient solution. Soil Sci Am J 59:125–133
132. Lefebvre DD, Mild DBL, Laliberte JF (1987) Mammalian metallothionein functions in plants. BiofTechnoI 5:1053–1056
133. Maiti LB, Wagner GJ, Hunt AG (1991) Light inducible and tissue specific expression of a chimeric mouse metallothionein cDNA gene in tobacco. Plant Sci 76:99–107
134. Macek T, Kotrba P, Svatos A, Novakova M, Demnerova K, Macková M (2007) Novel roles for genetically modified plants in environmental protection. Trends Biotech 26:146–152
135. Kamnev AA, van der Lelie D (2000) Chemical and biological parameters as tools to evaluate and improve heavy metal phytoremediation. Biosci Rep 20:239–258
136. Meagher RB, Rugh CL, Kandasamy MK, Gragson G, Wang NJ (2000) Engineered phytoremediation of Hg pollution in soil and water using bacterial genes. In: Terry N, Banuelos G (eds) Phytoremediation of contaminated soil and water. Lewis, Boca Raton, pp 201–221
137. Nedelkoska TV, Doran PM (2000) Hyperaccumulation of Cd by hairy roots of Thlaspi caerulescens. Biotechnol Bioeng 67:607–615
138. Cunningham SD, Berti WR, Huang JW (1995) Phytoremediation of contaminated soils. Tibtech 13:393–397
139. Fulekar MH, Singh A, Bhaduri AM (2008) Genetic engineering strategies for enhancing phytoremediation of heavy metals. Afr J Biotechnol 8(4):529–535
140. Mello-Farias PCD, Chaves ALS, Lencina CL (2011) Transgenic plants for enhanced phytoremediation—physiological studies. In: Alvarez M (ed.) Genetic transformation. InTech. ISBN: 978-953-307-364-4.http://www.intechopen.com/books/genetictransformation/transgenic-plants-for-enhanced-phytoremediation-physiological-studies
141. Eapen S, D'Souza SF (2005) Prospects of genetic engineering of plants for phytoremediation of toxic metals. Biotechnol Adv 23:97–114

142. Mello-Farias PC, Chaves ALS (2008) Biochemical and molecular aspects of toxic metals phytoremediation using transgenic plants. In: Tiznado-Hernandez ME, Troncoso-Rojas R, Rivera-Domínguez MA (eds) Transgenic approach in plant biochemistry and physiology. Research Signpost, Kerala, pp 253–266
143. Yang X, Jin XF, Feng Y, Islam E (2005) Molecular mechanisms and genetic bases of heavy metal tolerance/hyperaccumulation in plants. Journal of Integrative PlantBiology 47(9):1025–1035
144. Ehrlich HL (1997) Microbes and metals. Appl Microbiol Biotechnol 48:687–692
145. de la Fuente JM, Ramírez-Rodríguez V, Cabrera-Ponce JL, Herrera-Estrella L (1997) Aluminum tolerance in transgenic plants by alteration of citrate synthesis. Science 276:1566–1568
146. Zhu Y, Pilon-Smits EAH, Jouanin L, Terry N (1999) Overexpression of glutathione synthetase in *Brassica juncea* enhances cadmium tolerance and accumulation. Plant Physiol 119:73–79
147. Zhu Y, Pilon-Smits EAH, Tarun A, Weber SU, Jouanin L, Terry N (1999) Cadmium tolerance and accumulation in Indian tase. Plant Physiol 121:1169–1177
148. Evans KM, Gatehouse JA, Lindsay WP, Shi J, Tommey AM, Robinson NJ (1992) Expression of the pea metallothionein- like gene PsMTA in *Escherichia coli* and *Arabidopsis thaliana* and analysis of trace metal ion accumulation: Implications for gene PsMTA function. Plant Mol Biol 20:1019–1028
149. Hasegawa I, Terada E, Sunairi M, Wakita H, Shinmachi F, Noguchi A, Nakajima M, Yazaki J (1997) Genetic improvement of heavy metal tolerance in plants by transfer of the yeast metallothionein gene (*CUP I*). Plant Soil 196:277–281
150. Goto F, Yoshihara T, Shigemoto N, Toki S, Takaiwa F (1999) Iron fortification of rice seed by the soybean ferritin gene. Nat Biotechnol 17:282–286
151. Samuelsen AI, Martin RC, Mok DWS, Machteld CM (1998) Expression of the yeast FRE genes in transgenic tobacco. Plant Physiol 118:51–58
152. Arazi T, Sunkar R, Kaplan B, Fromm H (1999) A tobacco plasmamembrane calmodulin-binding transporter confers Ni_2-tolerance and Pb2-hypersensitivity in transgenic plants. Plant J 20:171–182
153. Van der Zaal BJ, Neuteboom LW, Pinas JE, Chardonnens AN, Schat H, Verkleij JAC, Hooykaas PJJ (1999) Overexpres sion of a novel *Arabidopsis* gene related to putative zinc-transporter genes from animals can lead to enhanced zinc resistance and accumulation. Plant Physiol 119:1047–1055
154. Curie C, Alonso JM, Le Jean V, Ecker JR, Briat JF (2000) Involvement of Nramp1 from *Arabidopsis thaliana* in iron trans port. Biochem J 347:749–755
155. Hirschi KD, Korenkov VD, Wilganowski NL, Wagner GJ (2000) Expression of *Arabidopsis* CAX2 in tobacco. Altered metal accumulation and increased manganese tolerance. Plant Physiol 124:125–133
156. Bennett LE, Burkhead JL, Hale KL, Terry N, Pilon M, Pilon-Smits EAH (2003) bioremediation and biodegradation. analysis of transgenic Indian mustard plants for phytoremediation of metal-contaminated mine tailings. J Environ Qual 32:432–440
157. Pilon-Smits E, Pilon M (2002) Phytoremediation of metals using transgenic plants. Crit Rev Plant Sci 21(5):439–456
158. MacNair MR, Tilstone GH, Smith SE (2000) The genetics of metal tolerance and accumulation in higher plants. In: Terry N, Bañuelos G (eds) Phytoremediation of contaminated soil and water. Lewis, Boca Raton, pp 235–250
159. Wu L (1990) Colonization and establishment of plants in contaminated sites. In: Shaw AJ (ed) Heavy metal tolerance in plants: evolutionary aspects. CRC Press, Boca Raton, pp 269–284
160. Seth CS (2011) A review on mechanisms of plant tolerance and role of transgenic plants in environmental clean-up. Botanical Review 78:9092. doi:10.1007/s12229-011-9092-x
161. Misra S, Gedamu L (1989) Heavy metal tolerant transgenic *Brassica napus* L. and *Nicotiana tabacum* L. plants. Theor Appl Genet 78:16–18

162. Pan A, Yang M, Tie F, Li L, Chen Z, Ru B (1994) Expression of mouse metallothionein-I gene confers cadmium resistance in transgenic tobacco plants. Plant Mol Biol 24:341–351
163. Dorlhac de Borne F, Elmayan T, De Roton C, De Hys L, Tepfer M (1998) Cadmium partitioning in transgenic tobacco plants expressing a mammalian metallothionein gene. Molecul Breeding 4:83–90
164. Pavlikova D, Macek T, Mackova M, Sura M, Szakova J, Tlustos P (2004) The evaluation of cadmium, zinc and nickel accumulation ability of transgenic tobacco bearing different transgenes. Plant Soil Environ 50:513–517
165. Macek T, Macková M, Pavlíková D, Száková J, Truksa M, Singh Cundy A, Kotrba P, Yancey N, Scouten H (2002) Accumulation of cadmium by transgenic tobacco. Acta Biotechnol 22:101–106
166. Suh MC et al (1998) Cadmium resistance in transgenic tobacco plants expressing the *Nicotiana glutinosa* L. metallothionein-like gene. Mol Cells 8:678–684
167. Elmayan T, Tepfer M (1994) Synthesis of a bifunctional metallothionein β-glucuronidase fusion protein in transgenic tobacco plants as a means of reducing leaf cadmium levels. Plant J 6:433–440
168. Stefanov I et al (1997) Effect of cadmium treatment on the expression of chimeric genes in transgenic seedlings and calli. Plant Cell Rep 16:291–294
169. Mejáre M, Bülow L (2001) Metal-binding proteins and peptides in bioremediation and phytoremediation of heavy metals. Trends Biotechnol 19(2):67–73
170. Balasundaram U, Venkataraman G, George S, Parida A (2014) Metallothioneins from a hyperaccumulating plant *Prosopis juliflora* show difference in heavy metal accumulation in transgenic tobacco. Int J Agric Environ Biotechnol 7(2):241–246, 10.5958/2230-732X.2014.00240.X
171. Xiang C, Werner BL, Christensen EM, Oliver DJ (2001) The biological functions of glutathione revisited in *Arabidopsis* transgenic plants with altered glutathione levels. Plant Physiol 126:564–574
172. Harada E, Choi YE, Tsuchisaka A, Obata H, Sano H (2001) Transgenic tobacco plants expressing a rice cysteine synthase gene are tolerant to toxic levels of cadmium. J Plant Physiol 158:655–661
173. Reisinger S, Schiavon M, Terry N, Pilon-Smits EAH (2008) Heavy metal tolerance and accumulation in Indian mustard (Brassica juncea L.) expressing bacterial gamma-glutamylcysteine synthetase or glutathione synthetase. Int J Phytoremediat 10:440–454
174. GuoJ XW, Maa M (2012) The assembly of metals chelation by thiols and vacuolar compartmentalization conferred increased tolerance to and accumulation of cadmium and arsenic in transgenic Arabidopsis thaliana. J Hazard Mater 199–200:309–313. doi:10.1016/j.jhazmat.2011.11.008
175. Ezaki B, Gardner RC, Ezaki Y, Matsumoto H (2000) Expression of aluminum-induced genes in transgenic *Arabidopsis* plants can ameliorate aluminum stress and/or oxidative stress. Plant Physiol 122:657–665
176. Marrs KA (1996) The functions and regulation of glutathione S-transferase in plants. Annu Rev Plant Physiol Plant Mol Biol 47:127–158
177. López-Bucio J, Martinez de la Vega O, Guevara-García A, Herrera-Estrella L (2000) Enhanced phosphorus uptake in transgenic tobacco plants that overproduce citrate. Nat Biotechnol 18:450–453
178. Guerinot ML (2001) Improving rice yields—ironing out the details. Nature Biotechnol 19:417–418
179. Huang JW, Blaylock MJ, Kapulnik Y, Ensley BD (1998) Phytoremediation of uranium-contaminated soils: role of organic acids in triggering uranium hyperaccumulation in plants. Environ Sci Technol 32:2004–2008
180. Lasat MM (2002) Phytoextraction of toxic metals: a review of biological mechanisms. J Environ Qual 31:109–120
181. Han YY, Zhang WZ, Zhang BL, Zhang SS, Wang W, Ming F (2009) One novel mitochondrial citrate synthase from Oryza sativa L. can enhance aluminum tolerance in transgenic tobacco. Mol Biotechnol 42:299–305

182. Higuchi K, Suzuki K, Nakanishi H, Yamaguchi H, Nishizawa NK, Mori S (1999) Cloning of nicotianamine synthase genes, novel genes involved in the biosynthesis of phytosiderophores. Plant Physiol 119:471–479
183. Meda AR, Scheuermann EB, Prechsl UE, Erenoglu B, Schaaf G, Hayen H, Weber G, von Wirén N (2007) Iron acquisition by phytosiderophores contributes to cadmium tolerance. Plant Physiol 143:1761–1773
184. Sappin-Didier V, Vansuyts G, Mench M, Briat JF (2005) Cadmium availability at different soil pH to transgenic tobacco overexpressing ferritin. Plant Soil 270:189–197
185. Takahashi M, Nakanishi H, Kawasaki S, Nishizawa NK, Mori S (2001) Enhanced tolerance of rice to low iron availability in alkaline soils using barley nicotianamine aminotransferase genes. Nature Biotechnol 19:466–469
186. Goto F, Yoshihara T, Saiki H (1998) Iron accumulation in tobacco plants expressing soyabean ferritin gene. Transgenic Res 7:173–180
187. Persans MW, Nieman K, Salt DE (2001) Functional activity and role of cation-efflux family members in Ni hyperaccumulation in *Thlaspi goesingense*. Proc Natl Acad Sci U S A 98:9995–10000
188. Shaul O, Hilgemann DW, de Almedia-Engler J, Van Montagu M, Inze D, Galili G (1999) Cloning and characterization of a novel Mg^{2+}/H^+ exchanger. EMBO J 18:3973–3980
189. Sunkar R, Kaplan B, Bouche N, Arazi T, Dolev D, Talke IN, Maathuis FJM, Sanders D, Bouchez D, Fromm H (2000) Plant J 24:533–542
190. Delhaize E (1996) A metal accumulator mutant of *Arabidopsis thaliana*. Plant Physiol 111:849–855
191. Robinson NJ, Procter CM, Connolly EL, Guerinot ML (1999) A ferric-chelate reductase for iron uptake from soils. Nature 397:694–697
192. Thomine S, Wang R, Ward JM, Crawford NM, Schroeder JI (2000) Cadmium and iron transport by members of a plant metal transporter family in *Arabidopsis* with homology to Nramp genes. Proc Natl Acad Sci U S A 97:4991–4996
193. Rogers EE, Eide DJ, Guerinot ML (2000) Altered selectivity in an *Arabidopsis* metal transporter. Proc Natl Acad Sci U S A 97:12356–12360
194. Kiyono M, Oka Y, Sone Y, Nakamura R, Sato MH, Sakabe K, Pan-Hou H (2013) Bacterial heavy metal transporter MerC increases mercury accumulation in *Arabidopsis thaliana*. Biochem Eng J 71:19–24
195. Mouhamada R, Ibrahimb K, Alib N, Ghanemc I, Al-Daoudec A (2014) Determination of heavy metal uptake in transgenic plants harbouring the rabbit CYP450 2E1 using X-ray fluorescence analysis. Int J Environ Stud 71(3):292–300. doi:10.1080/00207233.2014.909681
196. Summers AO (1986) Organization, expression, and evolution of genes for mercury resistance. Annu Rev Microbiol 40:607–634
197. Bizily SP, Rugh CL, Meagher RB (2000) Phytodetoxification of hazardous organomercurials by genetically engineered plants. Nature Biotechnol 18:213–217
198. Rugh CL, Bizily SP, Meagher RB (2000) Phytoreduction of environmental mercury pollution. In: Raskin I, Ensley BD (eds) Phytoremediation of toxic metals—using plants to clean up the environment. Wiley, New York, pp 151–171
199. Dhankher OP, Li YJ, Rosen BP, Shi J, Salt D, Senecoff JF, Sashti NA, Meagher RB (2002) Engineering tolerance and hyperaccumulation of arsenic in plants by combining arsenate reductase and γ-glutamylcysteine synthase expression. Nat Biotechnol 20:1140–1150
200. Chen L et al (1998) Expression and inheritance of multiple transgenes in rice plants. Nature Biotechnol 16:1060–1064
201. Brewer EP, Saunders JA, Angle JS, Chaney RL, McIntosh MS (1999) Somatic hybridization between the zinc accumulator *Thlaspi caerulescens* and *Brassica napus*. Theor Appl Genet 99:761–771
202. Oberschall A, Deak M, Torok K, Sass L, Vass I, Kovacs I, Feher A, Dudits D, Horvath GV (2000) A novel aldose/aldehyde reductase protects transgenic plants against lipid peroxidation under chemical and drought stress. Plant J 24:437–446

203. Pilon-Smits EAH, Zhu YL, Sears T, Terry N (2000) Overexpression of glutathione reductase in *Brassica juncea*: effects on cadmium accumulation and tolerance. Physiol Plant 110:455–460
204. Grichko VP, Filby B, Glick BR (2000) Increased ability of transgenic plants expressing the bacterial enzyme ACC deaminase to accumulate Cd, Co, Cu, Ni, Pb, and Zn. J Biotechnol 81:45–53
205. Eriksson ME, Israelsson M, Olsson O, Moritz T (2000) Increased gibberellins biosynthesis in transgenic trees promotes growth, biomass production and xylem fiber length. Nat Biotechnol 18:784–788
206. Gisbert C, Ros R, De Haro A, Walker DJ, Bernal MP, Serrano R, Navarro-Avino J (2003) A plant genetically modified that accumulates Pb is especially promising for phytoremediation. Biochem Biophys Res Commun 303:440–445
207. Rugh CL, Seueoff JF, Meagher RB, Merkle SA (1998) Development of transgenic yellow poplar for mercury phytoremediation. Nat Biotechnol 16:925–928
208. Pilon-Smits EAH, Hwang S, Lytle CM, Zhu Y, Tai JC, Bravo RC, Chen Y, Leustek T, Terry N (1999) Over expression of ATP sulfurylase in Indian mustard leads to increased selenate uptake, reduction, and tolerance. Plant Physiol 119:123–132
209. Li TQ, Yang XE, Long XX (2004) Potential of using *Sedum alfredii* for phytoremediating multi-metal contaminated soils. Journal of Soil Water Conservation 18:79–83
210. Mohammadi M, Chalavi V, Novakova-Sura M, Laliberte JF, Sylvestre M (2007) Expression of bacterial biphenyl-chlorophenyl dioxygenase genes in tobacco plants. Biotechnology Bioengineering 97:496–505
211. Wolfenbarger LL, Phifer PR (2000) The ecological risks and benefits of genetically engineered plants. Science 290:2088–2093
212. Glass DJ (1999) U.S. and international markets for phytoremediation, 1999–2000. D. Glass, Needham
213. Lin Z-Q, Schemenauer RS, Cervinka V, Zayed A, Lee A, Terry N (2000) Selenium volatilization from the soil—Salicornia bigelovii Torr treatment system for the remediation of contaminated water and soil in the San Joaquin Valley. J Environ Qual 29:1048–1056
214. Herbik A, Koch G, Mock HP, Dushkov D, Czihal A, Thielmann J, Stephan UW, Bäumlein H (1999) Isolation, characterization and cDNA cloning of nicotianamine synthase from barley — a key enzyme for iron homeostasis in plants. Eur J Biochem 265:231–239
215. Ling HQ, Koch G, Bäumlein H, Ganal MW (1999) Map-based cloning of chloronerva, a gene involved in iron uptake of higher plants encoding nicotianamine synthase. Proc Natl Acad Sci U S A 96:7098–7103
216. Takahashi M, Yamaguchi H, Nakanishi H, Shioiri T, Nishizawa NK, Mori S (1999) Cloning two genes for nicotianamine aminotransferase, a critical enzyme in iron acquisition (strategy II) in graminaceous plants. Plant Physiol 121:947–956
217. Clemens S, Kim EJ, Neumann D, Schroeder JI (1999) Tolerance to toxic metals by a gene family of phytochelatin synthases from plants and yeast. EMBOJ 18:3325–3333
218. Ha SB, Smith AP, Howden R, Dietrich WM, Bugg S, O'Connell MJ, Goldsbrough PB, Cobbett CS (1999) Phytochelatin synthase genes from *Arabidopsis* and the yeast *Schizosaccharomyces pombe*. Plant Cell 11:1153–1164
219. Vatamaniuk OK, Mari S, Lu YP, Rea PA (1999) AtPCS1, a phytochelatin synthase from *Arabidopsis*: isolation and in vitro reconstitution. Proc Natl Acad Sci U S A 96:7110–7115
220. Lu YP, Li ZS, Rea PA (1997) AtMRP1 gene of *Arabidopsis* encodes a glutathione S-conjugate pump: isolation and functional definition of a plant ATP-binding cassette transporter gene. Proc Natl Acad Sci U S A 94:8243–8248
221. Persans MW, Yan X, Patnoe JML, Krämer U, Salt DE (1999) Molecular dissection of the role of histidine in nickel hyperaccumulation in *Thlaspi goesingense* (Hálácsy). Plant Physiol 121:1117–1126
222. Krämer U, Chardonnens AN (2001) The use of transgenic plants in the bioremediation of soils contaminated with trace elements. Appl Microbiol Biotechnol 55:661–672

223. Wallace A, Mueller RT, Wood RA (1980) Arsenic phytotoxicity and interactions in bush bean plants grown in solution culture. J Plant Nutr 2:111–113
224. Förstner U (1995) Land contamination by metals: global scope and magnitude of problem. In: Allen HE, Huang CP, Bailey GW, Bowers AR (eds) Metal speciation and contamination of soil. CRC Press, Boca Raton, pp 1–33
225. Li HF, Gray C, Mico C, Zhao FJ, McGrath SP (2009) Phytotoxicity and bioavailability of cobalt to plants in a range of soils. Chemosphere 75:979–986
226. McCutcheon SC, Schnoor JL (2003) Phytoremediation. Wiley, Hoboken
227. Terry N, Bañuelos GS (2000) Phytoremediation of contaminated soil and water. CRC, Boca Raton
228. Visoottiviseth P, Francesconi K, Sridokchan V (2002) The potential of Thai indigenous plant species for the phytoremediation of arsenic contaminated land. Environ Pollut 118:453–461
229. Zayed AM, Terry N (2003) Chromium in the environment: factors affecting biological remediation. Plant Soil 249:139–156
230. Robinson BH, Brooks RR, Howes AW, Kirkman JH, Gregg PEH (1997) The potential of the high-biomass nickel hyperaccumulator Berkheya coddii for phytoremediation and phytomining. J Geochem Explor 60:115–126
231. Moradi AB, Swoboda S, Robinson B, Prohaska T, Kaestner A, Oswald SE, Wenzel WW, Schulin R (2010) Mapping of nickel in root cross-sections of the hyperaccumulator plant Berkheya coddii using laser ablation ICP-MS. Environ Exp Bot 69:24–31
232. Becerra-Castro C, Monterroso C, Garcia-Leston M, Prieto-Fernandez A, Acea MJ, Kidd PS (2009) Rhizosphere microbial densities and trace metal tolerance of the nickel hyperaccumulator Alyssum serpyllifolium subsp. lusitanicum. Int J Phytoremed 11:525–541
233. Barzanti R, Colzi I, Arnetoli M, Gallo A, Pignattelli S, Gabbrielli R, Gonnelli C (2011) Cadmium phytoextraction potential of different *Alyssum* species. J Hazard Mater 196:66–72
234. Jaffre T, Brooks RR, Lee J, Reeves RD (1976) Sebertia acuminata: a hyperaccumulator of nickel from New Caledonia. Science 193:579–580
235. Perrier N (2004) Nickel speciation in Sebertia acuminata, a plant growing on a lateritic soil of New Caledonia. Geoscience 336:567–577
236. Reeves RD, Baker AJM, Borhidi A, Berazain R (1999) Nickel hyperaccumulation in the serpentine flora of Cuba. Ann Bot 83:29–38
237. Reeves RD, Adiguzel N, Baker AJM (2009) Nickel hyperaccumulation in Bornmuellera kiyakii and associated plants of the Brassicaceae from Kızıldaäÿ Derebucak (Konya). Turkey Turk J Bot 33:33–40
238. Küpper H, Mijovilovich A, Götz B, Kroneck PMH, Küpper FC, Meyer-Klaucke W (2009) Complexation and toxicity of copper in higher plants (I): characterisation of copper accumulation, speciation and toxicity in Crassula helmsii as a new copper hyperaccumulator. Plant Physiol 151:702–714
239. Wang H, Zhong G (2011) Effect of organic ligands on accumulation of copper in hyperaccumulator and nonaccumulator Commelina communis. Biol Trace Elem Res 143:489–499
240. Oven M, Grill E, Golan-Goldhirsh A, Kutchan TM, Zenk MH (2002) Increase of free cysteine and citric acid in plant cells exposed to cobalt ions. Phytochemistry 60:467–474
241. Galeas ML, Zhang LH, Freeman JL, Wegner M, Pilon-Smits EAH (2007) Seasonal fluctuations of selenium and sulfur accumulation in selenium hyperaccumulators and related nonaccumulators. New Phytol 173:517–525
242. Freeman JL, Tamaoki M, Stushnoff C (2010) Molecular mechanisms of selenium tolerance and hyperaccumulation in Stanleya pinnata. Plant Physiol 153:1630–1652
243. Hladun KR, Parker DR, Trumble JT (2011) Selenium accumulation in the floral tissues of two Brassicaceae species and its impact on floral traits and plant performance. Environ Exp Bot 74:90–97
244. Kupper H, Kochian LV (2010) Transcriptional regulation of metal transport genes and mineral nutrition during acclimatization to cadmium and zinc in the Cd/Zn hyperaccumulator, *Thlaspi caerulescens* (Ganges population). New Phytol 185:114–129

245. Kubota H, Takenaka C (2003) Arabis gemmifera is a hyperaccumulator of Cd and Zn. Int J Phytoremed 5:197–201
246. Tang YT, Qiu RL, Zeng XW, Ying RR, Yu FM, Zhou XY (2009) Lead, zinc cadmium accumulation and growth simulation in Arabis paniculata Franch. Env Exp Bot 66:126–134
247. Sun Q, Ye ZH, Wang XR, Wong MH (2005) Increase of glutathione in mine population of Sedum alfredii: a Zn hyperaccumulator and Pb accumulator. Phytochemistry 66:2549–2556
248. Zhao FJ, Lombi E, Breedon T, McGrath SP (2000) Zinc hyperaccumulation and cellular distribution in Arabidopsis halleri. Plant Cell Environ 3:507–514
249. Du RJ, He EK, Tang YT, Hu PJ, Ying RR, Morel JL, Qiu RL (2011) How phytohormone IAA and chelator EDTA affect lead uptake by Zn/Cd hyperaccumulator Picris divaricata. Int J Phytoremed 13:1024–1036
250. Sahi SV, Bryant NL, Sharma NC, Singh SR (2002) Characterization of a lead hyperaccumulator shrub. Sesbania drummondii Environ Sci Technol 36:4676–4680
251. Sharma NC, Gardea-Torresday JL, Parson Sahi SV (2004) Chemical speciation of lead in Sesbania drummondii. Environ Toxicol Chem 23:2068–2073
252. Chandra Sekhar K, Kamala CT, Chary NS, Balaram V, Garcia G (2005) Potential of Hemidesmus indicus for phytoextraction of lead from industrially contaminated soils. Chemosphere 58:507–514
253. Bech J, Duran P, Roca N, Poma W, Sánchez I, Barceló J, Boluda R, Roca-Pérez L, Poschenrieder C (2011) Shoot accumulation of several trace elements in native plant species from contaminated soils in the Peruvian Andes. J Geochem Explor. doi:10.1016/j.gexplo.2011.04.007
254. Bidwell SD, Woodrow IE, Batianoff GN, Sommer-Knudsen J (2002) Hyperaccumulation of manganese in the rainforest tree *Austromyrtus bidwillii (Myrtaceae)* from Queensland. Australia Funct Plant Biol 29:899–905
255. Pollard AJ, Stewart HL, Roberson CB (2009) Manganese hyperaccumulation in Phytolacca americana L. from the southeastern united states. Northeastern Natur 16:155–162
256. Fernando DR, Bakkaus EJ, Perrier N, Baker AJM, Woodrow IE, Batianoff GN, Collins RN (2006) Manganese accumulation in the leaf mesophyll of four tree species: a PIXE/EDAX localization study. New Phytol 171:751–758
257. Fernando DR, Woodrow IE, Bakkaus EJ, Collins RN, Baker AJM, Batainoff GN (2007) Variability of Mn hyperaccumulation in the Australian rainforest tree Gossia bidwillii (Myrtaceae). Plant Soil 293:145–152
258. Fernando DR, Woodrow IE, Jaffre T, Dumontet V, Marshall AT, Baker AJM (2008) Foliar manganese accumulation by Maytenus founieri (Celastraceae) in its native New Caledonian habitats: populational variation and localization by X-ray microanalysis. New Phytol 177:178–185
259. Dahmani-Muller H, van Oort F, Gelie B, Balabane M (2000) Strategies of heavy metal uptake by three plant species growing near a metal smelter. Environ Poll 109:231–238
260. Bert V, Bonnin I, Saumitou-Laprade P, de Laguerie P, Petit D (2002) Do *Arabidopsis halleri* from nonmetallicolous populations accumulate zinc and cadmium more effectively than those from metallicolous populations? New Phytol 155:47–57
261. Sun Y, Zhou Q, Wang L, Liu W (2009) Cadmium tolerance and accumulation characteristics of Bidens pilosa L. as a potential Cd-hyperaccumulator. J Hazardous Mat 161:808–814
262. Gardea-Torresday JL, De la Rosa G, Peralta-Videa JR, Montes M, Cruz-Jimenez G, Cano-Aguilera I (2005) Differential uptake and transport of trivalent and hexavalent chromium by tumble wed (Salsola kali). Arch Environ Contam Toxicol 48:225–232
263. Zhang XH, Liu J, Huang HT, Chen J, Zhu YN, Wang DQ (2007) Chromium accumulation by the hyperaccumulator plant Leersia hexandra Swartz. Chemosphere 67:1138–1143
264. Mongkhonsin B, Nakbanpote W, Nakai I, Hokura A, Jearanaikoon N (2011) Distribution and speciation of chromium accumulated in Gynura pseudochina (L.) DC. Environ Exp Bot 74:56–64

265. Leblanc M, Petit D, Deram A, Robinson B, Brooks RR (1999) The phytomining and environmental significance of hyperaccumulation of thallium by Iberis intermedia from southern France. Econ Geol 94:109–113
266. Al-Najar H, Kaschl A, Schulz R, Romheld V (2005) Effects of thallium fractions in the soil and pollution origin in thallium uptake by hyperaccumulator plants: a key factor for assessment of phytoextraction. Intern J Phytoremed 7:55–67
267. Dhankher OP, Doty SL, Meagher RB, Pilon-Smits E (2011) Biotechnological approaches for phytoremediation. In: Altman A, Hasegawa PM (eds) Plant biotechnology and agriculture. Academic, Oxford, pp 309–328

Chapter 13
Role of Phytochelatins in Phytoremediation of Heavy Metals Contaminated Soils

Neerja Srivastava

13.1 Introduction

Metal contamination of soils is ubiquitous around the globe. Metals enter the soil due to anthropogenic activities such as the use of sewage sludge, urban composts, fertilisers, pesticides, sewage irrigation, incineration of municipal waste, auto vehicle exhausts, industrial emissions, and metal mining and smelting [1–3]. These metals include iron (Fe), copper (Cu), manganese (Mn), zinc (Zn), cadmium (Cd), lead (Pb), chromium (Cr), mercury (Hg), and nickel (Ni) [4]. Metals accumulate in the soil to toxic levels that may lead to accumulation of metals in plants to unacceptable levels. Metal accumulation is a subject of serious concern due to threat to plant growth, soil quality, animal, and human health [2]. Cleaning up soils to remove metals is a sign of the times, but it is a challenging task. Different technologies being used nowadays are ex situ which lead to destruction of soil structure, thus leaving it unusable with poor vegetative cover [5]. Growing plants to clean up the soils is a cost-effective and environmentally friendly alternative [6]. Phytoremediation seems attractive due to non-invasive and non-destructive technologies which leave the soil intact and biologically productive [7]. Plants respond differentially to metal contamination in soils and can be classified into different categories, depending upon their responses to metal contamination in their rooting medium. Plants can be classified into accumulators, indicators, or excluders depending upon absorption and translocation of metals by the plants to above-ground parts [8]. Accumulators can survive by maintaining high concentration of metals in their tissues. Indicator plants are reported to have mechanisms that control translocation of metals from roots to shoots and excluders restrict the entry of metals into plants at root level [9]. Plants use different adaptive mechanisms to accumulate or exclude metals and thus

---

N. Srivastava (✉)
Department of Biochemistry, IBSBT, CSJM University, Kanpur, UP, India
e-mail: neerjas70@gmail.com

A.A. Ansari et al. (eds.), *Phytoremediation*, DOI 10.1007/978-3-319-40148-5_13

maintain their growth. Accumulation and tolerance of metals by the plants is a complex phenomenon. Movement of metals across the root membrane, loading and translocation of metals through xylem and sequestration and detoxification of metals at the cellular and whole plant levels are important mechanisms adopted by accumulator plants [10]. Indicator plants absorb the metals from the soils and then restrict their movement to the shoots while excluders restrict the entry of metals into the plant roots. Understanding the mechanisms involved in phytoremediation is necessary to effectively use this technique on metal-contaminated soils [11].

Several plants have been reported to synthesise low molecular weight thiols, under metal stress, and have high binding affinity to cadmium and arsenic [12–14]. The most important low molecular weight biological thiols are NP-SH, GSH, and PCs. Glutathione is a sulphur-containing tripeptide thiol (γ-glutamatecysteine-glycine) and is a precursor for PCs synthesis [15, 16]. Phytochelatins are small metal-binding peptides with the structure (γ-Glu-Cys)n-Gly, where value of n varies from 2 to 11, and their synthesis is induced by several toxic metals, particularly by Cd [17, 18]. The functional significance of PCs can be attributed to the presence of thiol (-SH) group making a co-ordination bond with several toxic heavy metals [14, 15, 19, 20]. In general, while vacuolar compartmentalisation keeps heavy metals away from metal-sensitive metabolic centres in the cytoplasm, sequestration ligands seem to safeguard them from readily moving by reducing their chemical selectivity. For an effective and efficient internal metal tolerance, both roles are important. While the mechanism of low molecular weight endogenous or induced organic acids, particularly citrate, may be employed by plants as a strategy to detoxify the low-level exposure of heavy metals, an additional mechanism of producing large molecules and more specific compounds such as PCs may be employed by plants to combat high-level exposure to heavy metals. Although production of PCs may be a mechanism that plants employ in internal detoxification of HM, yet it is unlikely that such is the only mechanism in HM tolerance. This is because induction of PCs is associated with exposure to high external metal concentrations. Apparently, at relatively low metal exposure, plants employ mechanism(s) other than PC production to tolerate the metal stress internally.

13.2 Phytoremediation

Phytoremediation is the use of plants and their associated microbes for environmental cleanup [21–23]. This technology makes use of the naturally occurring processes by which plants and their microbial rhizosphere flora degrade and sequester organic and inorganic pollutants. Phytoremediation is an efficient cleanup technology for a variety of organic and inorganic pollutants. Organic pollutants in the environment are mostly manmade and xenobiotic to organisms. Many of them are toxic, some carcinogenic. Organic pollutants are released into the environment via spills (fuel, solvents), military activities (explosives, chemical weapons), agriculture (pesticides, herbicides), industry (chemical, petrochemical), wood treatment, etc. Depending on their properties, organics may be degraded in the root zone of plants or taken up, followed by degradation, sequestration, or volatilisation. Organic pollutants that have been successfully phytoremediated include organic solvents such as TCE (the most common pollutant of groundwater) [24, 25]; herbicides such as atrazine [26]; explosives such as TNT [27]; petroleum hydrocarbons such as oil, gasoline, benzene, toluene, and PAHs [28–30]; the fuel additive MTBE [31–33] and polychlorinated biphenyls (PCBs) [34]. Inorganic pollutants occur as natural elements in the earth's crust or atmosphere, and human activities such as mining, industry, traffic, agriculture, and military activities promote their release into the environment, leading to toxicity [35]. Inorganics cannot be degraded, but they can be phytoremediated via stabilisation or sequestration in harvestable plant tissues. Inorganic pollutants that can be phytoremediated include plant macronutrients such as nitrate and phosphate [36], plant trace elements such as Cr, Cu, Fe, Mn, Mo, and Zn [37], non-essential elements such as Cd, Co, F, Hg, Se, Pb, V, and W [36, 38], and radioactive isotopes such as 238U, 137Cs, and 90Sr [39–42].

Heavy metals degrade soil and water resources and thus pose a serious threat to human and animal health. This threat is further aggravated due to the persistent and non-biodegradable nature of metals [43]. Accumulation of metals in the bodies of animals and humans after entering the food chain has serious implications for health as some metals are known to damage DNA and cause cancer due to their mutagenic abilities [44]. Remediating the soils contaminated with metals is thus necessary for safe use of such soils and several in situ and ex situ technologies are used for this purpose. Phytoremediation is considered environmentally friendly, non-invasive, and cost-effective technology to clean up the metal-contaminated soils. Plants adopt different mechanisms to grow in the metal-contaminated soils without adverse effects on their growth. Some plants exclude the metals from metabolically active sites by restricted uptake or root to shoot transfer of metals [45]. Some other plants can tolerate high metal concentrations in their tissues through binding of metals with organic compounds, metal compartmentalisation at cellular and sub-cellular levels, and metabolic alterations [45–47]. Heavy metals tolerance in plants may be defined as the ability of plants to survive in a soil that is toxic to other plants [48]. Phytoremediation can be classified into phytoextraction, phytostabilisation, and phytovolatilisation [49, 50], in addition to various other classes of phytoremediation [11].

13.3 Types of Phytoremediation Strategies

Phytoremediation consists of five main processes as presented in Table 13.1 [51].

13.3.1 Rhizofiltration

It is defined as the use of plants, both terrestrial and aquatic, to absorb, concentrate, and precipitate contaminants from polluted aqueous sources with low contaminant concentration in their roots. Rhizofiltration can partially treat industrial discharge,

Table 13.1 Phytoremediation includes the following processes and mechanisms of contaminant removal

No.	Process	Mechanism	Contaminant
1	Rhizofiltration	Rhizosphere accumulation	Organics/Inorganics
2	Phytostabilisation	Complexation	Inorganics
3	Phytoextraction	Hyperaccumulation	Inorganics
4	Phytovolatilisation	Volatilisation by leaves	Organics/Inorganics
5	Phytotransformation	Degradation in plant	Organics

agricultural runoff, or acid mine drainage. It can be used for lead, cadmium, copper, nickel, zinc, and chromium, which are primarily retained within the roots [9, 52]. The advantages of rhizofiltration include its ability to be used as in situ or ex situ applications and species other than hyperaccumulators can also be used. Plants like sunflower, Indian mustard, tobacco, rye, spinach, and corn have been studied for their ability to remove lead from effluent, with sunflower having the greatest ability. Indian mustard has proven to be effective in removing a wide concentration range of lead (4–500 mg/L) [53]. The technology has been tested in the field with uranium (U)-contaminated water at concentrations of 21–874 µg/L; the treated U concentration reported by Dushenkov was <20 µg/L before discharge into the environment [54].

13.3.2 *Phytoextraction*

It is the best approach to remove the contamination primarily from soil and isolate it, without destroying the soil structure and fertility. It is also referred as phytoaccumulation [52]. As the plant absorb, concentrate, and precipitate toxic metals and radionuclide from contaminated soils into the biomass, it is best suited for the remediation of diffusely polluted areas, where pollutants occur only at relatively low concentration and superficially [55]. Several approaches have been used but the two basic strategies of phytoextraction, which have finally developed are (1) chelate-assisted phytoextraction or induced phytoextraction, in which artificial chelates are added to increase the mobility and uptake of metal contaminant. (2) Continuous phytoextraction in this the removal of metal depends on the natural ability of the plant to remediate; only the number of plant growth repetitions are controlled [22, 56]. Discovery of hyperaccumulator species has further boosted this technology. In order to make this technology feasible, the plants must extract large concentrations of heavy metals into their roots, translocate the heavy metals to surface biomass, and produce a large quantity of plant biomass. There moved heavy metal can be recycled from the contaminated plant biomass [57]. Factors such as growth rate, element selectivity, resistance to disease, method of harvesting are also important [58, 59]. However, slow growth, shallow root system, small biomass production, and final disposal limit the use of hyperaccumulator species [60].

13.3.3 Phytovolatilisation

Phytovolatilisation involves the use of plants to take up contaminants from the soil, transforming them into volatile form and transpiring them into the atmosphere. Phytovolatilisation occurs as growing trees and other plants take up water and the organic and inorganic contaminants. Some of these contaminants can pass through the plants to the leaves and volatilise into the atmosphere at comparatively low concentrations [61]. Phytovolatilisation has been primarily used for the removal of mercury; the mercuric ion is transformed into less toxic elemental mercury. The disadvantage is mercury released into the atmosphere is likely to be recycled by precipitation and then redeposited back into ecosystem [62]. Some plants growing in high Selenium media produce volatile selenium in the form of dimethylselenide and dimethyldiselenide [63]. Phytovolatilisation has been successful in tritium (3H), a radioactive isotope of hydrogen; it is decayed to stable helium with a half-life of about 12 years as reported by Dushenkov [39].

13.3.4 Phytodegradation

In phytoremediation of organics, plant metabolism contributes to the contaminant reduction by transformation, breakdown, stabilisation, or volatilising contaminant compounds from soil and groundwater. Phytodegradation is the breakdown of organics taken up by the plant to simpler molecules that are incorporated into the plant tissues [9]. Plants contain enzymes that can breakdown and convert ammunition wastes, chlorinated solvents such as trichloroethylene and other herbicides. The enzymes are usually dehalogenases, oxygenases, and reductases [64]. Rhizodegradation is the breakdown of organics in the soil through microbial activity of the root zone (rhizosphere) and is a much slower process than phytodegradation. Yeast, fungi, bacteria, and other microorganisms consume and digest organic substances like fuels and solvents. All phytoremediation technologies are not exclusive and may be used simultaneously, but the metal extraction depends on its bioavailable fraction in soil [51].

13.4 Phytoextraction of Heavy Metals

Phytoextraction is the main and most useful phytoremediation technique for removal of heavy metals and metalloids from polluted soils, sediments, or water [65–67]. It is the most promising for commercial application [68]. The efficiency of phytoextraction depends on many factors like bioavailability of the heavy metals in soil, soil properties, speciation of the heavy metals, and plant species concerned. Plants suitable for phytoextraction should ideally have the following characteristics [69–73]:

1. High growth rate.
2. Production of more above-ground biomass.
3. Widely distributed and highly branched root system.
4. More accumulation of the target heavy metals from soil.
5. Translocation of the accumulated heavy metals from roots to shoots.
6. Tolerance to the toxic effects of the target heavy metals.
7. Good adaptation to prevailing environmental and climatic conditions.
8. Resistance to pathogens and pests.
9. Easy cultivation and harvest.
10. Repulsion to herbivores to avoid food chain contamination [74].

The phytoextraction potential of a plant species is mainly determined by two key factors, i.e. shoot metal concentration and shoot biomass [75]. Two different approaches have been tested for phytoextraction of heavy metals: (1) The use of hyperaccumulators, which produce comparatively less above-ground biomass but accumulate target heavy metals to a greater extent. (2) The application of other plants, such as *Brassica juncea* (Indian mustard), which accumulate target heavy metals to a lesser extent but produce more above-ground biomass so that overall accumulation is comparable to that of hyperaccumulators due to production of more biomass [76, 77]. According to Chaney et al. [78], hyperaccumulation and hypertolerance are more important in phytoremediation than high biomass. Use of hyperaccumulators will yield a metal-rich, low-volume biomass, which is economical and easy to handle in case of both metal recovery and safe disposal. On the other hand, use of non-accumulators will yield a metal-poor, large-volume biomass, which will be uneconomical to process for recovery of metals and also costly to safely dispose. Plants, which offer multiple harvests in a single growth period (like *Trifolium* spp.), can have a great potential for phytoextraction of heavy metals [74]. Grasses are more preferable for phytoextraction than shrubs or trees because of their high growth rate, more adaptability to stress environment and high biomass [79]. Some researchers have evaluated the use of crops (such as maize and barley) for phytoextraction of heavy metals. In this case, several croppings are required to lower heavy metal contamination to acceptable levels. However, the use of crops for phytoextraction of heavy metals suffers from the disadvantage of contamination of food chain. According to Vamerali et al. [80], the use of field crops for phytoremediation purposes should not consider the use of products for animal feed or direct human consumption [74].

13.4.1 Types of Phytoextraction

13.4.1.1 Natural Phytoextraction

In the natural setting, certain plants have been identified which have the potential to uptake heavy metals. At least 45 families have been identified to have hyperaccumulate plants; some of the families are *Brassicaceae, Fabaceae, Euphorbiaceae, Asteraceae*, *Lamiaceae*, and *Scrophulariaceae* [23, 39]. Among the best-known hyperaccumulators is *Thlaspi caerulescens* commonly known as alpine pennycress [81], without showing injury it accumulated up to 26,000 mg kg^{-1} Zn; and up to 22 % of soil exchangeable Cd from contaminated site [82, 83]. *Brassica juncea*, commonly called Indian mustard, has been found to have a good ability to transport lead from the roots to the shoots. The phytoextraction coefficient for *Brassica juncea* is 1.7 and it has been found that a lead concentration of 500 mg/L is not phytotoxic to *Brassica* species [62]. Phytoextraction coefficient is the ratio of the metal concentration found within the surface biomass of the plant over the metal concentration found in the soil. Some calculations indicate that *Brassica juncea* is capable of removing 1550 kg of lead per acre [62]. On a worldwide basis, concentrations >1000 mg kg^{-1} are known for Ni in more than 320 plant species (sps.), Co (30 sps.), Cu (34 sps.), Se (20 sps.), Pb (14 sps.), and Cd (one sp.). The species involved in hyperaccumulation have recently been tabulated by Reeves and Baker [84], substantial number of these species are from Congo and Zaire. Concentration exceeding 10,000 mg kg^{-1} has been recorded for Zn (11 sps.) and Mn (10 sps.). The hyperaccumulation threshold levels of these elements have been set higher because their normal range in plants (20–500 mg kg^{-1}) is much higher than for the other heavy metals [85]. Aquatic plants such as the floating *Eichhornia crassipes* (water hyacinth), *Lemna minor* (duckweed), and *Azolla pinnata* (water velvet) have been investigated for use in rhizofiltration, phytodegradation, and phytoextraction [56]. Farago and Parsons [86] reported the bioremoval of platinum using *Eichhornia crassipes*. Many aquatic plants are used in the bioremoval of heavy metals, e.g. *Azolla filliculoides*, *A. pinnata*, *Typha orientalis*, and *Salvinia molesta*. Jin-Hong et al. in their study of twelve wetland species reported, *Polygonum hydropiperoides* Michx (smartweed) as the best for heavy metal phytoremediation, due to its faster growth and high plant density [87]. Recently, a fern *Pteris vitatta* has been shown to accumulate as much as 14,500 mg kg^{-1} arsenic in fronds without showing symptoms of toxicity [51, 88].

13.4.1.2 Induced Phytoextraction or Chelate-Assisted Phytoextraction

Within the plant cell heavy metal may trigger the production of oligopeptide ligands known as phytochelatins (PCs) and metallothioneins (MTs) [15]. These peptides bind and form stable complex with the heavy metal and thus neutralise the toxicity of the metal ion [89]. Phytochelatin (PCs) is synthesised with glutathione as building blocks resulting in a peptide with structure Gly–(y–Glu–Cys–)n (where, $n = 2–11$). Appearance of phytochelating ligands has been reported in hundreds of plant species exposed to heavy metals [90]. Metallothioneins (MTs) are small gene encoded, Cys-rich polypeptides. PCs are functionally equivalent to MTs [89]. Chelators have been isolated from plants that are strongly involved in the uptake of heavy metals and their detoxification. Chelating agents like ethylenediamine tetra acetic acid (EDTA) are applied to Pb-contaminated soils that increase the amount of bioavailable lead in the soil and a greater accumulation in plants is observed [91]. The addition of chelates to a lead-contaminated soil (total soil Pb 2500 mg kg^{-1})

increased shoot lead concentration of *Zea mays* (corn) and *Pisum sativum* (pea) from less than 500 mg kg^{-1} to more than 10,000 mg kg^{-1}. This was achieved by adding synthetic chelate EDTA to the soil, similar results using citric acid to enhance uranium uptake have been documented. These results indicate that chelates enhanced or facilitated Pb transport into the xylem and increased lead translocation from roots to shoots. For the chelates tested, the order of effectiveness in increasing Pb desorption from the soil was EDTA > Hydroxyethylethylene-diaminetriacetic acid (HEDTA) > Diethylenetriaminepentaaceticacid (DTPA) > Ethylenediamine di(o-hyroxyphenylacetic acid) EDDHA [91]. Vassil et al. [92] reported that *Brassica juncea* exposed to Pb and EDTA in hydroponic solution was able to accumulate up to 55 mM kg^{-1} Pb in dry shoot tissue (1.1 % [w/w]). This represents a 75-fold concentration of lead in shoot over that in solution. A threshold conc. of EDTA (0.25 mM) was required to stimulate this dramatic accumulation of both lead and EDTA in shoots.

Despite the possible usefulness of chelating agents, some concerns have been expressed regarding the potential inherent risk of leaching of metals to groundwater. The addition of chelates to a metal-contaminated matrix can increase the levels of the water extractable metals. For example, the use of EDTA to a metal-contaminated soil has been reported to increase significantly the concentrations of Cd, Zn, and Pb in soil solution [93, 94]. A similar trend was also found for EDDS, but this had a reduced risk of metal leaching than that of EDTA, in groundwater of contaminated soils [95–98]. It is reported that Zn concentration in water extracts of soils collected at the time of plant harvest was significantly increased by addition of EDTA or EDDS, by up to 4.0- and 3.1-fold, respectively [98]. Additionally, synthetic chelating agents at high concentrations can also be toxic to plants. Chlorosis, necrosis, and impairment of plant growth have been reported for plants growing in soils amended with EDTA, EDDS, and NTA [14, 95, 97, 99]. Moreover, the presence of these chelates can reduce the occurrence and number of microorganisms in the rhizosphere [98]. Chelates, especially EDTA, can greatly reduce the number of microbivorous nematodes and increases the stress index of microbial populations [100]. Further, Marques et al. [98] observed that addition of EDTA to Zn-contaminated soils promoted a decrease in root colonisation of *Solanum nigrum* by arbuscular mycorrhizal fungi (AMF). It is also reported that addition of EDTA and EDDS to a metal-contaminated soil reduced AMF colonisation of *Zea mays* and *Nicotiana tabacum* [95, 97, 99, 101, 102].

13.4.1.3 Types of Metal-Binding Peptides

The most important peptides/proteins involved in metal accumulation and tolerance are phytochelatins (PCs) and metallothioneins (MTs). Plant PCs and MTs are rich in cysteine sulfhydryl groups, which bind and sequester heavy metal ions in very stable complexes [103].

13.4.1.4 Phytochelatins

PCs are small glutathione-derived, enzymatically synthesised peptides, which bind metals and are principal part of the metal detoxification system in plants [104–107]. They have the general structure of (c-glutamyl-cysteinyl)n-glycine where $n = 2–11$ [108].

They are produced by the enzyme phytochelatin synthase [109]. PC synthase is activated by various heavy metal ions with in vivo induction of PCs [15]. Mutants of *Arabidopsis thaliana* that lack PC-synthase are unable to synthesise PCs and are hypersensitive to Cd and Hg [110, 111]. The accumulation of Pb^{2+} in the aquatic fern *Salvinia minima* caused changes in the expression of the SmPCS gene. Consequently, in vivo PCS (phytochelatin synthase) activity and PC production were increased in roots and to a lower extent in leaves [112]. MTs are gene-encoded, low molecular weight, metal-binding proteins, which can protect plants against the effects of toxic metal ions [105, 107, 113, 114]. By overexpression of natural chelators (PCs, MTs, and organic acids), not only metal ions' entrance into plant cell but also translocation through xylem is facilitated [115].

Modification or overexpression of GSH (glutathione) and PCS gene has significant potential for increasing heavy metal accumulation and tolerance in plants [20]. Studies are in progress to identify, isolate, and characterise the biomolecules involved in the cross membrane transport and vacuolar sequestration of heavy metals in plants. Advancement in such molecular studies will greatly help to improve our understanding of the complete mechanism of metal uptake, translocation, and tolerance in plants, which in turn will help to enhance the efficiency of phytoremediation [74].

13.4.1.5 PC Biosynthesis

The understanding of the PCs biosynthetic pathway is an important aspect for the improvement of phytoremediation technology. It is evident that regulation of PCS activity is likely to be the most important regulatory mechanisms of PCs biosynthetic pathway [116].

PCs are structurally related to glutathione (GSH; γ-GluCysGly), and numerous physiological, biochemical, and genetic studies have confirmed that GSH (or, in some cases, related compounds) is the substrate for PC biosynthesis (14, 58, 59, 84). In particular, genetic studies have confirmed that GSH-deficient mutants of *S. pombe* as well as *Arabidopsis* are PC deficient and hypersensitive to Cd. A list of mutants that identify a role for particular genes in PC biosynthesis or function is shown in Table 13.2.

It was observed that PCs biosynthesis occurred within minutes of exposure to Cd and was independent of *de novo* protein synthesis in the cell cultures of *Silene cucubalis* [117] and *Arabidopsis* [118]. The most important thing is that PCs biosynthesis probably varies between different plant species, being regulated at transcriptional or posttranscriptional levels, or both and this suggests that PCS activity is regulated differently in different plant species. In this direction it was observed in transgenic of Indian mustard that an increase in the expression of enzymes of the -GSH biosynthetic

Table 13.2 Mutants affected in phytochelatin biosynthesis and function

Phytochelatin biosynthesis			
Gene locus	Activity/function	Organism	References
Gsh1	γ-Glutamylcysteine synthetase/GSH biosynthesis	*S. pombe*	Mutoh and Hayashi [171], Glaser et al. [172]
CAD2/RML1	γ-Glutamylcysteine synthetase/GSH biosynthesis	*A. thaliana*	Cobbett [15],Vernoux et al. [173],
Gsh2	Glutathione synthetase/GSH biosynthesis	*S. pombe*	Mutoh and Hayashi [171], Glaser et al. [172]
CAD1	Phytochelin synthetase/ Phytochelatin biosynthesis	*A. thaliana*	Howden et al. [118]
Pcs1	Phytochelin synthetase/ Phytochelatin biosynthesis	*S. pombe*	Clemens et al. [122], Ha et al. [124]
Pcs1	Phytochelin synthetase/ Phytochelatin biosynthesis	*Candida albicans*	Vatamaniuk [129]
Phytochelatin function			
Hmt1	PC-Cd vacuolar membrane ABC-type transporter	*S. pombe*	Qadir et al. [140], Ortiz et al. [141]
Ade2, 6, 7, 8	Metabolism of cysteine sulfinate to products involved in sulphide biosynthesis; also required for adenine biosynthesis	*S. pombe*	Juang [165], Speiser et al. [164]
Hmt2	Mitochondrial sulphide: quinone oxidoreductase/detoxification of sulphide	*S. pombe*	van Hoof et al. [174]
Hem2	Porphobilinogen synthase/siroheme biosynthesis (cofactor for sulphite reductase)	*Candida albicans*	Hunter et al. [166]

pathways led to an increase in PCs biosynthesis and Cd tolerance [119]. However, wild-type Indian mustard responded in different way, where increased Cd exposure, led to the increased levels of γ-glutamylcysteine synthetase (γ-ECS) transcript, which codes for the first enzyme in the PCs biosynthetic pathway [120]. Further, the identification of PCS activity has been carried out about a decade ago but the identification of PCS gene remained elusive. Phytochelatin synthetase genes were first isolated almost simultaneously by two research groups. The first group identified an *Arabidopsis* cDNA, named *AtPCS1* and expression of *AtPCS1* protein helped in significant level of Cd accumulation in plants [121]. This observation is also suggesting a possible role of *AtPCS1* in Cd chelation. A second group of researchers [122] identified a wheat cDNA, named *TaPCS*, which increased Cd resistance in wild-type yeast. Similar to *AtPCS1*, the expression of *TaPCS1* was -GSH dependent and the Cd resistance mediated by *TaPCS1* was associated with an increase in Cd accumulation. It was found that both *AtPCS1* and *TaPCS1* function only via -GSH-dependent manner and helpful in Cd tolerance even in vacuole-deficient mutants in yeast [119]. The first evidence about the role of PCs in metal accumulation comes from a transgenic of Indian mustard and bacteria in which metal hyperaccumulation comes from the overexpression of a bacterial glutathione synthetase, which is an enzyme for -GSH synthesis [119]. These transgenics were capable for synthesis of higher amount of -GSH and PCs relatively to controls. It was also reported that overexpression of PCS gene in transgenic yeast mediated the enhanced level of Cd tolerance and accumulation [121]. Based on earlier studies, it is evident that PCs are playing very important role in phytoremediation technology because they are responsible for metal accumulation, tolerance, and detoxification in plants. It is reported that PCs not only have high metal-binding capacity but also strong scavenging activity against ROS such as $H_2O_2$and $O_2^{\cdot-}$ [123]. They have a higher metal-binding capacity than do MTs on a per-cysteine basis [117]. Therefore, modification or overexpression of -GSH and PCS gene has significant potential for increasing the tolerance and accumulation of toxic heavy metals in plants [102].

13.4.1.6 Identification of PC Synthase Genes

The enzyme catalysing the biosynthesis of PCs from GSH, phytochelatin synthase, was first characterised by Grill et al. [117] in 1989. However, it was not until 1999 that the cloning of PC synthase genes was described. The PC synthase gene was first identified genetically in Arabidopsis. Cd-sensitive, *cad1*, mutants are PC deficient but have wild-type levels of GSH. They also lack PC synthase activity, suggesting a defect in the PC synthase gene [118]. PC synthase genes were isolated simultaneously by three research groups using different approaches. Two groups used expression of Arabidopsis and wheat cDNA libraries in *S. cerevisiae* to identify genes (*AtPCS1* [121] and *TaPCS1* [122], respectively) conferring increased Cd resistance. The third group identified *AtPCS1* through the positional cloning of the *CAD1* gene of Arabidopsis [124]. A similar sequence was identified in the genome of *S. pombe*, and targeted deletion mutants of that gene are, like Arabidopsis *cad1* mutants, Cd sensitive and PC deficient, confirming the analogous function of the two genes in the different organisms. Heterologous expression of the *CAD1*/*AtPCS1* and *SpPCS* genes (25) or purification of epitope-tagged derivatives of SpPCS [121] and AtPCS1 [120] was used to demonstrate that both were necessary and sufficient for GSH-dependent PC biosynthesis in vitro. This combination of genetic, molecular, and biochemical data was a conclusive demonstration that these genes encode PC synthase. There is a second PC synthase gene, *AtPCS2*, in Arabidopsis with significant identity to *CAD1*/*AtPCS1* [124]. This was an unexpected finding because PCs were not detected in a *cad1* mutant after prolonged exposure to Cd, suggesting the presence of only a single active PC synthase in wild type [117]. *AtPCS2* is transcribed, and expression experiments have demonstrated it encodes a functional PC synthase enzyme. The physiological function of this gene remains to be determined. In most tissues *AtPCS2* is expressed at a relatively low level compared with *AtPCS1*. However, because AtPCS2 has been preserved as a functional PC synthase through evolution, it must presumably be the predominant PC synthase in some tissue(s) or environmental conditions, thereby conferring a selective advantage. Full-length or partial cDNA clones encoding presumptive PC synthases have also been isolated from other plant species, including *Brassica juncea* and rice (Table 13.3).

Table 13.3 Characterisation of PC synthase gene in plants

Phytochelatin synthase gene characterised	MW (kDa)	Number of Cys residues	Organism
AtPCS1	54.4	10	*Arabidopsis thaliana*
Atpcs2	51.5	7	*Arabidopsis thaliana*
BjPCS1	54.3	11	*Brassica juncea*
TaPCS1	55.0	14	Wheat
OsPCS1	55.6	12	Rice
SpPCS1	46.7	6	*Schizosaccharomyces pombe*
CaPCS1	42.1	11	*Caenorhabditis elegans*
DdPCS1	70.5	9	*Dictyostelium discoideum*

13.4.1.7 Some Animals Express a PC Synthase

Through the history of studies of heavy metal detoxification in animals there has been no evidence for the presence of PCs. Thus, it came as a surprise when database searches identified similar genes in the nematodes *Caenorhabditis elegans* and *C. briggsae* and in the slime mould *Dictyostelium discoideum*. In addition, using polymerase chain reaction, investigators have identified partial sequences with homology to the plant and yeast PC synthase genes from the aquatic midge, *Chironomus*, and earthworm species. Recent work has demonstrated that the *C. elegans* and *D. discoideum* genes encode PC synthase activity. *CePCS1* was able to rescue either a Cd-sensitive *ycf1* mutant of *S. cerevisiae* or a PC synthase-deficient mutant of *S. pombe* and catalysed PC biosynthesis in vivo in both heterologous hosts and in vitro [103, 125]. Similarly, expression of the *D. discoidium* PC synthase in *S. cerevisiae* is also able to catalyse PC biosynthesis in vivo and confer increased Cd resistance. Significantly, the suppression of *CePCS1* expression by using the double-stranded RNA interference technique resulted in Cd sensitivity, thereby demonstrating an essential role for PCs in heavy metal detoxification in *C. elegans* [125]. This shows clearly that PCs play a wider role in heavy metal detoxification in biology than previously expected. In contrast, it appears that some organisms do not (or probably do not) express a PC synthase. There is, for example, no evidence for PC synthase—homologous sequences in the *S. cerevisiae*, *Drosophila melanogaster*, or mouse and human genomes. One view of the limited selection of species in which such sequences have been identified is that organisms with an aquatic or soil habitat are more likely to express PCs. However, the recent report of partial PC synthase–homologous ESTs in, for example, the mosquito-borne parasitic nematode *Brugia malayi* [125] undermines this simplistic categorisation [104].

13.4.1.8 PC Synthase Enzymes and Their Regulation

The predicted molecular weights of various PC synthase enzymes deduced from DNA sequences are given in Table 13.3 and range from 42 to 70 kDa, and comparison of the deduced amino acid sequences shows that the N-terminal regions of the plant, yeast, and animal PC synthases are very similar (40–50 % identical), whereas the C-terminal sequences show little apparent conservation of amino acid sequence. The most apparent common feature of the C-terminal regions is the occurrence of multiple Cys residues, often as adjacent pairs or near pairs. The arrangements of Cys residues are reminiscent of those found in MTs. The C-terminal regions of the *Arabidopsis* and *S. pombe* PC synthase proteins, for example, have 10 and 7 Cys residues, respectively, of which 4 and 6, respectively, are as adjacent pairs. However, there is no apparent conservation of the positions of these Cys residues relative to each other. In contrast, monocot (TaPCS1) and dicot (AtPCS1) plant PC synthase sequences can be aligned across their entire length (55 % identity) [121]. The former contains 14 Cys residues, including two pairs, in the C-terminal domain. The *S. pombe* and *D. discoideum* sequences also contain N-terminal extensions, which in the latter also contains clusters of Cys residues that may play a role similar to the C-terminal Cys clusters. When a PC synthase activity was first identified (from cultured cells of *Silene cucubalis*) it was characterised as a γ-GluCys dipeptidyl transpeptidase (EC2.3.2.15) [116]. It catalysed the transpeptidation of the γ-GluCys moiety of GSH onto a second GSH molecule to form PC_2 or onto a PC molecule to produce a +1 oligomer. The enzyme was described as a tetramer of Mr 95,000 with a K_m for GSH of 6.7 mM. The MW of this purified enzyme seems inconsistent with the MWs of the PC synthase sequences deduced from both dicot and monocot plant genes (Table 13.3). Furthermore, there is no evidence that cloned plant PC synthase enzymes are multimeric. This suggests a protein mixture may have been purified from *S. cucubalis* and that the PC synthase activity was not the major component detected in MW determinations. PC synthase activities have also been detected in pea [126], tomato [127], and *Arabidopsis* [117].

In vivo studies have shown that PC synthesis can be induced by a range of metal ions in *S. pombe* and in both intact plants and plant cell cultures [128]. Kinetic studies using plant cell cultures demonstrated that PC biosynthesis occurs within minutes of exposure to Cd and is independent of de novo protein synthesis. The enzyme appears to be expressed independently of heavy metal exposure. It has been detected in extracts of plant cell cultures or tissues grown in the presence of only trace levels of essential heavy metals. Together, these observations indicate that PC synthase is primarily regulated by activation of the enzyme in the presence of heavy metals. In vitro, the partially purified enzyme from *S. cucubalis* was active only in the presence of a range of metal ions. The best activator tested was Cd, followed by Ag, Bi, Pb, Zn, Cu, Hg, and Au cations. This result has been mirrored by in vitro studies of PC synthase expressed in *E. coli* or in *S. cerevisiae*, where the enzyme was activated to varying extents by Cd, Cu, Ag, Hg, Zn, and Pb ions [120, 121, 124]. Early models for the activation of PC synthase assumed a direct interaction between metal ions and the enzyme but raised the question of how the enzyme might be activated by such a wide range of metals. A significant recent study has provided evidence for an alternative model that provides a solution to this dilemma [129].

With the cloning of PC synthase genes, the expression and purification of tagged recombinant derivatives of the enzyme have led to a more comprehensive understanding of the mechanisms of enzyme activation and catalysis. Vatamaniuk et al.

[129] demonstrated that, in contrast to earlier models of activation, metal binding to the enzyme, per se, is not responsible for catalytic activation. Although AtPCS1 binds Cd ions at high affinity ($K_d = 0.54 \pm 0.20$ μM) and high capacity (stoichiometric ratio = 7.09 ± 0.94) [120], it has a much lower affinity for other metal ions, such as Cu, which are equally effective activators. The kinetics of PC synthesis are consistent with a mechanism in which heavy metal glutathione thiolate (e.g. Cd.GS2) and free GSH act as γ-Glu-Cys acceptor and donor. First, modelling using the known binding constants of GSH and Cd showed that, in the presence of physiological concentrations of GSH and ^{1}M concentrations of Cd, essentially all of the Cd would be in the form of a GSH thiolate. Second, S-alkylglutathiones can participate in PC biosynthesis in the absence of heavy metals. These observations are consistent with a model in which blocked glutathione molecules (metal thiolates or alkyl substituted) are the substrates for PC biosynthesis. Thus, the role of metal ions in enzyme activation is as an integral part of the substrate, rather than interacting directly with the enzyme itself. In this way, any metal ions that form thiolate bonds with GSH may have the capacity to activate PC biosynthesis, subject to possible steric constraints in binding at the active site of the enzyme. Early work suggested that PC biosynthesis in vitro was ultimately terminated by the PC products chelating the activating metal ions (or could be prematurely terminated by the addition of a metal chelator such as EDTA) [130], which provides a mechanism to autoregulate the biosynthesis of PCs. Viewed from a perspective where the metal ion forms part of the substrate, termination of the reaction results simply from exhaustion of substrate. The conserved N-terminal domain of PC synthase is presumed to be the catalytic domain. The *cad1-5* mutation of Arabidopsis is a non-sense mutation that would result in premature termination of translation downstream of the conserved domain [124]. The truncated polypeptide would lack 9 of the 10 Cys residues in the C-terminal domain. Of all the *cad1* mutants characterised, *cad1-5* is the least sensitive to Cd and makes the highest residual level of PCs on exposure to Cd [117]. This suggests that the C-terminal domain is not absolutely required for catalysis. Because the work of Vatamaniuk et al. [129] suggests that heavy metal "activation" of PC synthase is in fact an integral component of catalysis, what then is the role of the multiple Cys residues in the variable C-terminal domain? Because the truncation of the *cad1-5* mutant polypeptide produces a mutant phenotype, the C-terminal domain clearly has some role in activity.

This domain probably acts to enhance activity by binding metal glutathione complexes, bringing them into closer proximity to the catalytic domain. Studies indicating PC synthase is expressed constitutively and levels of enzyme are generally unaffected by exposure of cell cultures or intact plants to Cd suggest the induction of PC synthase gene expression is unlikely to play a significant role in regulating PC biosynthesis. This is supported by analysis of the expression of *AtPCS1*/*CAD1* that showed that levels of mRNA were not influenced by exposure of plants to Cd and other metals, thus suggesting an absence of regulation at the level of transcription [117, 129]. In contrast, analysis of *TaPCS1* expression in roots indicated increased levels of mRNA on exposure to Cd [121]. This suggests that, in some species, PC synthase activity may be regulated at both the transcriptional and posttranslational levels. Little is known about the tissue specificity of PC synthase expression and/or PC biosynthesis. In a study in tomato, activity was detected in the roots and stems of tomato plants but not in leaves or fruits [104, 127].

13.4.1.9 Modification in the Biosynthesis of PCs

With the aim of creating plants that can tolerate and accumulate high levels of toxic metals, much effort has been focused on introducing mammalian MTs that are known to be involved in the detoxification of metals in mammals into plants. However, in plants PCs are the main detoxifiers by chelation of the metal. Modification or overexpression of the enzymes that are involved in the synthesis of glutathione and PCs might be a good approach to enhance heavy metal tolerance and accumulation in plants [69].

13.5 Role of Phytochelatins in Phytoremediation

No doubt the HMs exerts adverse effects on all living cells, but at the same time, they are also essential for the normal growth and development. The excess of metal ions causes death of cells, and thus can be overcome by homeostasis (regulate the metals within the cell). Plant cells have developed one general mechanism to achieve this goal, i.e. through synthesis of PCs. The process involves the chelation of metal ions by specific high-affinity ligands that reduce the concentration of free metal ions, and consequently decrease their phytotoxicity. A number of metal binding ligands have now been recognised in plants [15, 69]. PC formation is selected as biomarkers for cellular metal sequestration [131–134], because the genetic analysis has provided the direct evidence for PC involvement in metal detoxification [124, 135, 136]. PCs found in cytosol possess great affinity for binding with different metals, especially Cd. These complexes formed in the vacuole undergo sequestration, i.e. they keep the metals away from enzymes that are susceptible [137]. This system provides an environmentally well-conserved mechanism to deal with metal toxicity [15]. The major detoxification mechanism(s) in plants is based on vacuolar compartmentalisation and ligand complexation. Prolonged cadmium exposures cause a significant increase in PC synthetase (enzyme for synthesis of PC in leaves of *B. juncea*) [138]. High levels of PCs were identified in the phloem sap of *B. napus* within 24 h of Cd exposure using combined mass spectrometry and fluorescence high-performance liquid chromatography (HPLC) analysis [139]. In general, while vacuolar compartmentalisation keeps HMs away from metal sensitive metabolic centres in the cytoplasm, sequestration ligands seem to safeguard them from readily moving by reducing their chemical selectivity. For an effective and efficient internal metal tolerance, both roles are important. While the mechanism of low molecular weight endogenous or induced organic acids, particularly citrate, may be employed by plants as a strategy to detoxify the low-level exposure of HMs, an additional mechanism of producing large molecules and more specific compounds such as PCs may be employed by plants to combat high-level exposure to HMs. Although production of PCs may be a mechanism that plants employ in internal detoxification of HM, yet it is unlikely that such is the only mechanism in HM tolerance. This is because induction of PCs is associated with exposure to high external metal concentrations. Apparently, at relatively low metal exposure, plants employ mechanism(s) other than PC production to tolerate the metal stress internally [140].

13.5.1 Sequestration to the Vacuole

In both plant and yeast, PC–Cd complexes are sequestered to the vacuole. In *S. pombe*, this process has been most clearly demonstrated through studies of the Cd-sensitive mutant *hmt1*. In extracts of *S. pombe*, two PC–Cd complexes (referred to as HMW and LMW) can be clearly resolved using gel-filtration chromatography. The *hmt1* mutant is unable to form the HMW complexes. The *Hmt1*gene encodes a member of the family of ATP-binding cassette (ABC) membrane transport proteins that is located in the vacuolar membrane [140]. Both HMT1 and ATP are required for the transport of LMW PC–Cd complexes into vacuolar membrane vesicles [141]. In *S. cerevisiae*, which appears not to express a PC synthase, YCF1 is also a member of the ABC family of transporters and carries (GSH)2Cd complexes to the vacuole [142]. Interestingly, in *C. elegans*, various mutations affecting ABC transporter proteins also confer heavy metal sensitivity [143]. It is possible that these transporters are involved in the sequestration of PC metal complexes in *C. elegans*. The site of such sequestration is still unidentified. In plants, sequestration of PCs to the vacuole has also been observed. In mesophyll protoplasts derived from tobacco plants exposed to Cd, almost all of both the Cd and PCs accumulated was confined to the vacuole [144]. An ATP-dependent, proton gradient-independent activity, similar to that of HMT1, capable of transporting both PCs and PC–Cd complexes has been identified in oat roots [145]. Nonetheless, a plant gene encoding this function has not yet been identified. A recent inventory [146] of the ABC transporter protein genes in the Arabidopsis genome has not revealed a clearly identifiable homologue of HMT1 [105].

13.5.2 Formation of Metal Complex by Phytochelatins

Chelation of HMs in the cytosol by high-affinity ligands is potentially a very important mechanism of HM detoxification and tolerance in plants under HM stress. PCs are synthesised from GSH; the metal binds to the constitutively expressed enzyme γ-glutamylcysteinyl dipeptidyl transpeptidase (PC synthase), thereby activating it to catalyse the conversion of GSH to phytochelatin [147]. The biosynthesis of PCs is induced by many HMs, including Cd, Hg, Ag, Cu, Ni, Au, Pb, As, and Zn; however, Cd is by far the strongest inducer [89, 117]. PCs complex Cd ions through the thiolic group(-SH) of Cys and the PC–Cd complexes are accumulated in the vacuole through the activity of ABC transporters, thus limiting the circulation of free Cd^{2+} inside the cytosol [148]. Additionally, plants are not able to metabolise or eliminate Cd. Rather, they adopt the strategy of making Cd-GSH and Cd–PCs complexes to sequester Cd within vacuoles efficiently [148, 149] and also to transport Cd over a long distance through xylem and phloem vessels [139]. Further confirmation of the induction of PCs under HM stress was provided by the fact that PC production was found to be positively correlated with HM accumulation in both above-ground and below-ground tissues. Hossain et al. [150] studied water hyacinth (*Eichhornia crassipes*) with Cd stress and found that Cd^{2+} taken up by water hyacinth roots was present as Cd-binding complexes whereas, in the absence of Cd^{2+}, no such complexes were observed [151].

This indicates that these Cd^{2+}-binding complexes are formed in response to Cd^{2+} and play an important role in the accumulation of Cd in water hyacinth. Next, they characterised that the latter Cd-binding complex with a molecular weight of 4000 was composed of two cadystins A, (γ-Glu-Cys)3-Gly, two cadystins B, (γ-Glu-Cys) 2-Gly, and inorganic sulphur, which is identical to the fission yeast Cd-BPI [152]. Iglesia-Turiño et al. [153] studied Hg accumulation in rape (*Brassica napus*) plants grown under an Hg concentration gradient (0–1,000 μM) and found that Hg accumulation was strongly correlated with PC2 concentration. PCs are also involved in the homeostasis of Zn^{2+} and Cu^{+}/Cu^{2+} by providing a transient storage form for the ions [154, 155]. The induction of PCs by the anion arsenate has been observed in a survey for peptide-inducing metal ions [89] and suggests a unique mode of PC synthase activation. Nouairi et al. [156] observed that Cd treatment registered a consistent increase in PC synthesis up to 50 μM (94.66 $\mu mol\ g^{-1}$ FW) in *Brassica juncea* leaves with respect to the control (10.57 $\mu mol\ g^{-1}$ FW). However, after a 15-day exposure, the PC content in *B. napus* leaves was not significantly different to the control at any concentration of Cd. In addition, phytochelatin synthase (PCS) gene expression studies in garlic (*Allium sativum* L.) plants exposed to HMs [157] and an in vitro analysis in the marine alga *Dunaliela tertiolecta* [123] also suggested a role of PCs in the detoxification of HMs and in the mitigation of oxidative stress. Both HM-resistant and HM-sensitive plants produce PCs; however, several reports have concluded that PCs are not primary responsible for the hyperaccumulation of Zn, Ni, or Pb [158–160]. The HM-detoxification process is not limited to the chelation of HM ions. After the activation of PC synthase by the HM ions and HM chelation by the PCs synthesised, the HM ion complex is transported to the vacuole and stabilised there by forming a complex with sulphides or organic acid [90]. It has also been shown that PCs have a role in HM transports [145], so that their detoxifying capabilities may actually be secondary or part of a more complex mechanism. Although it is clearly demonstrated that PCs can have an important role in HM detoxification and accumulation in higher plants, formation of HM complexes provides insufficient explanation for either the HM specificity or species specificity of hyperaccumulation [161]. Therefore, it remains to be determined what exact role PCs play in the HM-tolerance mechanism at the cellular level and this requires more thorough research [150].

13.5.3 Sulphide Ions and PC Function

In some plants and yeasts *S. pombe* and *Candida glabrata*, sulphide ions play an important role in the efficacy of Cd detoxification by PCs. HMW PC–Cd complexes contain both Cd and acid-labile sulphide. The incorporation of sulphide into the HMW complexes increases both the amount of Cd per molecule and the stability of the complex. Some complexes with a comparatively high ratio of S2¡:Cd consist of aggregates of 20 Å-diameter particles, which themselves consist of a CdS crystallite core coated with PCs [162, 163]. The analysis of Cd-sensitive mutants of *S. pombe* deficient in PC–Cd complexes has provided evidence for the importance of sulphide in the function of PCs. These include some mutants affected in steps in the adenine biosynthetic pathway [164]. Juang et al. [165] have shown that these enzymes, in addition to catalysing the conversion of aspartate to intermediates in adenine biosynthesis, could also utilise cysteine sulfinate, a sulphur-containing analogue of aspartate, to form other sulphur containing compounds, which may be intermediates or carriers in the pathway of sulphide incorporation into HMW complexes. Whether sulphide is involved in the detoxification of other metal ions by PCs is unknown. Using other Cd-sensitive mutants of *S. pombe* and *Candida glabrata*, investigators have identified additional functions important in sulphide metabolism. In *C. glabrata*, the *hem2* mutant is deficient in porphobilinogen synthase, which is involved in siroheme biosynthesis [166]. Siroheme is a cofactor for sulphite reductase required for sulphide biosynthesis. This deficiency may contribute to the Cd-sensitive phenotype. However, additional studies are required to establish the precise influence of this pathway on PC function. In *S. pombe*, the *hmt2* mutant hyperaccumulates sulphide in both the presence and absence of Cd [167]. The *HMT2* gene encodes a mitochondrial sulphide:quinone oxidoreductase, which was suggested to function in the detoxification of endogenous sulphide. The role of HMT2 in Cd tolerance is uncertain, but one possibility is that it detoxifies excess sulphide generated during the formation of HMW PC–Cd complexes after Cd exposure [105].

13.5.4 Metals Other than Cd

Although both induction of PCs in vivo and activation of PC synthase in vitro are conferred by a range of metal ions, there is little evidence supporting a role for PCs in the detoxification of such a wide range of metal ions. For metals other than Cd, there are few studies demonstrating the formation of PC–metal complexes either in vitro or in vivo. PCs can form complexes with Pb, Ag, and Hg in vitro (for example, see [90, 168]). Maitani et al. [169] used inductively coupled plasma-atomic emission spectroscopy in combination with HPLC separation of native

PC-metal complexes in the roots of *Rubia tinctorum*. PCs were induced to varying levels by a wide range of metal ions tested. The most effective appeared to be Ag, arsenate, Cd, Cu, Hg, and Pb ions. However, the only PC complexes identified in vivo were with Cd, Ag, and Cu ions. PC complexes formed in response to Pb and arsenate, but these complexes contained copper ions and not the metal ion used for induction of synthesis. This seems to conflict with the model for PC synthase activity whereby a metal-GSH thiolate is the substrate for PC-metal biosynthesis. It may indicate that some metals in complexes with PCs can be exchanged for others. In contrast, however, Schmöger et al. [170] have clearly demonstrated the formation of PC–As complexes in vivo and in vitro. The clearest evidence for the role of PCs in heavy metal detoxification comes from characterisation of the PC synthase–deficient mutants of *Arabidopsis* and *S. pombe*. A comparison of the relative sensitivity of the *Arabidopsis* and *S. pombe* mutants to different heavy metals revealed a similar but not identical pattern [124].

In both organisms, PC-deficient mutants are highly sensitive to Cd and arsenate. For other metals, including Cu, Hg, Ag, Zn, Ni, and selenite ions, the mutants showed little or no sensitivity. Suppression of PC synthase in *C. elegans* also resulted in a Cd-sensitive phenotype, but the effect on responses to other metals has not been reported [125]. Thus, PCs play a clear role in Cd and arsenate detoxification. Cu, for example, is a strong activator of PC biosynthesis both in vivo and in vitro, yet PC-deficient mutants show relatively little sensitivity to Cu. PCs also form complexes with Cu in vivo. It is possible, nonetheless, that PC–Cu complexes are relatively poorly sequestered to the vacuole, that they are comparatively transient, or that there is an alternative, more effective, mechanism for Cu detoxification [105].

13.6 Conclusions

Phytoremediation technology is especially beneficial in remediating the heavy metal-contaminated soil as plants can grow in large areas, provide aesthetic value to the landscape of the contaminated sites, and may have potential of economic returns which would offset the cost involved, which is already low. Moreover, the process is environment friendly because plants uptake and accumulate the environmental contaminants within their tissues.

The potential for the use of plants for the detoxification or “phytoremediation” of polluted environments is being increasingly examined. The manipulation of PC expression is one potential mechanism for increasing the capacity of plants for phytoremediation. Understanding the effect of the overexpression, possibly in a tissue-specific manner, of the genes of the GSH/PC biosynthetic pathway on metal tolerance and accumulation will soon lead to indications as to their usefulness in this endeavour. Here too, genes controlling other aspects of PC function may be required.

References

1. Hussain SI, Ghafoor A, Ahmad S, Murtaza G, Sabir M (2006) Irrigation of crops with raw sewage: Hazard assessment of effluent, soil and vegetables. Pak J Agric Sci 43:97–102
2. McGrath S, Zhao F, Lombi E (2001) Plant and rhizosphere processes involved in phytoremediation of metal-contaminated soils. Plant and Soil 232:207–214
3. Murtaza G, Ghafoor A, Qadir M, Owens G, Aziz M, Zia M (2010) Disposal and use of sewage on agricultural lands in Pakistan: A review. Pedosphere 20:23–34
4. McIntyre T (2003) Phytoremediation of heavy metals from soils, Phytoremediation. Springer, Berlin, pp 97–123
5. He Z, Yang X (2007) Role of soil rhizobacteria in phytoremediation of heavy metal contaminated soils. J Zhejiang Univ Sci B 8:192–207
6. Yang X, Feng Y, He Z, Stoffella PJ (2005) Molecular mechanisms of heavy metal hyperaccumulation and phytoremediation. J Trace Elem Med Biol 18:339–353
7. Wenzel WW (2009) Rhizosphere processes and management in plant-assisted bioremediation (phytoremediation) of soils. Plant and Soil 321:385–408
8. Baker AJ (1981) Accumulators and excluders-strategies in the response of plants to heavy metals.J. Plant Nutr 3:643–654
9. Chaudhry TM, Hayes WJ, Khan AG, Khoo CS (1998) Phytoremediation—focusing on accumulator plants that remediate metal- contaminated soils. Austr J Ecotoxicol 4:37–51
10. Lombi E, Tearall KL, Howarth JR, Zhao FJ, Hawkesford MJ, McGrath SP (2002) Influence of iron status on cadmium and zinc uptake by different ecotypes of the hyperaccumulator *Thlaspi caerulescens*. Plant Physiol 128:1359–1367
11. Sabir M, Waraich EA, Hakeem KR (2015) Öztürk M. Ahmad HR, Shahid M. Soil Remediation and Plants, http://dx.doi.org/10.1016/B978-0-12-799937-1.00004-8
12. Wu FB, Chen F, Wei K, Zhang KB (2004) Effect of cadmium on free amino acid, glutathione and ascorbic acid concentrations in two barley genotypes (*Hordeum vulgare* L.) differing in cadmium tolerance. Chemosphere 57:447–454
13. Seth CS, Chaturvedi PK (2007) MisraV. Toxic effect of arsenic and cadmium alone and in combination on Giant duckweed (*Spirodela polyrrhiza* L.) in response to its accumulation. Environ Toxicol 22:539–549
14. Seth CS, Chaturvedi PK, Misra V (2008) The role of phytochelatins and antioxidants in tolerance to Cd accumulation in *Brassica juncea* L. Ecotoxicol Environ Saf 71:76–85
15. Cobbett CS (2000) Phytochelatins and their roles in heavy metal detoxification. Plant Physiol 123:825–832
16. Wang C, Wang LY, Sun Q (2010) Response of phytochelatins and their relationship with cadmium toxicity in a floating macrophyte *Pistia stratiotes* L. at environmentally relevant concentrations. Water Environ Res 82:147–154
17. Grill E, Winnacker E-L, Zenk MH (1991) Phytochelatins. Methods Enzymol 205:333–341
18. Mishra S, Srivastava S, Tripathi RD, Kumar R, Seth CS, Gupta DK (2006) Lead detoxification by coontail (*Ceratophyllum demersum* L.) involves induction of phytochelatins and antioxidant system in response to its accumulation. Chemosphere 65:1027–1039
19. Hall JL (2002) Cellular mechanism of heavy metal detoxification and tolerance. J Exp Bot 53:1–11
20. Seth CS, Misra V, Chauhan LKS (2012) Accumulation, Detoxification, and Genotoxicity of Heavy Metals in Indian Mustard (Brassica Juncea L.). Int J Phytoremediation 14(1):1–13. doi :10.1080/15226514.2011.555799
21. Raskin I, Kumar PBAN, Dushenkov S, Salt DE (1994) Bioconcentration of heavy metals by plants. Curr Opin Biotechnol 5:285–290
22. Salt DE, Blaylock M, Kumar NPBA, Dushenkov V, Ensley BD (1995) Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants. Biotechnology 13:468–474

23. Salt DE, Smith RD, Raskin I (1998) Phytoremediation. Annu Rev Plant Physiol Plant Mol Biol 49:643–668
24. Newman LA, Strand SE, Choe N, Duffy J, Ekuan G (1997) Uptake and biotransformation of trichloroethylene by hybrid poplars. Environ Sci Technol 31:1062–1067
25. Shang TQ, Newman LA, Gordon MP (2003) Fate of tricholorethylene in terrestrial plants. In: McCutcheon SC, Schnoor JL (eds) Phytoremediation: Transformation and Control of Contaminants. Wiley, New York, pp 529–560
26. Burken JG, Schnoor JL (1997) Uptake and metabolism of atrazine by poplar trees. Environ Sci Technol 31:1399–1406
27. Hughes JB, Shanks J, Vanderford M, Lauritzen J, Bhadra R (1997) Transformation of TNT by aquatic plants and plant tissue cultures. Environ Sci Technol 31:266–271
28. Aprill W, Sims RC (1990) Evaluation of the use of prairie grasses for stimulating polycyclic aromatic hydrocarbon treatment in soil. Chemosphere 20:253–265
29. Olson PE, Reardon KF, Pilon-Smits EAH (2003) Ecology of rhizosphere bioremediation. In: McCutcheon SC, Schnoor JL (eds) Phytoremediation: Transformation and Control of Contaminants. Wiley, New York, pp 317–354
30. Schnoor JL, Licht LA, McCutcheon SC, Wolfe NL, Carreira LH (1995) Phytoremediation of organic and nutrient contaminants. Environ Sci Technol 29:318A–323A
31. Davis LC, Erickson LE, Narayanan N, Zhang Q (2003) Modeling and design of phytoremediation. In: McCutcheon SC, Schnoor JL (eds) Phytoremediation: Transformation and Control of Contaminants. Wiley, New York, pp 663–694
32. Hong MS, Farmayan WF, Dortch IJ, Chiang CY, McMillan SK, Schnoor JL (2001) Phytoremediation of MTBE from a groundwater plume. Environ Sci Technol 35:1231–1239
33. Winnike-McMillan SK, Zhang Q, Davis LC, Erickson LE, Schnoor JL (2003) Phytoremediation of methyl tertiary-butyl ether. In: McCutcheon SC, Schnoor JL (eds) Phytoremediation: transformation and control of contaminants. Wiley, New York, pp 805–828
34. Harms H, Bokern M, Kolb M, Bock C (2003) Transformation of organic contaminants by different plant systems. In: McCutcheon SC, Schnoor JL (eds) Phytoremediation: Transformation and Control of Contaminants. Wiley, New York, pp 285–316
35. Nriagu JO (1979) Global inventory of natural and anthropogenic emissions of trace metals to the atmosphere. Nature 279:409–411
36. Horne AJ (2000) Phytoremediation by constructed wetlands. In: Terry N, Bañuelos G (eds) Phytoremediation of contaminated soil and water. Lewis, Boca Raton, pp 13–40
37. Lytle CM, Lytle FW, Yang N, Qian JH, Hansen D, Zayed A, Terry N (1998) Reduction of Cr(VI) to Cr(III) by wetland plants: potential for in situ heavy metal detoxification. Environ Sci Technol 32:3087–3093
38. Blaylock MJ, Huang JW (2000) Phytoextraction of metals. In: Raskin I, Ensley BD (eds) Phytoremediation of toxic metals using plants to clean up the environment. Wiley, New York, pp 53–70
39. Dushenkov S (2003) Trends in phytoremediation of radionuclides. Plant Soil 249:167–175
40. Dushenkov S, Kapulnik Y (2000) Phytofiltration of metals. In: Raskin I, Ensley BD (eds) Phytoremediation of Toxic Metals using Plants to Clean up the Environment. Wiley, New York, pp 89–106
41. Negri MC, Hinchman RR (2000) The use of plants for the treatment of radionuclides. In: Ensley BD, Raskin I (eds) Phytoremediation of Toxic Metals. Using Plants to Clean up the Environment. Wiley, New York, pp 107–132
42. Pilon-Smits E (2005) Phytoremediation. Annu Rev Plant Biol 56:15–39
43. Gisbert C, Ros R, De Haro A, Walker DJ, Pilar Bernal M, Serrano R, Navarro-Aviñó J (2003) A plant genetically modified that accumulates Pb is especially promising for phytoremediation. Biochem Biophys Res Commun 303:440–445
44. Steinkellner H, Mun-Sik K, Helma C, Ecker S, Ma TH, Horak O, Kundi M, Knasmüller S (1998) Genotoxic effects of heavy metals: Comparative investigation with plant bioassays. Environ Mol Mutagen 31:183–191

45. Küpper H, Zhao FJ, McGrath SP (1999) Cellular compartmentation of zinc in leaves of the hyperaccumulator *Thlaspi caerulescens*. Plant Physiol 119:305–312
46. Peng K, Li X, Luo C, Shen Z (2006) Vegetation composition and heavy metal uptake by wild plants at three contaminated sites in Xiangxi area. China J Environ Sci Health A41:65–76
47. Wei S, Zhou Q, Wang X (2005) Identification of weed plants excluding the uptake of heavy metals. Environ Int 31:829–834
48. Macnair MR, Bert V, Huitson SB, Saumitou-Laprade P, Petit D (1999) Zinc tolerance and hyperaccumulation are genetically independent characters. Proc Biol Sci 266:2175–2179
49. Alkorta I, Hernández-Allica J, Becerril J, Amezaga I, Albizu I, Garbisu C (2004) Recent findings on the phytoremediation of soils contaminated with environmentally toxic heavy metals and metalloids such as zinc, cadmium, lead, and arsenic. Rev Environ Sci Biotechnol 3:71–90
50. Raskin I, Smith RD, Salt DE (1997) Phytoremediation of metals: Using plants to remove pollutants from the environment. Curr Opin Biotechnol 8:221–226
51. Ghosh M, Singh SP (2005) A Review on Phytoremediation of Heavy Metals and Utilization of It's by Products.*As. J.* Energy Env 6(04):214–231
52. United States Protection Agency Reports. Introduction to phytoremediation. EPA. 2000; 600/R-99/107.
53. Raskin I, Ensley BD (2000) Phytoremediation of Toxic Metals: Using Plants to Clean Up the Environment. Wiley, New York, pp 53–70
54. Dushenkov S, Vasudev D, Kapolnik Y, Gleba D, Fleisher D, Ting KC, Ensley B (1997) Environ Sci Tech 31(12):3468–3476
55. Rulkens WH, Tichy R, Grotenhuis JTC (1998) Remediation of polluted soil and sediment: perspectives and failures. Water Sci Technol 37:27–35
56. Salt DE, Pickering IJ, Prince RC, Gleba D, Dushenkov S, Smith RD, Raskin I (1997) Metal accumulation by aquacultured seedlings of Indian Mustard. Environ Sci Technol 31(6):1636–1644
57. Brooks RR, Chambers MF, Nicks LJ, Robinson BH (1998) Phytomining. Trends Plant Sci 1:359–362
58. Cunningham SD, Ow DW (1996) Promises and prospects of phytoremediation. Plant Physiol 110:715–719
59. Baker AJM, McGrath SP, Sidoli CMD, Reeves RD (1994) The possibility of in situ heavy metal decontamination of polluted soils using crops of metal accumulating plants. Resour Conserv Recycl 11:41–49
60. Brooks RR (1994) Plants that Hyperaccumulate Heavy Metals. In: Gargo ME (ed) Plants and Chemical Elements: Biochemistry, Uptake, Tolerance and Toxicity. VCH Verlagsgesellsschaft, Weinheim, pp 88–105
61. Mueller B, Rock S, Gowswami Dib, Ensley D (1999) Phytoremediation decision tree. Prepared by Interstate Technology and Regulatory Cooperation Work Group, pp 1–36
62. Henry JR (2000) An overview of phytoremediation of lead and mercury. In: NNEMS Report. Washington, DC, pp 3–9
63. Bañuelos GS (2000) Phytoextraction of selenium from soils irrigated with selenium-laden effluent. Plant and Soil 224(2):251–258
64. Black H (1995) Absorbing possibilities: Phytoremediation. Environ Health Perspect 103(12):1106–1108
65. Cluis C (2004) Junk-greedy greens: phytoremediation as a new option for soil decontamination. BioTech J 2:61–67
66. Cherian S, Oliveira MM (2005) Transgenic plants in phytoremediation: recent advances and new possibilities. Environ Sci Technol 39:9377–9390
67. Milic D, Lukovic J, Ninkov J, Zeremski-Skoric T, Zoric L, Vasin J, Milic S (2012) Heavy metal content in halophytic plants from inland and maritime saline areas. Cent Eur J Biol 7:307–317
68. Sun Y, Zhou Q, Xu Y, Wang L, Liang X (2011) The role of EDTA on cadmium phytoextraction in a cadmium-hyperaccumulator Rorippa globosa. J Environ Chem Ecotoxicol 3:45–51

69. Mejare M, Bulow L (2001) Metal-binding proteins and peptides in bioremediation and phytoremediation of heavy metals. Trends Biotechnol 19:67–73
70. Tong YP, Kneer R, Zhu YG (2004) Vacuolar compartmentalization: a second-generation approach to engineering plants for phytoremediation. Trends Plant Sci 9:7–9
71. Adesodun JK, Atayese MO, Agbaje T, Osadiaye BA, Mafe O, Soretire AA (2010) Phytoremediation potentials of sunflowers (Tithonia diversifolia and Helianthus annuus) for metals in soils contaminated with zinc and lead nitrates. Water Air Soil Pollut 207:195–201
72. Sakakibara M, Ohmori Y, Ha NTH, Sano S, Sera K (2011) Phytoremediation of heavy metal contaminated water and sediment by Eleocharis acicularis. Clean: Soil Air Water 39:735–741
73. Shabani N, Sayadi MH (2012) Evaluation of heavy metals accumulation by two emergent macrophytes from the polluted soil: an experimental study. Environmentalist 32:91–98
74. Ali H, Khan E, Sajad MA (2013) Phytoremediation of heavy metals—Concepts and applications. Chemosphere 91:869–881
75. Li JT, Liao B, Lan CY, Ye ZH, Baker AJM, Shu WS (2010) Cadmium tolerance and accumulation in cultivars of a high-biomass tropical tree (Averrhoa carambola) and its potential for phytoextraction. J Environ Qual 39:1262–1268
76. Robinson BH, Leblanc M, Petit D, Brooks RR, Kirkman JH, Gregg PEH (1998) The potential of Thlaspi caerulescens for phytoremediation of contaminated soils. Plant Soil 203:47–56
77. Tlustoš P, Száková J, Hrubý J, Hartman I, Najmanová J, Nedělník J, Pavlíková D, Batysta M (2006) Removal of As, Cd, Pb, and Zn from contaminated soil by high biomass producing plants. Plant Soil Environ 52:413–423
78. Chaney RL, Malik M, Li YM, Brown SL, Brewer EP, Angle JS, Baker AJM (1997) Phytoremediation of soil metals. Curr Opin Biotechnol 8:279–284
79. Malik RN, Husain SZ, Nazir I (2010) Heavy metal contamination and accumulation in soil and wild plant species from industrial area of Islamabad. Pakistan Pak J Bot 42:291–301
80. Vamerali T, Bandiera M, Mosca G (2010) Field crops for phytoremediation of metal-contaminated land. A review. Environ Chem Lett 8:1–17
81. Kochian L (1996) Mechanisms of heavy metal transport across plant cell membranes. In: International phytoremediation conference, May 8–10, Arlington. International Business Communications, Southborough
82. Brown SL, Chaney RL, Angle JS, Baker AJM (1995) Zinc and cadmium uptake by hyperaccumulator Thlaspi caerulescens grown in nutrient solution. Soil Sci Soc Am J 59:125–133
83. Gerard E, Echevarria G, Sterckeman T, Morel JLP (2000) Availability of Cd to three plant species varying in accumulation pattern. J Environ Qual 29:1117–1123
84. Reeves RD, Baker AJM (2000) Metal accumulating plants. In: Raskin I, Ensley B (eds) Phytoremediation of Toxic Metals: Using plants to clean up the environment. Wiley, New York., pp 193–229
85. Reeves RD (2003) Tropical hyperaccumulators of metals and their potential for phytoextraction. Plant and Soil 249:57–65
86. Farago ME, Parsons PJ (1994) The effects of various platinum metal species on the water plant Eichhornia crassipes (MART.). Chem. Spec. Bioavail 6:1–12
87. Qian JH, Zayed A, Zhu YL, Yu M, Terry N (1999) Phytoaccumulation of trace elements by wetland plants: III.Uptake and accumulation of ten trace elements by twelve plant species. J Environ Qual 28(5):1448–1455
88. Ma LQ, Komar KM, Tu C, Zhang W, Cai Y, Kenelley ED (2001) Bioremediation: A fern that hyperaccumulates arsenic. Nature 409:579
89. Grill E, Winnacker EL, Zenk MH (1987) Phytochelatins, a class of heavy-metal-binding peptides from plants, are functionally analogous to metallothioneins. Proc Natl Acad Sci U S A 84(2):439–443
90. Rauser WE (1999) Structure and function of metal chelators produced by plants: the case for organic acids, amino acids, phytin and metallothioneins. Cell Biochem Biophys 31:19–48
91. Huang JW, Chen J, Berti WR, Cunningham SD (1997) Phytoremediation of lead contaminated soils-Role of synthetic chelates in lead phytoextraction. Environ Sci Tech 31:800–806

92. Vassil AD, Kapulnik Y, Raskin I, Salt DE (1998) The role of EDTA in lead transport and accumulation by Indian mustard. Plant Physiol 117:447–491
93. Blaylock MJ, Salt DE, Dushenkov S, Zakharova O, Gussman C, Kapulnik Y, Ensley BD, Raskin I (1997) Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents. Environ Sci Technol 31:860–865
94. Lasat MM (2002) Phytoextraction of toxic metals: A review of biological mechanisms. J Environ Qual 31:109–120
95. Wu LH, Luo YM, Xing XR, Christie P (2004) EDTA-enhanced phytoremediation of heavy metal contaminated soil with Indian mustard and associated potential leaching risk. Agric Ecosyst Environ 102:307–318
96. Lombi E, Zhao FJ, Dunham SJ, McGrath SP (2001) Phytoremediation of heavy metal-contaminated soils: Natural hyperaccumulation versus chemically enhanced phytoextraction. J Environ Qual 30:1919–1926
97. Quartacci MF, Argilla A, Baker AJM, Navari-Izzo F (2006) Phytoextraction of metals from a multiply contaminated soil by Indian mustard. Chemosphere 63:918–925
98. Marques AP, Rangel AO, Castro PM (2009) Remediation of heavy metal contaminated soils:Phytoremediation as a potentially promising clean-up technology. Crit Rev Environ Sci Technol 39:622–654
99. Chen Y, Li X, Shen Z (2004) Leaching and uptake of heavy metal by ten different species of plants during an EDTA-assisted phytoextraction process. Chemosphere 57:187–196
100. Luo C, Shen Z, Li X, Baker AJM (2006) Enhanced phytoextraction of Pb and other metals from artificially contaminated soils through the combined application of EDTA and EDDS. Chemosphere 63:1773–1784
101. Chen BD, Li XL, Tao HQ, Christie P, Wong MH (2003) The role of arbuscular mycorrhizal in zinc uptake by red clover growing in a calcareous soil spiked with various quantities of zinc. Chemosphere 50:839–846
102. Seth CS (2012) A review on mechanisms of plant tolerance and role of transgenic plants in environmental clean-up. Bot Rev 78:32–62
103. Karenlampi S, Schat H, Vangronsveld J, Verkleij J, van der Lelie D, Mergeay M, Tervahauta A (2000) Genetic engineering in the improvement of plants for phytoremediation of metal polluted soils. Environ Pollut 107:225–231
104. Clemens S (2001) Developing tools for phytoremediation: towards a molecular understanding of plant metal tolerance and accumulation. Int J Occup Med Environ Health 14:235–239
105. Cobbett C, Goldsbrough P (2002) Phytochelatins and metallothioneins: roles in heavy metal detoxification and homeostasis. Annu Rev Plant Biol 53:159–182
106. Yurekli F, Kucukbay Z (2003) Synthesis of phytochelatins in Helianthus annuus is enhanced by cadmium nitrate. Acta Bot Croat 62:21–25
107. Fulekar M, Singh A, Bhaduri AM (2009) Genetic engineering strategies for enhancing phytoremediation of heavy metals. Afr J Biotechnol 8:529–535
108. Inouhe M (2005) Phytochelatins. Braz J Plant Physiol 17:65–78
109. Sarma H (2011) Metal hyperaccumulation in plants: a review focusing on phytoremediation technology. J Environ Sci Technol 4:118–138
110. Memon AR, Aktoprakligil D, Ozdemir A, Vertii A (2001) Heavy metal accumulation and detoxification mechanisms in plants. Turk J Bot 25:111–121
111. Memon AR, Schroder P (2009) Implications of metal accumulation mechanisms to phytoremediation. Environ Sci Pollut Res 16:162–175
112. Gomez NE, Cozatl DM, Sanchez RM, Mendoza DG, Perez OZ, Hernandez AM, Santamaria JM (2009) The Pb-hyperaccumulator aquatic fern Salvinia minima Baker, responds to Pb^{2+} by increasing phytochelatins via changes in SmPCS expression and in phytochelatin synthase activity. Aquat Toxicol 91:320–328
113. Jabeen R, Ahmad A, Iqbal M (2009) Phytoremediation of heavy metals: physiological and molecular mechanisms. Bot Rev 75:339–364
114. Sheoran V, Sheoran A, Poonia P (2011) Role of hyperaccumulators in phytoextraction of metals from contaminated mining sites: a review. Crit Rev Environ Sci Technol 41:168–214

115. Wu G, Kang H, Zhang X, Shao H, Chu L, Ruan C (2010) A critical review on the bio-removal of hazardous heavy metals from contaminated soils: issues, progress, eco-environmental concerns and opportunities. J Hazard Mater 174:1–8
116. Pinto E, Sigaud-Kutner TCS, Leitão MAS, Okamoto OK, Morse D, Colepicolo P (2003) Heavy metal-induced oxidative stress in algae. J Phycol 39:1008–1018
117. Grill E, Loftier S, Winnacker EL, Zenk MH (1989) Phytochelatins, the heavy-metal-binding peptides of plants are synthesized from glutathione by a specific γ-glutamyl cysteine dipeptidyl transpeptidase (phytochelatin synthase). Proc Natl Acad Sci U S A 86:6838–6842
118. Howden R, Goldsbrough PB, Andersen CR, Cobbett CS (1995) Cadmium-sensitive, cad1, mutants of *Arabidopsis thaliana* are phytochelatin deficient. Plant Physiol 107:1059–1066
119. Zhu XL, Pilon-Smits AH, Jouanin L, Terry N (1999) Over expression of glutathione synthetase in Indian mustard enhances cadmium accumulation and tolerance. Plant Physiol 119:73–79
120. Schafer HJ, Haag-Kerwer A, Rausch T (1998) cDNA cloning and expression analysis of genes encoding -GSH synthesis in roots of the heavy metal accumulator *Brassica juncea* L.: Evidence of Cd-induction of a putative mitochondrial γ-glutamylcysteine synthetase isoform. Plant Mol Biol 37:87–97
121. Vatamaniuk OK, Mari S, Lu YP, Rea PA (1999) AtPCS1, a phytochelatin synthase from *Arabidopsis*: isolation and *in vitro* reconstitution. Proc Natl Acad Sci U S A 96:7110–7115
122. Clemens S, Kim EJ, Neumann D, Schroeder JL (1999) Tolerance to toxic metals by a gene family of phytochelatin synthases from plants and yeast. EMBO Journal 18:3325–3333
123. Tsuji N, Hirayanagi N, Okada M, Miyasaka H, Hirata K, Zenk MH, Miyamoto K (2002) Enhancement of tolerance to heavy metals and oxidative stress in *Dunaliella tertiolecta* by Zn-induced phytochelatin synthesis. Biochem Biophys Research Commun 293:653–659
124. Ha SB, Smith AP, Howden R, Dietrich WM, Bugg S (1999) Phytochelatin synthase genes from *Arabidopsis* and the yeast Schizosaccharomyces pombe. Plant Cell 11:1153–1164
125. Vatamaniuk OK, Bucher EA, Ward JT, Rea PA (2001) A new pathway for heavy metal detoxification in animals-phytochelatin synthase is required for cadmium tolerance in *Caenorhabditis elegans*. J Biol Chem 276:20817–20820
126. Klapheck S, Schlunz S, Bergmann L (1995) Synthesis of phytochelatins and homophytochelatins in *Pisum sativum* L. Plant Physiol 107:515–521
127. Chen JJ, Zhou JM, Goldsbrough PB (1997) Characterization of phytochelatin synthase from tomato. Physiol Plant 101:165–172
128. Rauser WE (1995) Phytochelatins and related peptides: structure, biosynthesis, and function. Plant Physiol 109:1141–1149
129. Vatamaniuk OK, Mari S, Lu YP, Rea PA (2000) Mechanism of heavy metal ion activation of phytochelatin (PC)synthase-blocked thiols are sufficient for PC synthase-catalyzed transpeptidation of glutathione and related thiol peptides. J Biol Chem 275:31451–31459
130. Loeffler S, Hochberger A, Grill E, Winnacker EL, Zenk MH (1989) Termination of the phytochelatin synthase reaction through sequestration of heavy metals by the reaction product. FEBS Lett 258:42–46
131. Sylwia W, Anna R, Ewa B, Stephan C, Maria AD (2010) The role of subcellular distribution of cadmium and phytochelatins in the generation of distinct phenotypes of AtPCS1- and CePCS3- expressing tobacco. J Plant Physiol 167(12):981–988
132. Jiang W, Liu D (2010) Pb-induced cellular defense system in the root meristematic cells of *Allium sativum* L. BMC Plant Biol 10:40
133. Yadav S (2010) Heavy metals toxicity in plants: an overview on the role of glutathione and phytochelatins in heavy metal stress tolerance of plants. South Afr J Bot 76(2):167–179
134. Brunet J, Varrault G, Zuily-Fodil Y, Repellin A (2009) Accumulation of lead in the roots of grass pea (*Lathyrus sativus* L.) plants triggers systemic variation in gene expression in the shoots. Chemosphere 77(8):113–1120
135. Pal R, Rai JP (2010) Phytochelatins: peptides involved in heavy metal detoxification. Appl Biochem Biotechnol 160(3):945–963

136. Cobbett CS, May MJ, Howden R, Rolls B (1998) The glutathione-deficient, cadmium sensitive mutant, cad 2-1 of *Arabidopsis thaliana* is deficient in γ-glutamyl cysteine synthetase. Plant J 16:73–78
137. Rauser WE (1990) Phytochelatins. Annu Rev Biochem 59:61–86
138. Heiss S, Wachter A, Bogs J, Cobbett C, Rausch T (2003) Phytochelatin synthase (PCS) protein is induced in *Brassica juncea* leaves after prolonged Cd exposure. J Exp Bot 54(389):1833–1839
139. Mendoza-Cózatl DG, Butko E, Springer F, Torpey JW, Komives EA, Kehr J, Schroeder JI (2008) Identification of high levels of phytochelatins, glutathione and cadmium in the phloem sap of *Brassica napus*. A role for thiol peptides in the long-distance transport of cadmium and the effect of cadmium on iron translocation. Plant Cell 54(2):249–259
140. Qadir S, Hameed A, Nisa NT, Azooz MM, Wani MR, Hasannuzaman M, Kazi AG, Parvaiz A (2014) In: Ahmad P et al (eds) Brassicas: responses and tolerance to heavy metal stress. Improvement of crops in the era of climatic changes, vol 2. Springer Science+Business Media, New York doi:10.1007/978-1-4614-8824-8_1
141. Ortiz DF, Ruscitti T, MacCue KF, Ow DW (1995) Transport of metal-binding peptides by HMT1, a fission yeast ABC-type vacuolar membrane protein. J Biol Chem 270:4721–4728
142. Li ZS, Lu YP, Zhen RG, Szczypka M, Thiele DJ, Rea PA (1997) A new pathway for vacuolar cadmium sequestration in *Saccharomyces cerevisiae*: YCF1-catalyzed transport of *bis*(glutathionato) cadmium. Proc Natl Acad Sci U S A 94:42–47
143. Broeks A, Gerrard B, Allikmets R, Dean M, Plasterk RH (1996) Homologues of the human multidrug resistance genes MRP and MDR contribute to heavy metal resistance in the soil nematode *Caenorhabditis elegans*. EMBO J 15:6132–6143
144. Vogeli-Lange R, Wagner GJ (1990) Subcellular localization of cadmium and cadmium-binding peptides in tobacco leaves. Implication of a transport function for cadmium-binding peptides. Plant Physiol 92:1086–1093
145. Salt DE, Rauser WE (1995) MgATP-dependent transport of phytochelatins across the tonoplast of oat roots. Plant Physiol 107:1293–1301
146. Sanchez-Fernandez R, Davies TGE, Coleman JOD, Rae PA (2001) The *Arabidopsis thaliana* ABC protein superfamily, a complete inventory. J Biol Chem 276:30231–30244
147. Zenk MH (1996) Heavy metal detoxification in higher plants-a review. Gene 179:21–30
148. Sanità di Toppi L, Gabbrielli R (1999) Response to cadmium in higher plants. Environ Exp Bot 41(2):105–130
149. DalCorso G, Farinati S, Maistri S, Furini A (2008) How plants cope with cadmium: staking all on metabolism and gene expression. J Integr Plant Biol 50(10):1268–1280
150. Hossain MA, Piyatida P, Teixeira da Silva JA, Fujita M (2012) Molecular Mechanism of Heavy Metal Toxicity and Tolerance in Plants: Central Role of Glutathione in Detoxification of Reactive Oxygen Species and Methylglyoxal and in Heavy Metal Chelation. J Bot 872875:37. doi:10.1155/2012/872875
151. Fujita M (1985) The presence of two Cd-binding components in the roots of water hyacinth cultivated in a Cd^2+-containing medium. Plant Cell Physiol 26(2):295–300
152. Fujita M, Kawanishi T (1986) Purificatin and characterization of a Cd-binding complexes from the root tissues of various higher plants cultivated in Cd^{2+}-containing medium. Plant Cell Physiol 27(7):1317–1325
153. Iglesia-Turiño S, Febrero A, Jauregui O, Caldelas C, Araus JL, Bort J (2006) Detection and quantification of unbound phytochelatin 2 in plant extracts of *Brassica napus* grown with different levels of mercury. Plant Physiol 142(2):742–749
154. Grill E, Thumann J, Winnacker EL, Zenk MH (1988) Induction of heavy-metal binding phytochelatins by inoculation of cell cultures in standard media. Plant Cell Rep 7(6):375–378
155. Thumann J, Grill E, Winnacker EL, Zenk MH (1991) Reactivation of metal-requiring apoenzymes by phytochelatin- metal complexes. FEBS Lett 284(1):66–69

156. Nouairi I, Ben Ammar W, Ben Youssef N, Ben Miled DD, Ghorbal MH, Zarrouk M (2009) Antioxidant defense system in leaves of Indian mustard (*Brassica juncea*) and rape (*Brassica napus*) under cadmium stress. Acta Physiol Planta 31(2):237–247
157. Zhang H, Xu W, Guo J, He Z, Ma M (2005) Coordinated responses of phytochelatins and metallothioneins to heavy metals in garlic seedlings. Plant Sci 169(6):1059–1065
158. Krämer U, Cotter-Howells JD, Charnock JM, Baker AJM, Smith JAC (1996) Free histidine as a metal chelator in plants that accumulate nickel. Nature 379(6566):635–638
159. Shen ZG, Zhao FJ, McGrath SP (1997) Uptake and transport of zinc in the hyperaccumulator *Thlaspi caerulescens* and the non-hyperaccumulator *Thlaspi ochroleucum.*Plant. Cell Environ 20(7):898–906
160. Leopold I, Günther D, Schmidt J, Neumann D (1999) Phytochelatins and heavy metal tolerance. Phytochemistry 50(8):1323–1328
161. Baker AJM, McGrath SP, Reeves RD, Smith JAC (2000) Metal hyperaccumulator plants: a review of the ecology and physiology of a biological resource for phytoremedation of metal polluted soils. In: Terry N, Banuelos G (eds) Phytoremediation of Contaminated Soil and Water. Michael Lewis, Boca Raton, pp 85–107
162. Cogoni C, Macino G (2000) Post-transcriptional gene silencing across kingdoms. Genes Dev 10:638–643
163. Reese RN, White CA, Winge DR (1992) Cadmium sulfide crystallites in Cd-(γ-EC)*n*G peptide complexes from tomato. Plant Physiol 98:225–229
164. Speiser DM, Ortiz DF, Kreppel L, Ow DW (1992) Purine biosynthetic genesare required for cadmium tolerance in Schizosaccharomyces pombe. Mol Cell Biol 12:5301–5310
165. Juang RH, MacCue KF, Ow DW (1993) Two purine biosynthetic enzymes that are required for cadmium tolerance in *Schizosaccharomyces pombe* utilize cysteine sulfinate *in vitro*. Arch Biochem Biophys 304:392–401
166. Hunter TC, Mehra RK (1998) A role for *HEM2* in cadmium tolerance. J Inorg Biochem 69:293–303
167. Vande Weghe JG, Ow DW (1999) A fission yeast gene for mitochondrial sulfide oxidation. J Biol Chem 274:13250–13257
168. Mehra RK, Tran K, Scott GW, Mulchandani P, Sani SS (1996) Ag(I)-binding to phytochelatins. J Inorg Biochem 61:125–142
169. Maitani T, Kubota H, Sato K, Yamada T (1996) The composition of metals bound to class III metallothionein (phytochelatin and its desglycyl peptide) induced by various metals in root cultures of *Rubia tinctorum*. Plant Physiol 110:1145–1150
170. Schmöger MEV, Oven M, Grill E (2000) Detoxification of arsenic by phytochelatins in plants. Plant Physiol 122:793–802
171. Mutoh N, Hayashi Y (1988) Isolation of mutants of *Schizosaccharomyces pombe* unable to synthesize cadystin, small cadmium-binding peptides. Biochem Biophys Res Commun 151:32–39
172. Glaeser H, Coblenz A, Kruczek R, Ruttke I, Ebert-Jung A, Wolf K (1991) Glutathione metabolism and heavy metal detoxification in *Schizosaccharomyces pombe*. Isolation and characterization of glutathione-deficient, cadmium-sensitive mutants. Curr. Genet 19:207–213
173. Vernoux T, Wilson RC, Seeley KA, Reichheld JP, Muroy S (2001) The *ROOT MERISTEMLESS1/CADMIUMSENSITIVE2* gene defines a glutathione dependent pathway involved in initiation and maintenance of cell division during postembyronic root development. Plant Cell 12:97–109
174. van Hoof NALM, Hassinen VH, Hakvoort H, Ballintijn KF, Schat H (2001) Enhanced copper tolerance in *Silene vulgaris* (Moench) Garcke populations from copper mines is associated with increased transcript levels of a 2b-type metallothione in gene. Plant Physiol 126:1519–1527

Chapter 14
Role of Biochar in Remediating Heavy Metals in Soil

**Amina Hayyat, Maryam Javed, Iqra Rasheed, Shafaqat Ali,
Munazzam Jawad Shahid, Muhammad Rizwan, Muhammad Tariq Javed,
and Qasim Ali**

14.1 Introduction

Soil contamination in these days has become a worldwide problem of greatest magnitude. Despite comprehensive regulations, a huge increase in concentration of different elements and compounds from threshold level is observed in recent years [1]. The main reasons of contamination are natural processes like volcanic eruption and weathering of rocks, as well as anthropogenic activities such as smelting, mining, and overapplication of agrochemicals such as fertilizers and pesticide [1, 2]. Industrial and technological advancement increased pollutant intake into the environment. Heavy metals among these pollutants have great influence on fertility of agricultural soils [3]. Urbanization leads to promote the farmer's concern to use contaminated soil for the production of food crops [4]. Urban agricultural soils are contaminated because of waste water irrigation to increase the crop yield [5]. The wastewater used for irrigation is rich in toxic heavy metals which are major contributor to heavy metal pollution in the soils irrigated are amended with waste water and material [6, 7]. Heavy metals are toxic and stored in the environment because they cannot be broken down [8, 9]. Due to its persistent nature, heavy metals become part of the food chain and pose risks to human health and degrade soil quality. Generally soil chemistry and the chemical form of heavy metal are responsible for the existence of these metals in crops and plants. In soil, commonly the accessibility of all the metals surges due to acidic environments; the transfer factor of a particular

---

A. Hayyat • M. Javed • I. Rasheed • S. Ali (✉) • M.J. Shahid • M. Rizwan
Department of Environmental Sciences and Engineering, Government College University,
Allama Iqbal Road, 38000 Faisalabad, Pakistan
e-mail: shafaqataligill@yahoo.com

M.T. Javed • Q. Ali
Department of Botany, Government College University,
Allama Iqbal Road, 38000 Faisalabad, Pakistan

A.A. Ansari et al. (eds.), *Phytoremediation*, DOI 10.1007/978-3-319-40148-5_14

element to the plants is also affected by the crop's type, such as Cd which is mainly considered a toxic element in soil and fertilizers because of its highest transfer factor for any crop [10]. The transfer of heavy metals from soil to plant is one of the basic mechanisms of human exposure to heavy metals through the food chain [11].

Here the overview of heavy metal pollution due to Pb, Cd, Cr, As Ni, Zn, and Cu is described. The concentration of these heavy metals above threshold level is lethal to human health. Some elements such as Cd and Pb, without microbial degradation above optimum level of concentration, put the plant growth at risk due to permanent adulteration in soil [12]. The continuous use of inorganic and biological fertilizers increased heavy metal pollution in soil [1]. It is very necessary to minimize the accessibility and phyto-availability of heavy metals to plants along with the restoration of contaminated soil for safe and healthy food production [13]. In situ remediation techniques have been emphasized in various studies about remediation of heavy metals. In recent years researchers much investigated the use of biochar as in situ soil amendment, and it was found to be effective in reducing the mobility of heavy metals in soils [9, 14]. Biochar method is known as Terra Preta de Indio, introduced from the dark soil of the Amazon basin and is of high utility. Biochar have high chemical stability in contaminated soils and is a C-rich material. A lot of researches are initiated to explore the distinctive use of biochar for continuing C sequestration [14]. Biochar which is porous and has high C content is prepared by pyrolysis of organic waste [14]. Carter et al. [15] defined biochar as follows: "it is a porous carbonaceous solid material manufactured by the process of thermo-chemical decomposition under little supply of oxygen appropriate for the benign and continuing storage of carbon." The International Biochar Initiative (IBI) described biochar as a charcoal which is used as a tool for agricultural and environmental management [9].

Biochar has proved to be a very effective tool for treatment of contaminated soils due to these reasons: it effectively adsorbs heavy metals and decreases bioavailability and toxin-induced stress to plants and microorganisms [13, 16]. Biochar compounds are a good source of organic material and mineral nutrients for microbes. It promotes the beneficial microbes that promote remediation and protect them from predators [17]. Biochar improves the soil fertility and plant growth by improving physical and chemical properties of soil and also increases the availability of useful nutrients [14]. In soils the use of biochar has proved to raise the stable C pool and minimize the increasing concentration of atmospheric CO_2 [18].

14.2 Biochar Production and Properties

14.2.1 Biomass Pyrolysis

Biochar is a fine-grained porous and carbonaceous solid material synthesized from waste biomass residues under limited oxygen condition and low to medium temperatures (450–650 °C) by the slow pyrolysis [19, 20]. Biochar is manufactured from renewable resources such as green waste and chicken manure [21]. With recent advancements, biochar can be produced by thermal decomposition of various kinds of organic feedstocks such as crop biomass, wood, agricultural residues (cereal straw, hazelnut and peanut shell, wheat straw, etc.), and industrial organic waste (sewage sludge and de-inking paper sludge [1]. Forest-remain biochar has low metal content, so the frequent use of this biochar has no negative effect on the agricultural soils [22].

14.2.2 Properties of Biochar

Biochar, a carbon-rich material, is now well known because of its agronomic benefits and ability to moderate climate change by carbon sequestration potential [23–25]. The biochar proved to have an effective role as a soil conditioner and fertilizer [26]. The properties of biochar include highly porous structure, high surface area, pH, cation exchange capacity (CEC), adsorptive capacity, carbon content, organic matter content, and high water-holding capacity. It reduces CO_2 emission, retains nutrients and, pesticide [3, 12, 26, 27], making it a perfect soil amendment to remediate heavy metals and to recover the fertility. Activation of biochar is very effective to improve the adsorption capacity of biochar. The nutrient retention and uptake by plants are enhanced due to activation of biochar as compared to non-activated biochar [28]. Therefore, steam activation is an exciting opportunity for prospective biochar applications because it revealed to almost double the constructive effects of biochars in all illustrations.

14.2.3 Factors Affecting Biomass Properties

The properties of biochar are dependent upon the type of feedstock and the production procedure. Depending upon these two main parameters, the composition of organic and inorganic contaminants in the biochar also changed, and application of this biochar may cause adulteration in the soil [22]. The source material of biochar may affect the carbon sequestration and conditioning capacity of soil [20]. Other factors such as the type of soil, the type of metal, the nature of biomass, the thermal decomposition conditions (pyrolysis), and the quantity of biochar used [13] may also have prominent effect on properties of biochars.

14.3 Heavy Metal-Contaminated Soils

Mostly the heavy metals such as Cu(II), Cd(II), and Ni(II) are found together in contaminated soils. Oxidation-reduction and acid-base properties of heavy metal ions affect the mobility of these heavy metals. Zn(II) and Pb(II) retain in soil, while Cr(II), Cu(II), and Cd(II) move through soil pore water. Cd(II) and Cu(II) form a complex with available natural organic material in the soil [26]. Mobility and bioavailability of Cu and Pb are a worldwide matter of concern especially in polluted soils of mining, shooting, and industrialized areas [29]. The frequent use of sewage, municipal composts, manure, mining wastes, and copper-containing fungicides is the main cause of Cu contamination in soil [30]. Higher concentration of Cu has detrimental effect on soil and reduces the population of bacteria, fungi, earthworms, and plant organic content in soil. It also disrupts the nutrient cycle and activity of enzymes such as arylsulfatase, phosphatase, dehydrogenase, and β-glucosidase [16]. Higher concentrations of Cu in fruits and vegetables cause gastrointestinal cancer [31]. In areas rich in Pb and Zn rocks, Cd is obtained from lithogenic sources. Recently, the increased level of Cd in contaminated soils is due to the frequent application of fertilizers and sewage sludge on agricultural soils.

However, plants have high tolerance level for Cd as compared to animals and humans due to this reason: Cd is only toxic to plants at significantly higher concentrations. Disease itai-itai caused by high ingestion of Cd may also cause cancer and damage the kidney [2]. Municipal waste incineration; coal combustion; Pb, Cu, or Zn smelter; electroplating; nickel–cadmium batteries; and pigment production are the major anthropogenic sources of Cd in the environment (World Health Organization, http://www.euro.who.int/en/home). Consequently, Cd content is increased due to the usage of phosphatic fertilizers and sewage sludge [32]. Smelting processes are the main cause of Zn production. Among other sources, cosmetics, galvanized products, television, coating of metals, rubber and tire industries, and Zn alloys are prominent. However, as compared to Cd, Zn is less toxic. The presence of Zn in soil is affected by pH, organic content, and structure and nature of parent material. Zn is a transition metal and is an essential micronutrient for many biological processes, but it is toxic at higher concentrations; Zn is the most abundant trace heavy metal existing in agroecosystems [33]. Zn is also entering in the environment from sources such as municipal waste treatment plants and burning of coal and waste.

Municipal waste treatment plants and burning of coal and waste are also sources of zinc. The bioavailability of zinc in alkaline soil increases due to its solubility. Zn(II) also has the mobility in acidic soils [34]. The adverse effects of Zn on plant physiology are widely reported [35]. The solubility and bioavailability of Pb increase in soil due to weather and oxidation processes which modify the metallic Pb in soil. After its bioavailability, Pb promptly adsorbed on the sediment and soil particles, and it also tends to accumulate in plants and animal bodies [29]. Arsenic (As)-contaminated soils deteriorate the water quality because it leaches down from the soil and contaminated water sources such as the rivers and canals. The process of oxidative phosphorylation and synthesis of ATP in cells is also disturbed from high concentration of arsenic [3].

Arsenic also has detrimental effect on the activity of microbial population, soil biota, and nutrient cycles [36]. Ni originated in the environment both from the natural and anthropogenic process. The weathering of rocks and human activities such as smelting, plating, and mining are the main sources of Ni contamination of soil. For agricultural soils, application of organic waste material such as sewage sludge and fertilizer application are the main causes of contamination [1]. Chromium exists in the contaminated soil as Cr(III) and Cr (VI) ions. Chromium oxidation states are responsible for toxicity toward plants and animals, such as Cr(III) which is an essential nutrient and has less solubility in acidic and alkaline soils, whereas Cr(VI) is highly soluble in acidic and alkaline soils considered as carcinogen. Cr(VI) has a harmful effect and disturbs the biological activity of the soil. The soil properties play the main role in the availability of heavy metals to plants for uptake [10, 37]. In soil, the various enzymatic activities of bacteria are disrupted due to modification of soil environment by chromium (Cr).

14.3.1 Heavy Metal Remediation by Biochar

Namgay et al. [38] documented a decrease in the accessibility of heavy metals after the contaminated soil was amended with biochar, due to which plant absorption of the heavy metals is reduced. Unlike many other biological amendments, biochar having the ability to increase soil pH [39] might have improved sorption of these metals, consequently decreasing their bioavailability for plant uptake.

14.3.2 Heavy Metals Found in Soil

Generally Cu, Zn, As, Cr, Co, Ni, Sb, Hg, Th, Pb, Se, Si, and Cd are heavy metals that originate in soil which may be extremely harmful to human and plant life by contamination of soil and water. Heavy metals do not have the ability to biodegrade, so they can persist in polluted soils for a longer time [40]. There is a prerequisite to remove these metals, and the best convenient way is by environmental friendly techniques, i.e., biochar. From a long period, biochar is being applied to overcome the problem of heavy metal contamination and to improve soil fertility. Depending on the soil type, diverse types of biochar are used for different types of heavy metals, as demonstrated in Table 14.1.

Biochar has high pH and organic carbon content; higher concentrations of phosphorous, calcium, and magnesium; and low particular surface area than activated carbon [41]. That's why the addition of biochar brought a notable proliferation in soil cation exchange capability [42]. Biochar is considerably more active in restraining soil Pb than AC [41]. Biochar has excellent adsorption capacity due to its asymmetrical plates and porous structure (Fig. 14.1) [42].

The biochar produced from different sources showed similar results [43]. The maximum falloff of transferable Pb was achieved at 10 % application rate of biochars with steady reduction of 68 and 30 % for sugar cane biogases and orange peel, respectively [3]. Biochar is prosperous in nutrients, i.e., nitrogen, calcium, magnesium, and phosphorous in addition to carbon. Concentration of carbon and nitrogen are reduced with the increase in temperature, although Mg, Ca, and P were augmented by rise in temperature [43]. 93 % of lead was absorbed by biochar at 100,

Table 14.1 Heavy metal removal by different types of biochar

Contaminants	Biochar type	Matrix	Effects	References
As and Cu	Hardwood	Soil	Mobilization due to enhanced pH and DOC	Beesley et al. [67]
As, Cr, Cd, Cu, Ni, Pb, and Zn	Sewage sludge (500–550 °C)	Soil	Immobilization of As, Cr, Ni, and Pb due to rise in soil pH. Mobilization of Cu, Zn, and Cd due to highly available concentrations in biochar	Khan et al. [68]
Cd and Zn	Hardwood	Soil	Immobilization due to enhanced pH	Beesley et al. [67]
Cd, Cu, and Pb	Chicken manure and green waste (550 °C)	Soil	Immobilization due to partitioning of metals from exchangeable phase to less bioavailable organic-bond fraction	Park et al. [20]
Cu	Broiler litter (700 °C)	Soil	Cation exchange; electrostatic interaction; sorption on mineral ash content; complexation by surface functional groups	Uchimiya et al. [69]
Cu and Pb	Oakwood	Soil	Complexation with phosphorus and organic matter	Karami et al. [70]
Pb	Dairy manure (450 °C)	Soil	Immobilization by hydroxypyromorphite formation	Cao et al. [41]
Pb	Oakwood (400 °C)	Soil	Immobilization by rise in soil pH and adsorption on biochar	Ahmad et al. [29]
Pb	Rice straw	Soil	Nonelectrostatic adsorption	Jiang et al. [42]
Pb, Cu and Zn	Broiler litter (300 and 600 °C)	Soil	Stabilization of Pd and Cu	Uchimiya et al. [48]
Ni, Cu, Pb, and Cd	Cottonseed hulls (200–800 °C)	Soil	Surface functional groups of biochar-controlled metal sequestration	Uchimiya et al. [71]

while at 200 and 350, approximately the entire lead was removed from the soil solution [43]. The pH is the main parameter for disturbing adsorption and/or desorption of heavy metals in acidic soils. The amplified system pH by application of biochar increases the adsorption of Pb(II) in the soils. Biochar results in pH increase that flourishes the negative surface charges in the soil and enhanced the attraction for cations [44].pH rise is beneficial for heavy metal control in bulk soils. The increasing amount of biochar reduced the acid soluble Pb(II) and Cu(II) by 18.8–77.0 % and 19.7–100.0 %, respectively [42]. The uses of biochar, mussel shell, and cow bone reduced the lead phyto-availability by 55.50 %, 71.22 %, and 70.47 %, respectively, in army firing soil [45].

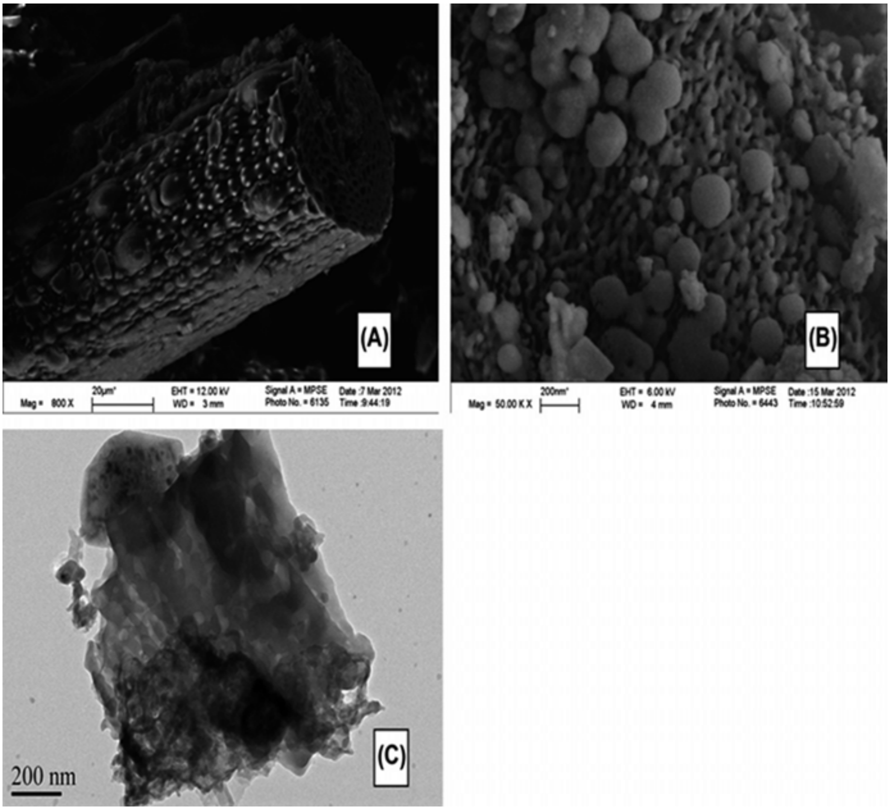


Fig. 14.1 SEM photographs of rice straw biochar at different magnifications: (**a**) ×800, (**b**) ×50,000, and (**c**) TEM photograph of the biochar

When the application rate of biochar produced from cow manure increased, the nutrient uptake, maize production, and photochemical properties of a dry land sandy soil considerably improved [46]. Sewage sludge-derived biochar successfully eradicates Pb^{2+} from acidic soil at early pH 5, 4, 3, and 2with the capabilities of 30.88, 24.80, 20.11, and 16.11 mg g^{-1}, respectively [47]. Biochar with phosphorus-rich manure serves as a fertilizer and is also used to remove heavy metal, specifically lead [48]. Historically, phosphorus in the biochar encouraged modification of less constant $PbCO_3$ to more stable $Pb_5(PO_4)_3OH$, liable for soil Pb restriction [41]. The application of biochar produced from cotton sticks put a positive impact on the cadmium-stressed soils by increasing the plant growth. It is due to the unique capacity of biochar that the metal ion is separated and the cadmium ion movement to the aerial tissue of plants is controlled [49].

The biochar synthesized from swine manure at 450 °C could contribute as a possible amendment for the control of heavy metals (Cd^{2+}) in sandy soil [9]. Biochar produced from green waste restrained lead, copper, and cadmium by 36.8 %, 22.9 %, and 30.3 %, respectively, for pointed soil and by 72.9, 0.901, and 42.7 % for naturally polluted soils [20]. Bamboo-derived biochar can adsorb nickel, chromium, copper, and mercury, from both water and soils, and cadmium from polluted soils [40].

Table 14.2 shows the effect of biochar application on the mobility of heavy metals in soils.

In basic soils, carbonaceous tools, irrespective of biomass and pyrolysis, improved the Cu(II) restriction than Ni(II) [26] while the Cu(II) adsorption amplified with pH rise (3.5–6.0). Cu(II) has an adsorption capability in the following order: canola straw char < soybean straw char < peanut straw char [50]. Organic segments of biochars and natural organic matter can stimulate Cu(II) extraction by basic soil because of more carboxyl contents [26]. The unsaturated biochar detached about 70 % of Cr(III) at equilibrium time, although only 30 % of As(V) in batch kinetic trials, suggesting that biochar is highly effective in eliminating cations than anions [51]. Biochar derived from chicken manure is more active in controlling metals as well as plant growth than biochar derived from green waste. So, biochar derived from chicken manure can be used to improve phyto-stabilization of metal tainted soils [20].

Jiang et al. [42] explored that biochar derived from rice straw influences the bioavailability and mobility of Pb(II), Cu(II), and Cd(II) in an Ultisol. When the amendment dosage of biochar increased, the acid removable Pb(II) and Cu(II) reduced by 18.8e77.0 % and 19.7e100 %, respectively. With the addition of 5 mmol kg^{-1} of these heavy metals, for treatments with 3 and 5 % biochar, the reducible Pb(II) was 2.0 and 3.0 times greater than the samples deprived of biochar. Pore structure of the biochars produced from crop straws (i.e., rice, corn, wheat, and cotton) is more developed as compared to wood char because wood char has greater lignin content. Biochars with

Table 14.2 Effect of biochar application on the mobility of heavy metals in soils

Feedstock	Production temperature (°C)	Contaminants	Effects	References
Bamboo	Not available	Cd	Combined effect of electrokinetics, removal of extractable Cd by 76.9 % within 12 days	Ma et al. [72]
Hardwood	450	As, Cu, Cd, and Zn	Reduction in Cd in soil pore water by tenfolds; Zn concentrations reduced 300- and 45-folds, respectively, in column leaching test	Beesley et al. [67]; Beesley and Marmiroli [73]
Hardwood	450	As, Cd, Cu, Pb, and Zn	Biochar surface mulch enhanced As and Cu mobility in the soil profile; little effect on Pb and Cd	Beesley and Dickinson [74]
Wood	200 and 400	Cd and Zn	Reduction in Cd and Zn leaching loss by >90 %	Debela et al. [75]

lower lignin content have larger surface areas and more developed pore volumes. In case of Cd the order of corn straw > cotton straw > wheat straw > rice straw > poplar shaving is for the sorption capacity of biochar that was not stringently reliable to the surface area of biochars [52]. Uchimiya et al. [53] reported that a biochar adsorbs Cd, Cu, Pb, and Ni and perceived that the tendency of the elimination order was Ni < Cd < Cu < Pb. The affinity for metal immobilization upsurges in the following order: Pb(II) > Cu(II) > Zn(II) > Cd(II) [26].

Table 14.3 shows the effect of biochar application on the bioavailability of heavy metals in soils.

Table 14.3 Effect of biochar application on the bioavailability of heavy metals in soils

Feedstock	Production temperature (°C)	Contaminant	Effects	References
Cotton stacks	450	Cd	Reduction of the bioavailability of Cd in soil by adsorption or Co precipitation	Zhou et al. [76]
Hardwood-derived biochar	450	As	Significant reduction of As in the foliage of *Miscanthus*	Hartley et al. [77]
Eucalyptus	550	As, Cd, Cu, Pb, and Zn	Decrease in As, Cd, Cu, and Pb in maize shoots	Namgay et al. [38]
Orchard prune residue	500	Cd, Cr, Cu, Ni, Pb, and Zn	Significant reduction of the bioavailable Cd, Pb, and Zn with Cd showing the greatest reduction; an increase in the pH, CEC, and water-holding capacity	Fellet et al. [78]
Chicken manure and green waste	550	Cd, Cu, and Pb	Significant reduction of Cd, Cu, and Pb accumulation by Indian mustard	Park et al. [20]
Chicken manure	550	Cr	Enhanced soil Cr(VI) reduction to Cr(III)	Choppala et al. [79]
Sewage sludge	500	Cu, Ni, Zn, Cd, Pb	Significant reduction in plant availability of the metals studied	Méndez et al. [80]
Rice straw	Not clear	Cd, Cu, and Pb	Significant reduction in concentrations of free Cu, Pb, and Cd in contaminated soils	Jiang et al. [42]
			Identification of functional groups on biochar with high adsorption affinity to Cu	
Quail litter	500	Cd	Reduction of the concentration of Cd in physic nut; greater reduction with the higher application rates	Suppadit et al. [81]
Oakwood	400	Pb	Bioavailability reduction by 75.8 %; bioaccessibility reduction by 12.5 %	Ahmad et al. [29]

14.4 Effects of Biochar on Soil

The increasing population of human and their activities put huge pressure on agriculture land to fulfill need of food. This overburden on cultivated land raised the problems of soil erosion and degradation and depletion of organic matter and vital nutrients from the soil. To overcome these problems, biochar can be used as an effective tool [54]. Biochar can be utilized to get agricultural, environmental, and economic benefits although the nature of these benefits can vary by type of biochar and nature of soil [55].

14.4.1 Agricultural Benefits

Application of biochar in soil results in the following positive impacts on the soil of agriculture lands.

14.4.1.1 Soil Fertility

The extreme use of fertilizers exacerbates the leaching of macronutrients from the agriculture lands leading to decrease in soil fertility, amplification of acidity, and increased requirements of fertilizers that resulted in ultimate low crop yield [56]. Biochar fulfills the nutrient requirement of the soil and improves the soil fertility and productivity that resulted in optimum yield of crops [57].

14.4.1.2 Crop Productivity

Biochar offers large surface area for soil microbes resulting in increase in microbial growth. It improves the degradation of organic matter and improves retention time of microbes in soil and increases availability of nutrients which are favorable for soil growth for better crop production [27, 49, 54]. The productivity of soil also increases due to the addition of biochar in soil because it increases the availability of C compound and minimizes the requirement of artificial fertilizers [58].

14.4.1.3 Water Holding Capacity

Biochar enhances the water-holding capacity of soil by improving the soil quality in terms of physical, biological, and chemical fertility and better plant root density [59].

14.4.1.4 Cation Exchange Capacity

The application of biochar causes the stabilization of heavy metal, increases CEC, and increases the availability of higher mineral essential for plant growth [48, 60].

14.4.1.5 pH of soil

The specific pH properties of biochar make the soil acidic for evaporation of metal ion in soil. The soils with low pH and CEC will also have the low adsorption caption for metals [61].

14.4.1.6 Uptake of Heavy Metals

The addition of biochar reduces the heavy metal uptake by plant roots and minimizes the chance of low productivity due to toxicity in plant body [59].

14.4.1.7 Waste Reduction

The production of biochar is also an innovative technique to make beneficial reuse of waste and to minimize the waste volume [41].

14.4.1.8 Moisture Holding Capacity

The moisture holding capacity is improved due to application of biochar due to retention of pollution for shorter or longer time duration [62].

14.4.1.9 Adsorbent Capacity

Biochar has high adsorbent capacity for adsorption of heavy metals and minerals in soil. Due to its high competency to adsorb organic contaminants for purification of soil from contaminants, it improves the soil fertility [63, 64].

14.4.1.10 Soil Respiration Rate

The respiration of soil improves by decomposition of biochar with the help of microbes (bacterias) resulting in C production which is used by microbes for microbial activity in soil [1].

14.4.1.11 Residing Capacity of Soil-Living Organisms

Biochar also improves the resistance capacity of living organisms residing in soil, i.e., earthworms (nematodes), insects, fungus, etc. [58].

14.4.1.12 Nutrient Availability

High concentration of ash in biochar increases the availability of nutrients which satisfies the need of soil for nutrients. In case of low ash concentration in biochar, compost or manure can be added to maintain the ratio of nutrients [65].

14.4.1.13 Rate of Germination

The rate of seed germination increased due to direct interaction of biochar with soil [58].

14.4.2 Environmental Benefits

Interaction of soil with biochar results in subsequent effects on environment:

14.4.2.1 Carbon Sequestration

Biochar has great resistance for biotic and biotic degradation in soil which emphasizes its importance in carbon cycle as carbon sink [66]. Carbon sequestration resulting in reduction of CO_2 in the atmosphere is due to the long-term availability of biochar in soil. It also has the ability to minimize the effect of climate change, decrease in GHG (greenhouse gas), and NO_x emissions by causing decline in C emission as output from burning fossil fuels. Methane and nitrous oxide produced from carbon cycle and nitrification/denitrification process, respectively, can be reduced by application of biochar, and thus biochar can play an important role to solve issues of global warming [54, 56, 57, 62].

14.4.3 Economic Benefits

Application of biochar is also beneficial for the economy.

14.4.3.1 Economically Beneficial

Biochar is desirable nowadays because of its low energy demand, ease of use, low cost, and no pretreatment for use in soil.

14.4.3.2 Environment Friendly

The eco-friendly nature of biochar made it desirable, and nowadays it is being widely used in various applications because it (1) can be reuseable, (2) can recycle the organic waste, and (3) can reduce waste quantity [64].

14.5 Conclusion

Global industrialization leads pollutants to the environment. Among these pollutants, heavy metals have the profound effect on fertility of soils. Biochar amendments remediate heavy metal toxicity in agricultural soils through different processes. Biochar is an effective tool for contaminated soil due to these different processes: (1) adsorption of heavy metals; (2) highly porous structure and high surface area; (3) CEC; (4) reduced CO2 emission; (5) high water-holding capacity; (6) retention of pesticides, PAHs, and PSBs; (7) reduction of bioavailability and toxin-induced stress to microorganisms and plants; (8) C-rich material having high chemical stability in the contaminated soils and mineral nutrients for microbes; (9) reduction of the mobility in soil; (10) protection of microbes from predators and introduction of beneficial microbes that promote remediation; and (11) improvement of soil fertility and plant growth by improving physical and biological properties of soil by provision and maintenance of nutrients.

However, these processes vary with biochar type, nature of soil, type of plants, type of metal toxicity, conditions of thermal decomposition (pyrolysis), and the quantity of biochar used. Therefore, we should use biochar according to soil contamination. However, the various facts of opinions founded on comprehensive point of views should not be snubbed. Variant consequences recommend that recent biochar application to soil is not a standard example, as an alternative extensive concern of the properties related to each specific biochar material and how those properties could cure a particular soil scarcity are mandatory [55]. Biochar use in soil has been suggested to increase the stable C pool and limit the growing concentration of atmospheric CO_2. In conclusion much more investigations are required to check the long-term environmental and economic feasibility of biochar application to remediate heavy metal-contaminated soils.

References

1. Mendez A, Ferreiro JP, Gasco G (2014) Biochar from pyrolysis of drinking paper sludge and its use in the treatment of a nickel polluted soil. J Anal Appl Pyrolysis 107:46–52
2. Lu H, Li Z, Fu S, Mendez A, Gasco G, Ferreiro JP (2014) Can biochar and phytoextractors be jointly used for cadmium remediation? PLoS One 9:1–7

3. Abdelhafez AA, Li J, Abbas MHH (2014) Feasibility of biochar manufactured from organic wastes on the stabilization of heavy metals in a metal smelter contaminated soil. Chemosphere 117:66–71
4. Nabulo G (2009) Assessing risks to human health from peri-urban agriculture in Uganda. Ph.D. thesis, University of Nottingham
5. Mahmood A, Malik RN (2014) Human health risk assessment of heavy metals via consumption of contaminated vegetables collected from different irrigation sources in Lahore, Pakistan. Arab J Chem 7:91–99
6. Singh KP, Mohon D, Sinha S, Dalwani R (2004) Impact assessment of treated/untreated wastewater toxicants discharge by sewage treatment plants on health, agricultural and environmental quality in wastewater disposal area. Chemosphere 55:227–255
7. Mapanda F, Mangwayana EN, Nyamangara J, Giller KE (2005) The effect of long-term irrigation using wastewater on heavy metal contents of soils under vegetables in Harare, Zimbabwe. Agr Ecosyst Environ 107:151–165
8. Anwar J, Shafique U, Zaman WU, Salman M, Dar A, Anwar S (2010) Removal of Pb(II) and Cd(II) from water by adsorption on peels of banana. Bioresour Technol 101:1752–1755
9. Xu D, Zhao Y, Sun K, Gao B, Wang Z, Jin J, Zhang Z, Wang S, YanY LX, Wu F (2014) Cadmium adsorption on plant- and manure-derived biochar and biochar-amended sandy soils: Impact of bulk and surface properties. Chemosphere 11:320–326
10. Javied S, Mehmood T, Choodhry MM, Tufail M, Irfan N (2009) Heavy metal pollution from phosphate rock used for the production of fertilizer in Pakistan. Microchem J 91:94–99
11. Khan S, Rehman S, Khan AZ, Khan MA, Shah MT (2010) Soil and vegetables enrichment with heavy metals from geological sources in Gilgit, northern Pakistan. Ecotoxicol Environ Saf 73:1820–1827
12. Kloss S, Zehetner F, Obergur E, Buecker J, Kitzler B, Wenzel WW, Wimmer B, Soja G (2014) Trace element concentrations in leachates and mustard plant tissue (Sinapis alba L.) after biochar application to temperate soils. Sci Total Environ 481:498–508
13. Al-Wabel MI, Usman ARA, El-Naggar AH, Aly AA, Ibrahim HM, Elmaghraby S, Al-Omran A (2014) Conocarpus biochar as a soil amendment for reducing heavy metal availability and uptake by maize plants. Saudi J Biol Sci 22:503–511
14. Houben D, Evrard L, Sonnet P (2013) Mobility, bioavailability and pH-dependent leaching of cadmium, zinc and lead in a contaminated soil amended with biochar. Chemosphere 92:1450–1457
15. Carter S, Shackley S, Sohi S, Suy TB, Haefele S (2013) The impact of biochar application on soil properties and plant growth of pot grown lettuce (Lactuca sativa) and cabbage (Brassica chinensis). Agronomy 3:404–418
16. Mackie KA, Marhan S, Ditterich F, Schmidt HP, Kandeler E (2015) The effects of biochar and compost amendments on copper immobilization and soil microorganisms in a temperate vineyard. Agric Ecosyst Environ 201:58–69
17. Rutigliano FA, Romano M, Marzaioli R, Baglivo I, Baronti S, Miglietta F, Castaldi S (2014) Effect of biochar addition on soil microbial community in a wheat crop. Eur J Soil Biol 60:9–15
18. Fang Y, Singh B, Singh BP (2015) Effect of temperature on biochar priming effects and its stability in soils. Soil Biol Biochem 80:136–145
19. Lehmann J, Joseph S (2009) Biochar for environmental management: an introduction. In: Lehmann J, Joseph S (eds) Biochar for environmental management-science and technology. Earthscan, London, pp 1–9
20. Park JH, Choppala GK, Bolan NS, Chung JW, Chuasavathi T (2011) Biochar reduces the bioavailability and phytotoxicity of heavy metals. Plant Soil 348:439–451
21. Hass A, Gonzalez JM, Lima IM, Godwin HW, Halvorson JJ, Boyer DG (2011) Chicken manure biochar as liming and nutrient source for acid Appalachian soil. J Environ Qual 41:1096–1106

22. Lucchini P, Quilliam RS, DeLuca TH, Vamerali T, Jones DL (2014) Does biochar application alter heavy metal dynamics in agricultural soil? Agric Ecosyt Environ 184:149–157
23. Marris E (2006) Putting the carbon back: black is the new green. Nature 442:624–626
24. Lehmann J (2007) A handful of carbon. Nature 447:143–144
25. Khan S, Reid BJ, Li G, Zhu YG (2014) Application of biochar to soil reduces cancer risk via rice consumption: A case study in Miaoqian village, Longyan, China. Environ Int 68:154–161
26. Uchimiya M, Lima IM, Klasson KT, Wartelle LH (2010) Contaminant immobilization and nutrient release by biochar soil amendment: Roles of natural organic matter. Chemosphere 80:935–940
27. Fellet G, Marmiroli M, Marchiol L (2014) Elements uptake by metal accumulator species grown on mine tailings amended with three types of biochar. Sci Total Environ 468–469:598–608
28. Borchard N, Wolf A, Laabs V, Aeckersberg R, Scherer HW, Moeller A, Amelung W (2012) Physical activation of biochar and its meaning for soil fertility and nutrient leaching-a greenhouse experiment. Soil Use Manage 28:177–184
29. Ahmad M, Lee SS, Yang JE, Ro HM, Lee YH, Ok YS (2012) Effects of soil dilution and amendments (mussel shell, cow bone, and biochar) on Pb availability and phytotoxicity in military shooting range soil. Ecotoxicol Environ Saf 79:225–231
30. Uchimiya M, Klasson KT, Wartelle LH, Lima IM (2011) Influence of soil properties on heavy metal sequestration by biochar amendment: 2. Copper desorption isotherms. Chemosphere 82:1438–1447
31. Turkdogan MK, Fevzi K, Kazim K, Ilyas T, Ismail U (2003) Heavy metals in soil, vegetables and fruits in the endemic upper gastrointestinal cancer region of Turkey. Environ Toxicol Pharm 13:175–179
32. Lu H, Li Z, Fu S, Mendez A, Gasco G, Ferriero JP (2015) Combining phytoextraction and biochar addition improves soil biochemical properties in a soil contaminated with Cd. Chemosphere 119:209–216
33. Baccio D, Kopriva S, Sebastiani L, Rennenberg H (2005) Does glutathione metabolism have a role in the defence of poplar against zinc excess. New Phytol 167:73–80
34. Cameron RE (1992) Guide to site and soil description for hazardous waste site characterization, EPA/600/4-91/029, vol 1, Metals. US Environmental Protection Agency, Washington, DC
35. Manivasagaperumal R, Balamurugan S, Thiyagarajan G, Sekar J (2011) Effect of zinc on germination, seedling growth and biochemical content of cluster bean (Cyamopsis tetragonoloba (L.) Taub). Curr Bot 2:11–15
36. Gregory SJ, Anderson CWN, Arbestain MC, McManus MT (2014) Response of plant and soil microbes to biochar amendment of an arsenic-contaminated soil. Agric Ecosyst Environ 191:133–141
37. Wyszkowska J (2002) Soil contamination by chromium and its enzymatic activity and yielding. Pollut Environ Stud 11:79–84
38. Namgay T, Singh B, Singh BP (2010) Influence of biochar application to soil on the availability of As, Cd, Cu, Pb, and Zn to maize (Zea mays L.). Soil Res 48:638–647
39. Novak JM, Busscher WJ, Laird DL, Ahmedna M, Watts DW, Niandou MAS (2009) Impact of biochar amendment on fertility of a south eastern coastal plain soil. Soil Sci 174:105–112
40. Zhang X, Wang H, He L, Lu K, Sarmah A, Li J, Bolan NS, Pei J, Huang H (2013) Using biochar for remediation of soils contaminated with heavy metals and organic pollutants. Environ Sci Pollut Res 20:8472–8483
41. Cao X, Ma L, Liang Y, Gao B, Harris W (2011) Simultaneous immobilization of lead and atrazine in contaminated soils using dairy-manure biochar. Environ Sci Technol 45:4884–4889
42. Jianga J, Xua R, Jianga T, Li Z (2012) Immobilization of Cu(II), Pb(II) and Cd(II) by the addition of rice straw derived biochar to a simulated polluted Ultisol. J Hazard Mater 229–230:145–150

43. Cao X, Harris W (2010) Properties of dairy-manure-derived biochar pertinent to its potential use in remediation. Bioresour Technol 101:5222–5228
44. Jiang T, Jiang J, Xu R, Li Z (2012) Adsorption of Pb (II) on variable charge soils amended with rice-straw derived biochar. Chemosphere 89:249–256
45. Ahmad M, Lee SS, Lim JE, Lee S, Cho JS, Moon DH, Hashimoto Y, Ok YS (2014) Speciation and phytoavailability of lead and antimony in a small arms range soil amended with mussel shell, cow bone and biochar: EXAFS spectroscopy and chemical extractions. Chemosphere 95:433–441
46. Uzoma KC, Inoue M, Andry H, Fujimaki H, Nishihara E, Zahoor A (2011) Effect of cow manure biochar on maize productivity under sandy soil condition. Soil Use Manage 27:205–212
47. Lu H, Zhang W, YangY HX, Wang S, Qiu R (2012) Relative distribution of Pb 2D sorption mechanisms by sludge-derived biochar. Water Res 46:854–862
48. Uchimiya M, Bannon DI, Wartell LH, Lima IM, Klasson KT (2012) Lead retention by broiler litter biochars in small arms range soil: impact of pyrolysis temperature. Agric Food Chem 60:5035–5044
49. Younis U, Shah MHR, Danish S, Malik SA, Ameer A (2014) Biochar role in improving biometric and growth attributes of S. oleracea and T. corniculata under cadmium stress. Int J Biosci 5:84–90
50. Tong X, Li J, Yuan J, Xu R (2011) Adsorption of Cu (II)by biochars generated from three crop straws. Chem Eng J 172:828–834
51. Agrafioti E, Bouras G, Kalderis D, Diamadopoulos E (2013) Biochar production by sewage sludge pyrolysis. J Anal Appl Pyrolysis 101:72–78
52. Sun J, Lian F, Liu Z, Zhu L, Song Z (2014) Biochars derived from various crop straws: characterization and Cd(II) removal potential. Ecotoxicol Environ Saf 106:226–231
53. Uchimiya M, Lima IM, Klasson KT, Chang SC, Wartelle LH, Rodgers JE (2010) Immobilization of heavy metals ions (CuII, CdII, NiII, and PbII) by broiler litter-derived biochar in water and soil. J Agric Food Chem 58:5538–5544
54. Fisher D, Glaser B (2012) Synergism between compost & biochar for sustainable soil amelioration. Intech Open Sci/Open Mind 32:167–198
55. Novak JM, Busscher WJ (2012) Selection and use of designer biochars to improve characteristics of southeastern USA Coastal Plain degraded soils. In: Lee JE (ed) Advanced biofuels and bioproducts. Springer, New York
56. Yao Y, Gao B, Zhang M, Inyang M, Zimmerman AR (2012) Effect of biochar amendment on sorption & leaching of nitrate, ammonium& phosphate in a sandy soil. Chemosphere 89:1467–1471
57. Jones DL, Murphy DV, Khalid M, Ahmed W, Jones GE, Deluca TH (2011) Short term biochar-induced increase in soil CO_2 release in both biotically & abiotically mediated. Soil Biol Biochem 43:1723–1731
58. Ahmad M, Rajapaksha AU, Lim JE, Zhang M, Bolan N, Mohan D, Vithanage M, Lee SS, Ok YS (2014) Biochar as a sorbent for contaminant management in soil and water: a review. Chemosphere 99:19–33
59. Younis U, Qayyum MF, Shahi MHR, Malik SA (2014) Nutrient shift modeling in Fenugreek (Trigonella corniculata L.) under biochar and cadmium treatment. Int J Biosci 5:64–74
60. Bolan NS, Kunhikrishnan A, Choppala GK, Thangarajan R, Chung JW (2012) Stabilization of carbon in composts and biochars in relation to carbon sequestration and soil fertility. Sci Total Environ 424:264–270
61. Jiang J, Xu RK (2013) Evaluating role of alkali and organic functional group in Cu(II) immobilization. Bioresour Technol 133:537–545
62. Kolodyriska D, Wnetrzak R, Leahy JJ, Hayes MHB, Kwapiriski W, Hubicki Z (2012) Kinetic and adsorption characterization of biochar in metal ions removal. Chem Eng J 197:295–305
63. Xu X, Cao X, Zhao L, Wang H, Yu H, Gao B (2013) Removal of Cu, Zn and Cd from aqueous solutions by the dairy manure-derived biochar. Environ Sci Pollut Res 20:358–368

64. Ahmad M, Lee SS, RajapakshaAU VM, Zhang M, Cho JS, Lee SE, Ok YS (2013) Trichloroethylene adsorption by pine needle biochars produces at various pyrolysis temperature. Bioresour Technol 143:615–622
65. Ska DK, Trzak RW, Leahy JJ, Hayes MHB, Ski WK, Hubicki Z (2012) Kinetic and adsorption characterization of biochar in metal ions removal. Chem Eng J 197:295–305
66. Harvey OR, Kuo LJ, Zimmerman AR, Louchouarn P, Amonette JE, Herbert BE (2012) An index based approach to assessing recalcitrance and soil carbon sequestration potential of engineered black carbons (biochar). Environ Sci Technol 46:1415–1421
67. Beesley L, Jiménez EM, Eyles JLG (2010) Effects of biochar and green waste compost amendments on mobility, bioavailability and toxicity of inorganic and organic contaminants in a multi-element polluted soil. Environ Pollut 158:2282–2287
68. Khan S, Chao C, Waqas M, Arp HPH, Zhu YG (2013) Sewage sludge biochar influence upon rice (Oryza sativa L.) yield, metal bioaccumulation and greenhouse gas emissions from acidic paddy soil. Environ Sci Technol 47:8624–8632
69. Uchimiya M, Wartelle LH, Klasson T, Fortier CA, Lima IM (2011) Influence of pyrolysis temperature on biochar property and function as a heavy metal sorbent in soil. J Agric Food Chem 59:2501–2510
70. Karami N, Clemente R, Moreno-Jiménez E, Lepp NW, Beesley L (2011) Efficiency of green waste compost and biochar soil amendments for reducing lead and copper mobility and uptake to ryegrass. J Hazard Mater 191:41–48
71. Uchimiya M, Klasson KT, Wartelle LH, Lima IM (2011) Influence of soil properties on heavy metal sequestration by biochar amendment: 1. Copper sorption isotherms and the release of cations. Chemosphere 82:1431–1437
72. Ma JW, Wang H, Luo QS (2007) Movement-adsorption and its mechanism of Cd in soil under combining effect of electrokinetics and a new type of bamboo charcoal. Environ Sci 28:1829–1834
73. Beesley L, Marmiroli M (2011) Theimmobilisation and retention of soluble arsenic, cadmium and zinc by biochar. Environ Pollut 159:474–480
74. Beesley L, Dickinson N (2011) Carbon and trace element fluxes in the pore water of an urban soil following greenwaste compost, woody and biochar amendments, inoculated with the earthworm Lumbricus terrestris. Soil Biol Biochem 43:188–196
75. Debela F, Thring RW, Arocena JM (2012) Immobilization of heavy metals by Co-pyrolysis of contaminated soil with woody biomass. Water Air Soil Pollut 223:1161–1170
76. Zhou JB, Deng CJ, Chen JL, Zhang QS (2008) Remediation effects of cotton stalk carbon on cadmium (Cd) contaminated soil. Ecol Environ 17:1857–1860
77. Hartley W, Dickinson NM, Riby P, Lepp NW (2009) Arsenic mobility in brownfield soils amended with green waste compost or biochar and planted with Miscanthus. Environ Pollut 157:2654–2662
78. Fellet G, MarchiolL DVG, Peressotti A (2011) Application of biochar on mine tailings: effects and perspectives for land reclamation. Chemosphere 83:1262–1297
79. Choppala GK, Bolan NS, Mallavarapu M, Chen Z, Naidu R (2012) The influence of biochar and black carbon on reduction and bioavailability of chromate in soils. J Environ Qual 41:1–10
80. Mendez A, Gomez A, Ferreiro JP, Gasco G (2012) Effects of sewage sludge biochar on plant metal availability after application to a Mediterranean soil. Chemosphere 89:1354–1359
81. Suppadit T, Kitikoon V, Phubphol A, Neumnoi P (2012) Effect of quail litter biochar on productivity of four new physic nut varieties planted in Cadmium-contaminated soil. Chilean J Agric Res 72:125–132

Chapter 15
Heavy Metal Uptake and Tolerance Mechanisms of Serpentine Flora: Implications for Phytoremediation

Mihiri Seneviratne, Sumedha Madawala, and Meththika Vithanage

15.1 Introduction

Soil derived from ultramafic rocks or serpentinite (metamorphosed ultramafic rock) are termed as serpentine soils. These rocks cover about 1 % of the earth's surface. The term 'Ultramafic' is generally used to rocks which contain more than 70 % ferromagnesian materials [1–3]. Different pedogenesis processes lead to the formation of serpentine soil from the serpentinite rocks and these soils can be red, green, blue, or black in color, due to their chemical composition. The ultramafic rocks are known to contain high concentrations of potentially harmful elements including Ni, Cr, Mn and Co [4, 5]. Due to varying climatic conditions, nature of the parental material, tectonic activity, time and weathering processes, the heavy metal content of serpentine soils may differ from location to location [6–9].

The rocky granular textured soil, lack of organic material, low soil water-holding capacity, low Ca/Mg ratio, low nutrient content, and heavy metal toxicity in serpentine soils create an unfavorable environment for plant growth and development [10, 11]. Interestingly, there are several mechanisms in plants to withstand the heavy metal toxicity in serpentine soil. Due to the restricted soil chemical and physical characteristics, several plant species are evolved as serpentine endemics [12, 13]. Moreover, due to the chemistry of these soils, the serpentine associated groundwater and vegetation will differ from site to site with possible health inferences for

---

M. Seneviratne
Department of Botany, Faculty of Natural Sciences, Open University of Sri Lanka,
Nawala, Nugegoda, Sri Lanka

S. Madawala
Department of Botany, University of Peradeniya, Peradeniya, Sri Lanka

M. Vithanage (✉)
Chemical and Environmental Systems Modeling Research Group, National Institute
of Fundamental Studies, Kandy, Sri Lanka
e-mail: meththikavithanage@gmail.com

A.A. Ansari et al. (eds.), *Phytoremediation*, DOI 10.1007/978-3-319-40148-5_15

humans [14]. Studies have conducted in order to estimate the impact of heavy metals release from the ultramafic rocks to soil and water [15, 16]. Due to the transport of weathered parent rock fragments from upslope ultramafic sources, high concentrations of lithogenic Ni and Cr were observed in agricultural top soils of Thiva Valley in Central Greece [17]. The serpentine soil contains higher Ni (up to 2640 mg/kg) compared to Cr (up to 856 mg/kg). Plants and soils were tested from central Euboea, for Cr (total), Cr(VI), Ni, Mn, Fe, and Zn. Cr(VI) concentrations in soils extracted by alkaline digestion solution were lower than phytotoxic levels. However, foliar contents of Cr and Ni in majority of plants were near or above toxicity levels [17]. Franciscan Complex of California produces serpentine soils containing high concentrations of Cr as well as Ni, Co, and Mn. It has recorded that chromium-bearing igneous and metamorphic silicates in the protolith and Cr-bearing clay minerals in the soil are the major sources of bioavailable Cr [18].

Since some of these areas are associated with agriculture, it may create hazardous effects to the biota. It has observed that the agricultural soil of the valley Atalanti in central Greece has affected by the physical and chemical weathering of mafic and ultramafic rocks outcropping in the surrounding mountainous area, giving rise to high concentrations of Cr, Ni, and Co [15]. Nickel concentrations in groundwater were significantly lower than Cr, especially in the alluvial aquifer of Atalanti, since primary silicate minerals containing Ni in ultramafic rocks are relatively unstable in the surface environment. However, the mobile fraction of Ni is subsequently incorporated into secondary clay minerals and Fe–Mn oxides. Both ligands and protons hasten the release of Ni and Mn from serpentine soil into surrounding environments. Nickel is the seventeenth element recognized as essential for plant growth and development (Liu 2001). Plants' Ni requirement is the lowest of all essential elements at < 0.5 mg per kg of dry weight, making it an essential plant micronutrient [9, 19].

Interestingly, even under such extreme conditions, plant life exists. Serpentine flora exhibits unique features to adapt to its extreme micro-environment. The endemics have developed molecular to macro-level features to withstand these unfavorable conditions [20]. These marvels of nature can be used for biotechnological applications in phytoremediation approaches. A comprehensive understanding of cellular and biochemical processes is essential to step forward in further research. Even though studies have been conducted on heavy metal chemistry and serpentine flora in different regions, there is a lack of research carried on Ni hyperaccumulation mechanisms, especially on Ni transport across the plasma membrane and sequestration, which can be used for development of phytoremediation via biotechnological modifications. Moreover, there are many other cellular processes that are yet to be revealed to answer why they uptake these heavy metals without developing mechanisms to avoid them. Furthermore, there is a lack of comprehensive reviews on the significance of plant life on serpentine soils. Hence, this chapter focuses a discussion on the serpentine flora distributed throughout the world with their metal uptake mechanisms and tolerance in the light of phytoremediation aspects.

15.2 Plants in Serpentine Soil

Due to high Ni, Cr, Mn, and Co contents in serpentine soils, plants possess several mechanisms to tolerate them. There are distinct differences between plants which are grown in serpentine and nonserpentine soils, even though in the same genus [21]. An extraordinary uptake of heavy metals has been observed in some plants grown in Ni-rich serpentine soils originated from ultramafic rocks in various parts of the world. It has been revealed that about 1000 times greater Ni accumulation has been reported in hyperaccumulators (about 190 species) grown in Ni-rich serpentine soils derived from ultramafic rocks in various parts of the world [22]. Family Buxaceae is recognized as a serpentine plant family in Cuba with 30 species of serpentine endemics [23]. Genus *Buxus* is an evergreen shrub or a small tree. Moreover, the genus *Buxus* is reported in serpentine areas in Japan as well. The largest community of *Buxus microphylla* is located in Sugashima Island in Japan. *Buxus microphylla* showed the highest Ni concentration among the plants collected with 400 mg kg^{-1} of Ni [24]. The genus *Leucocroton*, in family Euphorbiaceae is a common thorny shrub found in xeromorphic serpentine areas in Cuba and there are 27 *Leucocroton* serpentine endemic species in Cuba [22]. Almost all serpentine endemic species are identified as hyperaccumulators while nonserpentine plants in the same area possess lower Ni content [22].

Alyssum bertolonii is the first Ni hyperaccumulator plant discovered from Italy by Minguzzi and Vergnano [25]. They observed a 7900 mg kg^{-1} of Ni in *Alyssum* leaves. *Alyssum serpyllifolium* (Brassicaceae) shows an extensive growth on both limestone and serpentine soils of Iberian Peninsula and southern France. *Alyssum* species are also found in southern Europe, including Portugal, where several ultramafic outcrops are present [26]. Interestingly, the family Brassicaceae contains the highest number (90 taxa) of Ni hyperaccumulators in the temperate region [27]. Due to infertile soil conditions, grass species are prominent in most of the serpentine areas. *Agrostis pallens, Aira caryophyllea, Avena barbata, Bromus carinatus, Bromus madritensis, Cortaderia selloana, Cynosurus echinatus, Elymus glaucus, Festuca arundinacea, Festuca bromoides, Hordeum brachyantherumm, Hordeum marinum, Hordeum murinum, Melica Californcia, Polypogon monspeliensis, Stipa lepida*, and *Stipa lepida* are some of the grass species that have been found in California [28, 29].

15.3 Plant Heavy Metal Uptake Mechanisms in Serpentine Soil

15.3.1 Effect of pH on Plant Heavy Metal Uptake

Hyperaccumulators are the plants that are able to uptake high doses of heavy metals. According to [30], metal hyperaccumulator plants are able to uptake metals from the soil, translocate them to their shoots, and concentrate in a range of 100- to

1000-fold more than the nonhyperaccumulating plants. The xylem translocation of metals from the roots to aerial parts is a characteristic feature of hyperaccumulator phenotype [31]. Hyperaccumulator plants are able to accumulate heavy metals in their shoot tissues at high concentrations. Even though the soil contains a high or low amount of heavy metals, the hyperaccumulating plants show an extraordinary ability to uptake metals from soils [33]. The soil heavy metal content, pH, Eh, soil water content, soil organic matter, and other elements in the rhizosphere affect the bioavailability and plant uptake of heavy metals [34, 35].

Changes in pH and redox potential in the rhizosphere soil were studied in a Ni hyperaccumulator plant (*Alyssum murale*) and of a crop plant, radish (*Raphanus sativus*). Differences in rhizosphere pH and reducing activity were found between the lateral and the main roots of both species, but the pH changes in the rhizosphere were similar in both species. Changes in pH were associated with the relative uptake of cations and anions and the source of nitrogen. However, the reducing activity of *A. murale* was always smaller than that of radish. They conclude that the reduction of pH in the rhizosphere or the release of reductants from roots do not involve in the metal uptake mechanism in hyperaccumulators [36].

The organic acids secreted by both plant root system and rhizospheric organisms cause pH reduction thus leading to mobilization of heavy metals. The amount of low molecular weight organic acids (LMWOAs) present in the rhizosphere of durum wheat cultivars, *Arcola* (low Cd accumulator), and *Kyle* (high Cd accumulator) was investigated [37]. The plants were grown for two weeks in pot cultures and the amounts of organic acids were quantified in water extracts of rhizospheric soil. However, water extractable organic acids were unable to be found in the bulk soil. This indicates that the root exudates and/or microbial metabolites at the soil/root interface are the major sources of organic acids found in the rhizosphere soil. Acetic and succinic acids were prominent in the water extracts while oxalic, fumaric, succinic, L-malic, tartaric, citric, acetic, propionic, and butyric acids were found in minor quantities. The total amount of water extractable LMWOAs in the soils ranged from 50.6 to 5520.3 nmol g^{-1} dry weight. According to the study carried by [37], higher amounts of LMWOAs were present in the rhizosphere soil of high Cd-accumulating cultivar *Kyle* than the low accumulating cultivar *Arcola* irrespective of the soil type.

15.4 Heavy Metal Transport Through Plasma Membrane

The heavy metal transport across the plasma membrane is a significant feature of hyperaccumulators. Heavy metal uptake and transport to different tissues, delivery to metal-requiring proteins in different subcellular localizations, metal storage, and remobilization requires the assistance of transition metal transporters. Membrane transport proteins and intracellular high-affinity binding sites involve in the uptake of metals across the plasma membrane. The zinc-regulated transporter, iron-regulated transporter protein (ZIP) family, the cation diffusion facilitator (CDF) family, the

P_{1B}-type subfamily of P-type ATPases, the natural resistance-associated macrophage protein (NRAMP) family, the yellow-stripe 1-like (YSL) subfamily of the oligopeptide transporter (OPT) superfamily, and three subfamilies of ATP-binding cassette (ABC) transporters play a vital role in metal transport across membranes [8, 38, 39].

High-affinity Zn^{2+} uptake, as well as low-affinity Cd^{2+} uptake was observed with *ZNT1* with Zn/Cd-hyperaccumulating *Thlaspi caerulescens*. Moreover, it was found that ZIP transporter is expressed at very high levels in roots and shoots of the hyperaccumulators. Furthermore, increased Zn influx in roots of the hyperaccumulating *Thlaspi* species was detected with respect to plant Zn status [40]. As in Cd/Zn hyperaccumulator, *Alyssum halleri* concentration showed a decreasing uptake of Cd by roots when supplied with increasing Zn. With respect to *A. halleri* and numerous ecotypes of *T. caerulescens*, it reveals that Cd influx is largely influenced due to Zn with a strong preference for Zn over Cd [41]. Moreover, the serpentine and the non-metallicolous populations of *Thlaspi caerulescens* showed low-affinity for Cd and Zn over Ni. This may be due to the Ni hyperaccumulation of the serpentine population in its natural environment. Moreover, this suggests that a Zn transport system might also lead to Ni entrance into roots [42].

Table 15.1 shows the membrane transport systems of heavy metals.

15.5 Heavy Metal Translocation from Root–Shoot

Nonhyperaccumulator plants retain most of the heavy metals in their root cells via detoxifying them by chelation in the cytoplasm or accumulating in vacuoles. Furthermore, hyperaccumulators are characteristically translocating heavy metals to the shoot through the xylem in an efficient manner [43]. It has been observed that lower sequestration into root vacuoles enhances the As translocation in hyperaccumulator plant *Pteris* sp. (*Pteris vittata* and *Pteris cretica*) compared with nonhyperaccumulator species such as *Nephrolepis exaltata* [44]. Due to the low association constants of malate and citrate (organic acids) toward the metals, their complexes make less bioavailable under cytosolic pH values. Moreover, they could be considered only within the acidic vacuolar environment [43]. Interestingly, a significant role in heavy metal hyperaccumulation seems to be played by free amino acids, such as histidine and nicotinamine, due to the formation of stable complexes with divalent cations [43, 45].

Nicotianamine is a metal-chelating, nonproteinogenous amino acid produced by the trimerization of *S*-adenosylmethionine catalyzed by nicotianamine synthase, which is essential in plant metal homeostasis. As nicotianamine synthase gene of *Arabidopsis thaliana* was expressed in transgenic tobacco plants, it led to a significant increase in iron levels in leaves and an increase in the tolerance ability against Ni (upto 1 mM) [46]. Furthermore, enhanced chelation of Ni(II) by nicotianamine in the xylem has been suggested as a mechanism of Ni tolerance in the hyperaccumulator *Thlaspi caerulescens*. Furthermore, Ni–NA complexes have been detected in roots of Ni-treated Zn-hyperaccumulator *Thlaspi caerulescens* [46]. Transgenic *Arabidopsis*

Table 15.1 Membrane transport systems of heavy metals

Transport system	Heavy metal	Plant	Reference
ZIP family	Zn/Cd	*Thlaspi caerulescens*	Pence et al. [40]
	Zn	*Arabidopsis thaliana*	Grotz et al. [69]
ATPase	Cd	*Lactuca sativa*	Costa and Morel [70]
	Cd	*Beta vulgaris*	Lindberg and Wingstrand [71]
	Cd	*Thlaspi caerulescens*	Bernard et al. [72]
	Cd	*Lupines albus*	Costa and Morel [73]
	Cd	*Zea mays*	Astolfi et al. [74], Pál et al. [75]
	Ni/Cd	*Oryza sativa*	Ros et al. [76]
	Cd	*Arabidopsis thaliana*	Courbot et al. [77]
	Zn/Cd	*Oryza sativa*	Satoh-Nagasawa et al. [78]
Nramp	Cd and Fe	*Arabidopsis thaliana*	Thomine et al. [79]
	Cd and Mn	*Oryza sativa*	Sasaki et al. [80]
	Cd	*Thlaspi caerulescens*	Zhao et al. [41]
	Cd	*Arabidopsis thaliana*	Vacchina et al. [46]
	Cd	*Lycopersicon esculentum*	López-Millán et al. [81]
	Ni	*Thlaspi japonicum*	Mizuno et al. [82]
CDF	Ni/Zn	*Thlaspi goesingense*	Kim et al. [83]
	Ni	*Alyssum lesbiacum*, *Brassica juncea*	Kerkeb and Krämer [49]
	Cd	*Arabidopsis halleri*	Zhao et al. [84]
ABC transporters	Cd	*Arabidopsis thaliana*	Benavides et al. [85]
	Cd/Pb	*Arabidopsis thaliana*	Kim et al. [86]
	As	*Arabidopsis thaliana*	Song et al. [87]
	Cd/Hg	*Arabidopsis thaliana*	Park et al. [88]

and tobacco plants showed an enhanced biosynthesis which granted a pronounced tolerance of high Ni levels. Moreover under high Ni concentrations, the activities of nicotianamine synthase genes in *Arabidopsis* were increased. Furthermore, transgenic tobacco plants with a high level of nicotianamine grew well in a Ni enriched serpentine soil without developing any Ni toxicity symptoms [47].

Free histidine (His) has been considered as one of the most important Ni-binding ligand that increases xylem transport of Ni. Due to the presence of carboxylate, amine, and imadazole function it acts as a tridentate ligand. Histidine has a relatively high association constant (8.7 lg K) toward Ni. High Ni concentrations have shown an activation of free histidine production in a larger proportion with hyperaccumulator *Alyssum lesbiacum* while there was no such indication in the nonaccumulator *Alyssum montanum* [48]. Moreover, Ni tolerance, Ni transport from roots to the shoots was enhanced with foliar spray or by addition to the root medium to nonaccumulator *Alyssum montanum* [49]. Similarly, an enhanced release of Ni into the xylem is coupled with the release of histidine from free histidine pool in roots of both

Alyssum lesbiacum and *Brassica juncea* [49]. Similarly Ingle et al. [50] has shown that the nonaccumulator *B. juncea* increased the root-to-shoot mobility of Ni with exogenous histidine. Furthermore, it has suggested that ATP-phosphoribosyltransferase (ATP-PRT) expression plays a major role in regulating the free histidine content, which causes the exceptional Ni tolerance of hyperaccumulator *Alyssum* species. It has suggested that the overexpression of genes coding for transport systems in non-hyperaccumulators is the cause efficient root-to-shoot heavy metal translocation hyperaccumulator plants.

YSL (Yellow Strip1-Like) family members are also considered as an important group involved in heavy metal translocation in plants. YSL family members assist the xylem loading and unloading nicotianamine metal (Ni) chelates [51]. Three *YSL* genes in *Thlaspi caerulescens* have been identified in hyperaccumulation of several heavy metals. Which were TcYSL7, TcYSL5 and TcYSL3. They were highly expressed respectively in the flowers, shoots and in all the organs tested. The transport activity of NA-metal chelates, upregulation, and the localization of the expression of these *TcYSL* genes around the vascular tissues are high and efficient in all organs. This feature of the *YSL* genes in *T. caerulescens* is important for the long-distance transport of metal ions (mainly Ni). The capacity of TcYSL3 to catalyze the influx of Ni–NA is vital for the entry of Ni–NA into the symplastic transport in the root for delivery to the xylem and for the unloading of the Ni–NA complexes from the xylem in the leaves to the final storage sites [52].

15.6 Storage and Detoxification of Heavy Metals and Excess Mg

As the heavy metals are taken up, they are directed for detoxification and storage. Detoxification and storage are major mechanisms that are shown by hyperaccumulators. Since the free form of heavy metals is toxic for plant biochemical and physiological activities, hyperaccumulators lead them to concentrate in aboveground organs without causing any phytotoxic effect [53]. The heavy metal detoxification sites in leaves are located in epidermis, trichomes, cuticles, and as latex to cause a minimal damage to the photosynthetic machinery [54, 55]. Accumulation of Ni in vacuoles of epidermal cells in *Hybanthus floribundus* (a hyperaccumulator of Ni) leaves was investigated at relatively high spatial resolution using energy-dispersive X-ray microanalysis (EDAX). Moreover, it was observed that Ni is concentrated on the outside of cell walls throughout the leaves, indicating that apoplastic compartmentation is also involved in Ni tolerance and accumulation in this plant [53].

CDF (cation diffusion facilitator) family members are proteins, which mediate bivalent cation transfer from the cytosol, across plasma membrane/tonoplast. It has been observed that CDF proteins are involved in mediating the vacuolar storage of Ni in *Thlaspi goesingense* shoots [56]. Organic acids are small ligands, which play a major role in detoxification. Where these ligands lead the bound form of metal cations and accumulate them in vacuoles. However, citrate is the main ligand of Ni in leaves of *Thlaspi goesingense* [37]. Phytochelatins (PC) are heavy metal binding peptides and it is another plant heavy metal immobilization mechanism [57]. PC synthase-deficient mutants of *Arabidopsis* have been used to extract the apparent role of PCs in heavy metal detoxification. It has revealed that, PCs play an important role in Cd and arsenate detoxification and there is no significant role shown in the detoxification of Zn, Ni, and selenite ions. During the detoxification process with respect to PC, the metal ion activates PC synthase followed by chelation by the PCs synthesized, and then transported to the vacuole and immobilize in the vacuole with sulfide or organic acids. Moreover, Zn and Ni are relatively ineffective activators of PC synthase in vitro [58].

Due to the high Mg:Ca in serpentine soils, the serpentine plants show tolerance towards high Mg and Ca levels. Most plants show a huge Mg requirement for maximum growth and some plants have several mechanisms to remove Mg from their leaves. The genetic basis of adaptation to serpentine soils in *Mimulus guttatus* was studied by [59]. It was observed that none—serpentine plants are unable to survive on serpentine soils. There was a significant difference in tolerance to low Ca:Mg in serpentine and nonserpentine populations of *M. guttatus*. The ecotypic differences in survival and tolerance were characterized using the genetic basis of quantitative trait locus (QTL) mapping. A single major QTL that controls both the ability to survive on serpentine soils and tolerance to low Ca:Mg ratio was identified. It suggests that the major QTL consists of different serpentine tolerance alleles which are not functionally equivalent [60].

It was clearly observed that the growth of crop plants retarded considerably as soil Ca levels dropped. However, the native serpentine species were not significantly affected by changing Ca concentrations. Analysis of plant tissues revealed that as both the plants were grown in soils with low Ca:Mg ratios, native serpentine species absorbed more Ca and less Mg than the crop plants. Because serpentine-tolerant species are able to absorb an adequate amount of Ca without absorbing an excessive amount of Mg [61]. Even though these plants absorb more Mg it is not toxic to the plant. Analysis of *Agrostis* spp. growing on serpentine soils shows that, as they uptake an excess amount of magnesium/calcium without causing negative effects on their survival [61]. As Ca plays a major role in plant cell wall stability and cell-to-cell adhesion [62], Ca deficiency causes lessening the strength of cell wall extension, breakdown of cell walls, and tissue destruction [32]. These symptoms are mostly seen in actively growing tissues. Serpentine plants need specialized physiological mechanisms to maintain a sufficient internal concentration of Ca under the higher Ca:Mg (<1) in soil [60].

High substratum concentrations of Mg in the serpentine soil are antagonistic to plant Ca uptake [32]. Due to immobilization nature of Ca, it shows a unidirectional transport which is from the root elongation zone to the shoot. Hence, the growing root tips should obtain the required Ca from its immediate environment [32]. High concentration of Mg in the serpentine soil causes induction of Ca deficiency in growing root tips, which leads necrosis of root tip [63].

15.7 Use of Serpentine Flora for Heavy metal Remediation

A number of species in the genus Alyssum has been recorded as Ni hyperaccumulators [50]. *Alyssum bertolonii* is a hyperaccumulator plant, which is grown in Ni-rich serpentine soil. It has an extraordinary Ni accumulating ability, which contains about 1 % Ni with respect to the dry matter. Experiments have carried out for the use of *Alyssum bertolonii* in phytomining of Ni. It was observed that there was no relationship between age of the plant and its Ni content. It has suggested that *Alyssum bertolonii* and other Alyssum species could be used for phytomining in Mediterranean area, Western Australia, and Western United States [64].

A number of species in the genus Alyssum has been recorded as Ni hyperaccumulators [50]. Ni phytoextraction was assessed in A. lesbiacum with phenanthrene and chrysene (polycyclic aromatic hydrocarbons). According to the results Alyssum lesbiacum has the ability to uptake Ni in polycyclic aromatic hydrocarbons contaminated soils [65]. *Thalspi* is also a plant, which is principally grown in Ni-rich contaminated soil. However, it has been indicated that *Thlaspi* is able to hyperaccumulate more than one metal. Principally, *Thlaspi* grows on Ni contaminated sites and accumulates about 3 % of its dry weight. Moreover *T. caerulescens* and *T. rotundifolium* can accumulate Cd, Ni, Zn and also Pb whereas *T. ochroleucum* hyperaccumulate Ni and Zn. Thereby species in the *Thlaspi* genus can be used to remediate heavy metal-contaminated sites [48, 66]. Moreover, *Berkheya coddii* is an asteraceous perennial plant grown in serpentine soils. It has reported as a hyperaccumulator, which accumulates about 1.16 % foliar Ni concentrations. Its higher Ni uptaking ability, high biomass, tolerance to cool climates render the hyperaccumulation ability to this plant in contaminated sites [67]. Furthermore, it has been revealed that the Ni-hyperaccumulator *Streptanthus polygaloides* could yield a 100 kg/ha of sulfur-free Ni [68].

15.8 Remarks

Serpentine soils derived from ultramafic rock depositions have given rise to a specialized vegetation, which shows tolerance to the heavy metal-rich less nutritious soil. To withstand against these harsh conditions a specialized flora has evolved associated with the serpentine areas, which distributed as patches in all over the world. This endemic vegetation is highly specialized to the particular regions with numerous mechanisms of heavy metal uptake and storage. The metal uptake is the most crucial part of the hyperaccumulators where un-heavy metal accumulators do not allow. Though numerous research has come up with heavy metal (As, Cd) transporters during recent decade, there is a gap to fill with respect to Ni transport. Interestingly, the xylem transport and vacuolar sequestration have studied in detail with important results. Moreover, the knowledge of cell biology of these plants can be used as biotechnological tools for further research in genetic engineering and phytomining activities.

Not like in normal soils serpentine soil plants are able to live under a higher Mg:Ca ratio. However, the mechanism that allows for greater growth rates and higher biomass in serpentine plants despite low calcium availability is not clear.

- Shoot translocation is one of the major features in serpentine vegetation. However, more research is needed for the clear understanding of mechanisms in metal chelation in long-distance transport of metal.
- Though many studies are conducted under other heavy metals such as Cd and As, research on Ni transporters across the plasma membrane is much lesser.
- More studies are suggested with the involvement of organic acids in heavy metal transport through the xylem.
- It has been revealed that hyperaccumulators create a high heavy metal concentration in their cytoplasm to keep a high osmotic potential to avoid excess metal uptake. However, it is not clear that osmotic potential is created via free ions or with bound forms. Moreover if it is in free-ion form the mechanisms to avoid heavy metal toxicity should be further revealed.
- These patches of ecosystems, which are distributed worldwide, consist of a wide genetic diversity. Numerous endemics, which consist vital biochemical compounds could be used as pharmaceuticals. More studies should be carried toward the biochemistry and genetic of such vegetations.
- Even though it is hypothesized that phytoaccumulation of heavy metals causes allelopathic effects on neighboring plants, there is not enough body of knowledge to confirm it.
- A comprehensive understanding of the metal transport processes in plants is essential for formulating effective strategies to develop genetically engineered plants that can accumulate specific metals.

References

1. Farough A, Moore D, Lockner D, Lowell R (2016) Evolution of fracture permeability of ultramafic rocks undergoing serpentinization at hydrothermal conditions: an experimental study. Geochem Geophys Geosyst. doi:10.1002/2015GC005973
2. Kruckeberg AR (1969) Soil diversity and the distribution of plants, with examples from western North America. Madroño 20:129–154
3. Robinson BH, Brooks RR, Kirkman JH, Gregg PE, Alvarez HV (1997) Edaphic influences on a New Zealand ultramafic ("serpentine") flora: a statistical approach. Plant and Soil 188:11–20
4. Cheng C-H, Jien S-H, Iizuka Y, Tsai H, Chang Y-H, Hseu Z-Y (2011) Pedogenic chromium and nickel partitioning in serpentine soils along a toposequence. Soil Sci Soc Am J 75:659–668
5. Oze C, Fendorf S, Bird DK, Coleman RG (2004) Chromium geochemistry of serpentine soils. Int Geol Rev 46:97–126
6. Garnier J, Quantin C, Guimarães EM, Vantelon D, Montargès-Pelletier E, Becquer T (2013) Cr (VI) genesis and dynamics in Ferralsols developed from ultramafic rocks: the case of Niquelândia, Brazil. Geoderma 193:256–264
7. Hseu Z-Y, Iizuka Y (2013) Pedogeochemical characteristics of chromite in a paddy soil derived from serpentinites. Geoderma 202:126–133

8. Sadegh MJM, Heidari A, Sarmadian F (2012) The role of pedogenic processes and soil characteristics on nickel distribution in some Oxiaquic Paleudalfs. Int Res J Appl Basic Sci 3(5):1032–1039
9. Vithanage M, Rajapaksha AU, Tang X, Thiele-Bruhn S, Kim KH, Lee S-E, Ok YS (2014) Sorption and transport of sulfamethazine in agricultural soils amended with invasive-plant-derived biochar. J Environ Manage 141:95–103
10. Rajakaruna N, Bradfield GE, Bohm BA, Whitton J (2003) Adaptive differentiation in response to water stress by edaphic races of Lasthenia californica (Asteraceae). Int J Plant Sci 164:371–376
11. Seneviratne M, Seneviratne G, Madawala H, Iqbal M, Rajakaruna N, Bandara T, Vithanage M (2015) A preliminary study of the role of bacterial–fungal co-inoculation on heavy metal phytotoxicity in serpentine soil. Aust J Bot 63:261–268
12. Bayer RJ, Figura PJ (2015) Antennaria sawyeri (Asteraceae: Gnaphalieae), a new serpentine endemic species from the Klamath mountains of Northern California. Syst Bot 40:620–626
13. Brady KU, Kruckeberg AR, Bradshaw H Jr (2005) Evolutionary ecology of plant adaptation to serpentine soils. Annu Rev Ecol Evol Syst 36:243–266
14. Cox SF, Chelliah MC, McKinley JM, Palmer S, Ofterdinger U, Young ME, Cave MR, Wragg J (2013) The importance of solid-phase distribution on the oral bioaccessibility of Ni and Cr in soils overlying Palaeogene basalt lavas, Northern Ireland. Environ Geochem Health 35:553–567
15. Kanellopoulos C, Argyraki A, Mitropoulos P (2015) Geochemistry of serpentine agricultural soil and associated groundwater chemistry and vegetation in the area of Atalanti, Greece. J Geochem Explor 158:22–33
16. Shchegolikhina A, Guadagnini L, Guadagnini A (2015) Genesis and transport of hexavalent chromium in the system ophiolitic rocks-groundwater, EGU General Assembly Conference Abstracts, p 754
17. Megremi I (2010) Distribution and bioavailability of Cr in central Euboea, Greece. Open Geosci 2:103–123
18. Oze C, Fendorf S, Bird DK, Coleman RG (2004) Chromium geochemistry in serpentinized ultramafic rocks and serpentine soils from the Franciscan complex of California. Am J Sci 304:67–101
19. Rajapaksha AU, Vithanage M, Oze C, Bandara W, Weerasooriya R (2012) Nickel and manganese release in serpentine soil from the Ussangoda Ultramafic Complex, Sri Lanka. Geoderma 189:1–9
20. Reeves RD, Laidlaw WS, Doronila A, Baker AJ, Batianoff GN (2015) Erratic hyperaccumulation of nickel, with particular reference to the Queensland serpentine endemic Pimelea leptospermoides. Aust J Bot 63:119–127
21. Chathuranga P, Dharmasena S, Rajakaruna N, Iqbal M (2015) Growth and nickel uptake by serpentine and non-serpentine populations of Fimbristylis ovata (Cyperaceae) from Sri Lanka. Aust J Bot 63:128–133
22. Reeves R, Baker A, Bgrhidi A, Berazain R (1996) Nickel-accumulating plants from the ancient serpentine soils of Cuba. New Phytol 133:217–224
23. Reeves R, Baker A, Borhidi A, Berazain R (1999) Nickel hyperaccumulation in the serpentine flora of Cuba. Ann Bot 83:29–38
24. Mizuno T, Kirihata Y (2015) Elemental composition of plants from the serpentine soil of Sugashima Island, Japan. Aust J Bot 63(4):252–260
25. Minguzzi C, Vergnano O (1948) Il contenuto di nichel nelle ceneri di Alyssum bertolonii. Atti Soc Tosc Sci Natur 55:49–77
26. Freitas H, Prasad M, Pratas J (2004) Analysis of serpentinophytes from north–east of Portugal for trace metal accumulation—relevance to the management of mine environment. Chemosphere 54:1625–1642
27. Ghaderian S, Mohtadi A, Rahiminejad R, Reeves R, Baker A (2007) Hyperaccumulation of nickel by two Alyssum species from the serpentine soils of Iran. Plant and Soil 293:91–97
28. Harrison S, Rice K, Maron J (2001) Habitat patchiness promotes invasion by alien grasses on serpentine soil. Biol Conserv 100:45–53

29. Huenneke LF, Hamburg SP, Koide R, Mooney HA, Vitousek PM (1990) Effects of soil resources on plant invasion and community structure in Californian serpentine grassland. Ecology 71:478–491
30. Brooks R, Lee J, Reeves RD, Jaffré T (1977) Detection of nickeliferous rocks by analysis of herbarium specimens of indicator plants. J Geochem Explor 7:49–57
31. Clemens S, Palmgren MG, Krämer U (2002) A long way ahead: understanding and engineering plant metal accumulation. Trends Plant Sci 7:309–315
32. Marschner H (2011) Marschner's mineral nutrition of higher plants. Academic, Amsterdam
33. Clemens S, Ma JF (2016) Toxic heavy metal and metalloid accumulation in crop plants and foods. Annu Rev Plant Biol 67:517–534
34. Adamczyk-Szabela D, Markiewicz J, Wolf WM (2015) Heavy metal uptake by herbs. IV. Influence of soil pH on the content of heavy metals in Valeriana officinalis L. Water Air Soil Pollut 226:1–8
35. Martínez-Alcalá I, Bernal MP, de la Fuente C, Gondar D, Clemente R (2016) Changes in the heavy metal solubility of two contaminated soils after heavy metals phytoextraction with Noccaea caerulescens. Ecol Eng 89:56–63
36. Bernal M, McGrath S, Miller A, Baker A (1994) Comparison of the chemical changes in the rhizosphere of the nickel hyperaccumulator Alyssum murale with the non-accumulator Raphanus sativus. Plant and Soil 164:251–259
37. Cieśliński G, Van Rees K, Szmigielska A, Krishnamurti G, Huang P (1998) Low-molecular-weight organic acids in rhizosphere soils of durum wheat and their effect on cadmium bioaccumulation. Plant and Soil 203:109–117
38. Hall J, Williams LE (2003) Transition metal transporters in plants. J Exp Bot 54:2601–2613
39. McGrath SP, Zhao F-J (2003) Phytoextraction of metals and metalloids from contaminated soils. Curr Opin Biotechnol 14:277–282
40. Pence NS, Larsen PB, Ebbs SD, Letham DL, Lasat MM, Garvin DF, Eide D, Kochian LV (2000) The molecular physiology of heavy metal transport in the Zn/Cd hyperaccumulator Thlaspi caerulescens. Proc Natl Acad Sci U S A 97:4956–4960
41. Zhao FJ, Hamon RE, Lombi E, McLaughlin MJ, McGrath SP (2002) Characteristics of cadmium uptake in two contrasting ecotypes of the hyperaccumulator Thlaspi caerulescens. J Exp Bot 53:535–543
42. Assunção AG, Bleeker P, Wilma M, Vooijs R, Schat H (2008) Intraspecific variation of metal preference patterns for hyperaccumulation in Thlaspi caerulescens: evidence from binary metal exposures. Plant and Soil 303:289–299
43. Haydon MJ, Cobbett CS (2007) Transporters of ligands for essential metal ions in plants. New Phytol 174:499–506
44. Poynton CY, Huang JW, Blaylock MJ, Kochian LV, Elless MP (2004) Mechanisms of arsenic hyperaccumulation in Pteris species: root As influx and translocation. Planta 219:1080–1088
45. Callahan DL, Baker AJ, Kolev SD, Wedd AG (2006) Metal ion ligands in hyperaccumulating plants. J Biol Inorg Chem 11:2–12
46. Vacchina V, Mari S, Czernic P, Marquès L, Pianelli K, Schaumlöffel D, Lebrun M, Lobinski R (2003) Speciation of nickel in a hyperaccumulating plant by high-performance liquid chromatography-inductively coupled plasma mass spectrometry and electrospray MS/MS assisted by cloning using yeast complementation. Anal Chem 75:2740–2745
47. Kim S, Takahashi M, Higuchi K, Tsunoda K, Nakanishi H, Yoshimura E, Mori S, Nishizawa NK (2005) Increased nicotianamine biosynthesis confers enhanced tolerance of high levels of metals, in particular nickel, to plants. Plant Cell Physiol 46:1809–1818
48. Charnock J, JAC JBAS (1996) Free histidine as a metal chelator in plants that accumulate nickel. Nature 379:635–638
49. Kerkeb L, Krämer U (2003) The role of free histidine in xylem loading of nickel in Alyssum lesbiacum and Brassica juncea. Plant Physiol 131:716–724
50. Ingle RA, Mugford ST, Rees JD, Campbell MM, Smith JAC (2005) Constitutively high expression of the histidine biosynthetic pathway contributes to nickel tolerance in hyperaccumulator plants. Plant Cell 17:2089–2106

51. Colangelo EP, Guerinot ML (2006) Put the metal to the petal: metal uptake and transport throughout plants. Curr Opin Plant Biol 9:322–330
52. Gendre D, Czernic P, Conéjéro G, Pianelli K, Briat JF, Lebrun M, Mari S (2007) TcYSL3, a member of the YSL gene family from the hyper-accumulator Thlaspi caerulescens, encodes a nicotianamine-Ni/Fe transporter. Plant J 49:1–15
53. Bidwell S, Crawford S, Woodrow I, Sommer-Knudsen J, Marshall A (2004) Sub-cellular localization of Ni in the hyperaccumulator, Hybanthus floribundus (Lindley) F. Muell. Plant Cell Environ 27:705–716
54. Freeman JL, Zhang LH, Marcus MA, Fakra S, McGrath SP, Pilon-Smits EA (2006) Spatial imaging, speciation, and quantification of selenium in the hyperaccumulator plants Astragalus bisulcatus and Stanleya pinnata. Plant Physiol 142:124–134
55. Küpper H, Lombi E, Zhao F-J, McGrath SP (2000) Cellular compartmentation of cadmium and zinc in relation to other elements in the hyperaccumulator Arabidopsis halleri. Planta 212:75–84
56. Persans MW, Nieman K, Salt DE (2001) Functional activity and role of cation-efflux family members in Ni hyperaccumulation in Thlaspi. Proc Natl Acad Sci U S A 98:9995–10000
57. Clemens S (2001) Molecular mechanisms of plant metal tolerance and homeostasis. Planta 212:475–486
58. Cobbett CS (2000) Phytochelatins and their roles in heavy metal detoxification. Plant Physiol 123:825–832
59. Selby JP (2014) The genetic basis of local adaptation to serpentine soils in Mimulus guttatus. Duke University, Durham
60. Ghasemi R, Chavoshi ZZ, Boyd RS, Rajakaruna N (2015) Calcium: magnesium ratio affects environmental stress sensitivity in the serpentine-endemic Alyssum inflatum (Brassicaceae). Aust J Bot 63:39–46
61. Proctor J (1971) The plant ecology of serpentine: III. The influence of a high magnesium/calcium ratio and high nickel and chromium levels in some British and Swedish serpentine soils. J Ecol 59:827–842
62. Jarvis M, Briggs S, Knox J (2003) Intercellular adhesion and cell separation in plants. Plant Cell Environ 26:977–989
63. O'Dell RE, James JJ, Richards JH (2006) Congeneric serpentine and nonserpentine shrubs differ more in leaf Ca: Mg than in tolerance of low N, low P, or heavy metals. Plant and Soil 280:49–64
64. Robinson B, Chiarucci A, Brooks R, Petit D, Kirkman J, Gregg P, De Dominicis V (1997) The nickel hyperaccumulator plant Alyssum bertolonii as a potential agent for phytoremediation and phytomining of nickel. J Geochem Explor 59:75–86
65. Singer AC, Bell T, Heywood CA, Smith J, Thompson IP (2007) Phytoremediation of mixed-contaminated soil using the hyperaccumulator plant Alyssum lesbiacum: evidence of histidine as a measure of phytoextractable nickel. Environ Pollut 147:74–82
66. Robinson BH, Leblanc M, Petit D, Brooks RR, Kirkman JH, Gregg PE (1998) The potential of Thlaspi caerulescens for phytoremediation of contaminated soils. Plant and Soil 203:47–56
67. Robinson B, Brooks R, Howes A, Kirkman J, Gregg P (1997) The potential of the high-biomass nickel hyperaccumulator Berkheya coddii for phytoremediation and phytomining. J Geochem Explor 60:115–126
68. Anderson C, Brooks R, Chiarucci A, LaCoste C, Leblanc M, Robinson B, Simcock R, Stewart R (1999) Phytomining for nickel, thallium and gold. J Geochem Explor 67:407–415
69. Grotz N, Fox T, Connolly E, Park W, Guerinot ML, Eide D (1998) Identification of a family of zinc transporter genes from Arabidopsis that respond to zinc deficiency. Proc Natl Acad Sci U S A 95:7220–7224
70. Costa G, Morel J (1994) Efficiency of H^+-atpase activity on cadmium uptake by four cultivars of lettuce. J Plant Nutr 17:627–637
71. Lindberg S, Wingstrand G (1985) Mechanism for Cd^{2+} inhibition of (K^{++} Mg^{2+}) ATPase activity and K^+ ($86Rb^+$) uptake join roots of sugar beet (Beta vulgaris). Physiol Plant 63:181–186

72. Bernard C, Roosens N, Czernic P, Lebrun M, Verbruggen N (2004) A novel CPx-ATPase from the cadmium hyperaccumulator Thlaspi caerulescens. FEBS Lett 569:140–148
73. Costa G, Morel J (1993) Cadmium uptake by Lupinus albus (L.): cadmium excretion, a possible mechanism of cadmium tolerance. J Plant Nutr 16:1921–1929
74. Astolfi S, Zuchi S, Passera C (2005) Effect of cadmium on H^+ ATPase activity of plasma membrane vesicles isolated from roots of different S-supplied maize (Zea mays L.) plants. Plant Sci 169:361–368
75. Pál M, Horváth E, Janda T, Páldi E, Szalai G (2006) Physiological changes and defense mechanisms induced by cadmium stress in maize. J Plant Nutr Soil Sci 169:239–246
76. Ros R, Morales A, Segura J, Picazo I (1992) In vivo and in vitro effects of nickel and cadmium on the plasmalemma ATPase from rice (Oryza sativa L.) shoots and roots. Plant Sci 83:1–6
77. Courbot M, Willems G, Motte P, Arvidsson S, Roosens N, Saumitou-Laprade P, Verbruggen N (2007) A major quantitative trait locus for cadmium tolerance in Arabidopsis halleri colocalizes with HMA4, a gene encoding a heavy metal ATPase. Plant Physiol 144:1052–1065
78. Satoh-Nagasawa N, Mori M, Nakazawa N, Kawamoto T, Nagato Y, Sakurai K, Takahashi H, Watanabe A, Akagi H (2012) Mutations in rice (Oryza sativa) heavy metal ATPase 2 (OsHMA2) restrict the translocation of zinc and cadmium. Plant Cell Physiol 53:213–224
79. Thomine S, Wang R, Ward JM, Crawford NM, Schroeder JI (2000) Cadmium and iron transport by members of a plant metal transporter family in Arabidopsis with homology to Nramp genes. Proc Natl Acad Sci U S A 97:4991–4996
80. Sasaki A, Yamaji N, Yokosho K, Ma JF (2012) Nramp5 is a major transporter responsible for manganese and cadmium uptake in rice. Plant Cell 24:2155–2167
81. López-Millán A-F, Sagardoy R, Solanas M, Abadía A, Abadía J (2009) Cadmium toxicity in tomato (Lycopersicon esculentum) plants grown in hydroponics. Environ Exp Bot 65:376–385
82. Mizuno T, Usui K, Horie K, Nosaka S, Mizuno N, Obata H (2005) Cloning of three ZIP/Nramp transporter genes from a Ni hyperaccumulator plant Thlaspi japonicum and their Ni^{2+}-transport abilities. Plant Physiol Biochem 43:793–801
83. Kim D, Gustin JL, Lahner B, Persans MW, Baek D, Yun DJ, Salt DE (2004) The plant CDF family member TgMTP1 from the Ni/Zn hyperaccumulator Thlaspi goesingense acts to enhance efflux of Zn at the plasma membrane when expressed in Saccharomyces cerevisiae. Plant J 39:237–251
84. Zhao F, Jiang R, Dunham S, McGrath S (2006) Cadmium uptake, translocation and tolerance in the hyperaccumulator Arabidopsis halleri. New Phytol 172:646–654
85. Benavides MP, Gallego SM, Tomaro ML (2005) Cadmium toxicity in plants. Braz J Plant Physiol 17:21–34
86. Kim DY, Bovet L, Maeshima M, Martinoia E, Lee Y (2007) The ABC transporter AtPDR8 is a cadmium extrusion pump conferring heavy metal resistance. Plant J 50:207–218
87. Song W-Y, Park J, Mendoza-Cózatl DG, Suter-Grotemeyer M, Shim D, Hörtensteiner S, Geisler M, Weder B, Rea PA, Rentsch D (2010) Arsenic tolerance in Arabidopsis is mediated by two ABCC-type phytochelatin transporters. Proc Natl Acad Sci U S A 107:21187–21192
88. Park J, Song WY, Ko D, Eom Y, Hansen TH, Schiller M, Lee TG, Martinoia E, Lee Y (2012) The phytochelatin transporters AtABCC1 and AtABCC2 mediate tolerance to cadmium and mercury. Plant J 69:278–288

Chapter 16
Phytoremediation: Uptake and Role of Metal Transporters in Some Members of Brassicaceae

Ritu Chaturvedi, Mayank Varun, and Manoj S. Paul

16.1 Introduction

Phytoremediation (Greek *phyto*-plant, Latin *remedium*-to cure) is a generic term used for the treatment of environmental contamination through plants via their biological, chemical, or physical activities. Living, green plants can be used to treat soils, sludges, sediments, surface water, and ground water to remove, detoxify, or immobilize contaminants in the respective growth matrix since they are unique organisms in the sense that they have remarkable capacities for absorbing and transporting nutrients and contaminants from the growth matrix. They can be used effectively to remediate sites with shallow and low-to-moderate level of contamination.

The process involves planting the material in the contaminated area, growing it for required time, and harvesting it followed by disposing it as hazardous waste. As the conventional practices like dig and dump, pump and treat, and chemical methods are expensive and do not provide a permanent cure, *Phytoremediation* emerges as a cost-effective and solar energy-driven process. Phytoremediation consists of different plant-based technologies each having a different mechanism of action for the remediation of metal-polluted soil, sediment, or water. The various pathways of soil–metal–plant interaction have been elucidated in Fig. 16.1.

Phytodegradation utilizes the rhizospheric associations between plants and soil microorganisms to degrade organic-metal contaminant mixtures [1]. This approach is suitable for organic contaminants like TNT, PAHs, and petroleum hydrocarbons. *Phytoextraction* is the most commonly recognized of all phytoremediation technologies. It relies on metal-accumulating plants to transport and concentrate metals from soil into the harvestable aboveground shoots [2, 3]. Pollutants accumulated in stems and leaves are harvested and removed from the site. Such plants can accumulate As, Cu, Co, Cd, Mn, Ni, Se, Pb, and Zn up to levels that are 100–1000

R. Chaturvedi (✉) • M. Varun • M.S. Paul
Department of Botany, St. John's College, Agra 282 002, India
e-mail: rituchaturvedi88@gmail.com

A.A. Ansari et al. (eds.), *Phytoremediation*, DOI 10.1007/978-3-319-40148-5_16

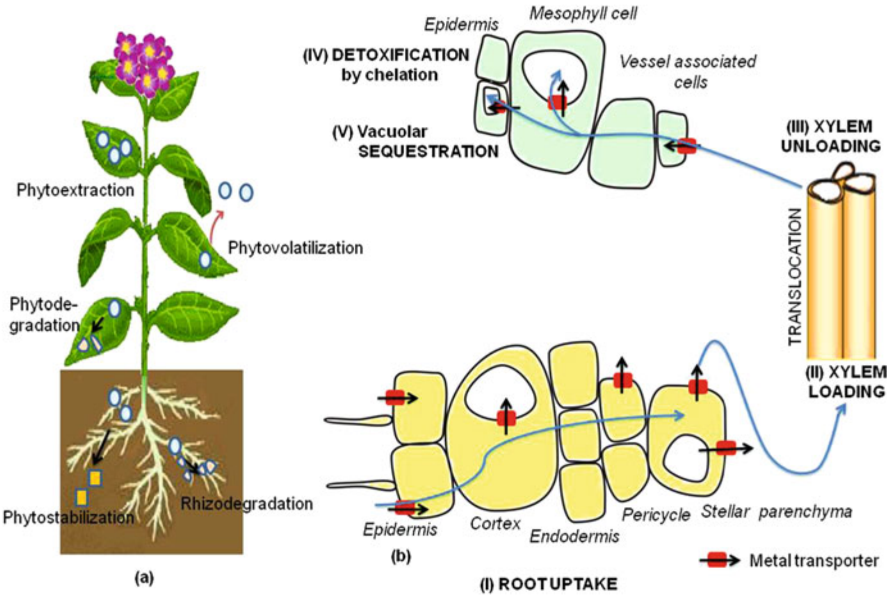


Fig. 16.1 (**a**) Phytoremediation strategies and (**b**) metal transporter and mechanism of uptake

times of those normally accumulated by plants grown under the same conditions [4–6]. *A. halleri* can hyperaccumulate Zn [7]. *Rhizofiltration* utilizes plant roots to take up and sequester metal contaminants and/or excess nutrients from aqueous growth substrates [8]. Plants can be used in constructed wetlands [9, 10] or in a hydroponic setup [11] acting as a biogeochemical filter, efficiently removing contaminants from wastewater. *Phytostabilization* utilizes tolerant plants to inhabit heavy metal contaminated environments where they prevent soil erosion, reduce heavy metal leaching, and minimize uptake, hence effectively containing and minimizing the spread of heavy metals [12–14].

Phytovolatilization uses the ability of plants to absorb and subsequently volatilize the contaminant into the atmosphere. This approach is suitable for remediating metal (loid)s that may exist in gaseous form in the environment such as As, Hg, and Se [15, 16]. Although it is a passive process, it may be maximized by using plants with higher transpiration rates, by overexpression of enzymes that mediates S/Se volatilization [17] and also by transferring gene for Se volatilization from hyperaccumulators to non-accumulators [18]. The environmental impact of the conventional technologies discussed before can be very high. Most of these are invasive and employ heavy, noisy, large construction equipment that is costly and can potentially spread the contamination [19]. Implementing phytoremediation as a natural environment can enhance or restore the physical appearance of a site.

Potential role of phytoremediation, especially in higher plants in remediation of metal-polluted soils has been studied by an increasing number of scientists from various disciplines including plant scientists, microbiologist, chemists, soil scientists, and geologists owing to its commercial importance [20, 21]. Some species recommended as phytoextractors are *Calotropis procera* for Zn and As; *Datura stramonium* for Mn, Cr, Cu, and As; *Chenopodium murale* for Zn, Cd, Pb, and Cu; *Lycopersicum esculentum* for Cd, Cr and As; and *Poa annua* for Pb, Cd and As; *Rumex dentatus* for Pb; *Amaranthus spinosus*, *Parthenium hysterophorus*, *Croton bonplandianum*, and *Ricinus communis* for As. While recommended phytostabilizers are *Calotropis procera* for Zn, Mn, Cd, Pb, and Cu; *Poa annua* for Mn, Cd, and As; *Gnaphalium luteo-album* for Mn and As; *Withania somnifera* for Cu; *Eclipta alba* for Mn, *Argemone mexicana* for Pb and *Heliotropium ellipticum* for As [22, 23].

Some examples of plants being used as effective remediators are: *Hordeum vulgare* and *Hydrangea* spp. for aluminum contaminated sites, *Brassica juncea* and *Brassica napus* for silver, chromium, selenium, zinc and lead contaminated sites, *Thlaspi caerulescens* for copper, chromium, lead, molybdenum, and zinc contaminated sites; *Azolla* spp. for chromium, copper, manganese, and lead contaminated sites; *Helianthus annuus* for copper, chromium, manganese, and lead contaminated sites; *Salix* spp. for silver, selenium, chromium, mercury, and zinc contaminated sites; *Lemna minor* for lead and copper contaminated sites.

16.2 How Plants Work as Remediators?

Plants absorb water and nutrients from the growth matrix by their root system. While water is absorbed passively through osmosis and passes from the cortex to the vascular bundles through apoplastic or symplastic pathways, minerals are not absorbed passively. Since the minerals are present in charged form in the matrix they cannot move across cell membranes. Moreover, concentration of minerals in the soil is usually lower than the concentration of minerals in roots. Hence, minerals need to be actively absorbed by the epidermal cells. Specific proteins in the membranes of root hairs actively pump ions from the soil to the epidermal cells. It is with these minerals that the heavy metal ions also find an entry or gateway in the plant system as these transporters are not too specialized. They are general in behavior in the way that they allow similar ions to pass through. This may lead to metal toxicity in plants for which plants have different stress mechanisms to cope with. Plants may use efflux pumps or signal transduction cascades to regulate gene expression and production of organic acids which help them survive in the presence of these abiotic stress-causing agents.

16.2.1 The Process

At the root level and subsequently at the shoot level, five stages are essential for phytoremediation to take place namely dissolution, chelation, transportation, storage, and damage curation. For the plant root to extract a metal from its matrix, the metal should be dissolved and available in a form that roots can absorb. Metals cannot be absorbed in their stable states in any organism. They need to be present in ionic states dissolved in a solution for uptake. Certain valence forms are highly preferred over others. Plant roots may affect the availability of nutrients at root–soil interface level by releasing root exudates (inorganic and organic compounds) in the rhizosphere which mobilize or immobilize the contaminants in the rhizosphere and also influence the plant-assisted microbial activity.

Once the metal ion is available and mobile, it gets absorbed and transported over the root cell wall by a specific metal transporter or carried over by a specific agent (Fig 16.1). After being absorbed, the water and minerals along with contaminants reach the xylem in the form of a sap and move along the transpiration stream. Metal ions are also transported in phloem. The sap moves against the gravitational force from source to sink, i.e., from root to shoot and circulates in the various parts of plant.

Transportation of heavy metals is a critical phenomenon as heavy metals damage the plant before they are stored. Storage in hyperaccumulators often takes place in the leaves. Transportation is regulated by metal transporters and these metal transporters are regulated by gene expression. Genes coding for metal transporters have been identified and are present in both hyperaccumulators and non-accumulators, but their activity is highly regulated. There is considerable evidence suggesting that hyperaccumulators have overexpressed their metal transporters in the presence of respective metal ions. Besides some amount of gene regulation is always present based on the presence or absence of metal ions in non-accumulator plants also.

16.2.2 Metal Availability and Uptake

In order to grow and carry their life cycle, plants need two kinds of nutrients—organic and inorganic. While organic nutrition focuses on the formation of carbon compounds through CO_2 and H_2O via photosynthesis; inorganic nutrition is dependent on acquisition of mineral elements from soil. Out of the 17 essential nutrient elements required for plant growth, 8 (Cl, B, Fe, Mn, Zn, Cu, Ni, Mo) are trace elements or micronutrients. Some of these (e.g., Fe, Mn, Zn, Cu, Ni) are heavy metals and are extremely toxic for both plants and environment. In spite of the apparent toxicity of micronutrients, many plant species have developed the capacity to tolerate extraordinarily high concentrations. For example, most plants are severely injured by nickel concentrations in excess of 5 $\mu g\ g^{-1}$ dry weight, but species of the genus *Alyssum* can tolerate levels in excess of 10,000 $\mu g\ g^{-1}$ dry weight. Different plants show different tolerance levels for a particular metal. The critical toxicity levels for manganese vary from 200 $\mu g\ g^{-1}$ dry weight for corn, to 600 $\mu g\ g^{-1}$ for soybean, and 5300 $\mu g\ g^{-1}$ for sunflower.

16.3 Metal Transporters and Their Role

Various metal transporters have been identified in *Arabidopsis* till date namely Zinc-regulated transporter (ZRT), Iron regulated transporter (IRT), ZRT-, IRT-like proteins (ZIP), Natural resistance-associated macrophage proteins (NRAMP), Cation diffusion facilitator (CDF)/Metal tolerance protein (MTP), and Heavy metal ATPase (HMA), Endomembrane-type CA-ATPase (ECA), Magnesium Proton Exchanger (MHX), and Detoxification (DTX); we searched for their orthologs in *A. halleri*, *B. oleracea*, and *B. rapa* bioinformatically and generated an inventory (Table 16.1). Sequences were downloaded from TAIR, Phytozome v 10.3, and Brassica Database for *A. thaliana*, *A. halleri*, and *Brassica* sp., respectively.

ZIP is a member of Fe(II) transporter isolog family involved in zinc ion transmembrane transport integral to plasma membrane. The family is characterized by the presence of eight putative transmembrane spanners. IRT is involved in Zn ion transmembrane transport integral to plasma membrane. The *Arabidopsis* genome consists of 15 members of ZIP family. Ten ZIP orthologs were found in *A. halleri* and nine in *B. rapa*. No ZIP orthologs were found in *B. oleracea* suggesting that some other transport proteins are present for the transport of Zn in these plants. Overexpression of *AtZIP1* in *Cassava* tuber has shown to increase Zn concentration in the storage organ [24]. Transporters such as ZIP1, ZIP3, ZIP4 ZIP6, ZIP9 ZIP10, ZIP12, and IRT3 have been shown to be expressed higher in *A. halleri* than *A. thaliana* [25–28].

Nramp is a novel family of related proteins involved in the transport of divalent metal ions. The Nramp gene family has been highly conserved during evolution and its homologs have been found in a wide range of living organisms. In *Arabidopsis*, six genes *AtNramp1-6* encode Nramp proteins. Six orthologs were found in *A. halleri* and *B. oleracea* and eleven in *B. rapa*. MTP encodes a golgi-localized manganese transporter that is involved in Mn tolerance. MTPs are metal efflux transporters from the cytoplasm transporting mainly Zn^{2+} but also Mn^{2+}, Fe^{2+}, Cd^{2+}, Co^{2+}, and Ni^{2+}. In Zn hyperaccumulator plants, the MTP1 protein is related to hypertolerance to elevated Zn concentrations [29]. Five MPT proteins are encoded in *A. thaliana* and three orthologs were found in *A. halleri* and *B. rapa* and one in *B. oleracea*.

HMA is involved in transport of Cu^{2+}, Zn^{2+}, Cd^{2+}, and Pb^{2+}. In *Arabidopsis*, eight genes *AtHMA1-8* encode HMA proteins. Five orthologs were found in *A. halleri*, fourteen in *B. rapa*, and twelve in *B. oleracea*. In recent studies, it has been shown that *AtHMA2* and *AtHMA4* orthologs in tobacco plants show reduced Zn and Cd concentration in shoots when silenced [30]. Employing *Arabidopsis* microarrays, cross-species transcriptomics identified a number of candidate genes that are more highly expressed in the Zn/Cd-hypertolerant metal hyperaccumulator *Arabidopsis halleri* than in the closely related non-tolerant non-accumulator species *Arabidopsis thaliana* [31]. *HMA3* is highly expressed in the Zn/Cd hyperaccumulators *Noccaea caerulescens* (previously named *Thlaspi caerulescens*) and *Arabidopsis halleri* [32, 33] suggesting it may play a positive role in Zn/Cd hyperaccumulation.

MHX encodes an magnesium/proton exchanger involved in Zn^{2+} and Fe^{2+}. *AtMHX1* gene encodes a Mg^{2+}/H+ exchanger in *Arabidopsis* genome. One homolog

Table 1 Inventory of heavy metal transporters in *A. thaliana* and their orthologs in *A. halleri*, *B. oleracea* and *B. rapa*

Gene family	Gene i.d.				Functional annotation
	A. thaliana	*A. halleri*	*B. oleracea*	*B. rapa*	
ZIP	AT3G12750.1	Araha.3837s0009.1		Bra020314	Zn2+/Fe2+ transporter
	AT5G59520.1	Araha.7283s0002.1		Bra005579	also transports Mn2+
	AT2G32270.1	Araha.17510s0004.1		Bra021776	Cd2+,Ni2+
	AT1G10970.1	Araha.24726s0001.1		Bra031470	
	AT1G05300.1	Araha.10595s0008.1		Bra018341	
	AT2G30080.1	Araha.17932s0003.1		Bra011406	
	AT2G04032.1	Araha.5635s0015.1		Bra023171	
	AT5G45105.1	Araha.25051s0001.1		Bra031470	
	AT4G33020.1	Araha.28434s0003.1		Bra027108	
	AT1G31260.1	Araha.0881s0001.1			
	AT1G55910.1				
	AT5G62160.1				
	AT4G19690.1				
	AT4G19680.1				
	AT1G60960.1				
Nramp	AT1G80830.1	Araha.6467s0014.1	Bol038570	Bra008437	Cd2+ transporter
	AT1G47240.1	Araha.15903s0001.1	Bol038186	Bra003587	
	AT2G23150.1	Araha.44080s0002.1	Bol045179	Bra035149	
	AT5G67330.1	Araha.28176s0001.1	Bol045810	Bra026089	
	AT4G18790.1	Araha.1484s0004.1	Bol015033	Bra033988	
	AT1G15960.1	Araha.1306s0004.1	Bol024410	Bra040791	
				Bra040711	
				Bra000573	
				Bra012154	
				Bra039185	
				Bra012577	

MTP	AT2G46800.1	Araha.11074s0002.1	Bol025344	Bra039979	Zn2+, Mn2+, Fe2+, Co2+
	AT3G61940.1	Araha.36627s0001.1		Bra017071	Cd2+, Ni2+ transporter
	AT2G29410.1	Araha.13755s0002.1		Bra005039	
	AT3G58810.1				
	AT2G39450.1				
HMA	AT4G37270.1	Araha.0482s0007.1	Bol028928	Bra011750	Cu2+ , Zn2+, Cd2+
	AT4G30110.1	Araha.1261s0006.1	Bol044715	Bra039194	Pb2+ transporter
	AT4G30120.1	Araha.12160s0001.1	Bol033637	Bra011164	
	AT2G19110.1	Araha.0854s0004.1	Bol020969	Bra024107	
	AT1G63440.1	Araha.26867s0001.1	Bol041580	Bra038842	
	AT4G33520.1		Bol020970	Bra024449	
	AT5G44790.1		Bol033641	Bra011165	
	AT5G21930.1		Bol030251	Bra024103	
			Bol030701	Bra027641	
			Bol032030	Bra003110	
			Bol004464	Bra027573	
			Bol035748	Bra025102	
				Bra002374	
				Bra011450	
MHX	AT2G47600.1	Araha.1610s0017.1		Bra004443	Na+/Ca2+ exchanger ; Zn2+, Fe2+, Mg2+

(continued)

Table 1 (continued)

Gene family	Gene i.d.				Functional annotation
	A. thaliana	*A. halleri*	*B. oleracea*	*B. rapa*	
ECA	AT1G07810.1	Araha.5007s0005.1	Bol007671	Bra031593	Mn2+ transporter
	AT1G10130.1	Araha.13184s0008.1	Bol006517	Bra029645	
			Bol011452	Bra037404	
			Bol031235	Bra031583	
			Bol036766	Bra031701	
			Bol041160	Bra019960	
DTX	AT2G04040.1	Araha.50900s0001.1	Bol028159	Bra013214	Cd2+ transport
			Bol042498	Bra015131	
			Bol009761	Bra015133	
			Bol028158	Bra030999	

was found in *A. halleri* and *B. rapa*. In *Arabidopsis*, there are two characterized proteins AtECA1 And AtECA3 localized in ER and golgi apparatus, respectively. They function as Mn^{2+} pumps that remove Mn^{2+} from the cytosol and deliver it to their endomembrane compartment. Two orthologs were found in *A. halleri* and five each in *B. rapa* and *B. oleracea*. DTX has been identified as a detoxifying efflux carrier for plant-derived antibiotics and other toxic compounds including Cd^{2+}. It is involved in Cd^{2+} transport and sequestration. One homolog was found in *A. halleri* and four each in *B. rapa* and *B. oleracea*.

16.4 Transporters Across Genomes

Throughout evolution and development some protein families have remained highly conserved as they are essential for survival. Genes encoding metal transporters are conserved across the plant kingdom and their regulation separates the hyperaccumulators from non-accumulators. Genes involved in hyperaccumulation are differently expressed and regulated, compared to non-hyperaccumulator species. Gene duplication may contribute to the ability of hyperaccumulation to express genes at very high level. Comparative transcript profiling of metal hyperaccumulator and non-accumulator show that transporters are overexpressed in hyperaccumulators as compared to non-accumulators [34]. Several species of the Brassicaceae family like *Arabidopsis halleri* and *Noccaea caerulescens* are metal hyperaccumulators. *N. caerulescens* was the first identified Zn hyperaccumulator and was reported to accumulate about 25,000–30,000 $\mu g\ g^{-1}$ dry weight (DW) of total Zn [35, 36]. *A. halleri* is a Zn/Cd hyperaccumulator [25, 37–40] and can accumulate >10,000 and >100 $\mu g\ g^{-1}$ DW of Zn and Cd, respectively. *A. halleri* is closely related to *A. thaliana*, a non-hyperaccumulator whose genome has been thoroughly explored. These species diverged about 5 million years ago and they show, on an average, around 94 % nucleotide identity within coding regions [41]. Thus, *A. halleri* is a good model system to study hyperaccumulation mechanisms. These mechanisms, if present in other plants, whether naturally or through genetic engineering, can render them the ability to accumulate metals and can be used as effective phytoremediators.

A way to study the evolutionary relationships among these species in terms of conservation of these metal transporters is through phylogeny. A phylogenetic tree is a diagram that depicts the lines of evolutionary descent of different species, organisms, or genes from a common ancestor. Phylogenetic trees are based on the similarities and differences in physical characteristics and genetic makeup. Hence, sequences of heavy metal transport proteins already identified and studied in model plant *A. thaliana* were downloaded and searched for their orthologs in genomes of plants under study. The sequences of the orthologs found were used to generate a phylogenetic tree to study the evolutionary relationships among all these members of the family Brassicaceae. Phylogenetic trees were generated using the software MEGA 4.0.2 with the help of full length amino acid sequences, by neighbor joining method (Figs. 16.2, 16.3, 16.4, 16.5, 16.6, 16.7, and 16.8). The scale corresponds to

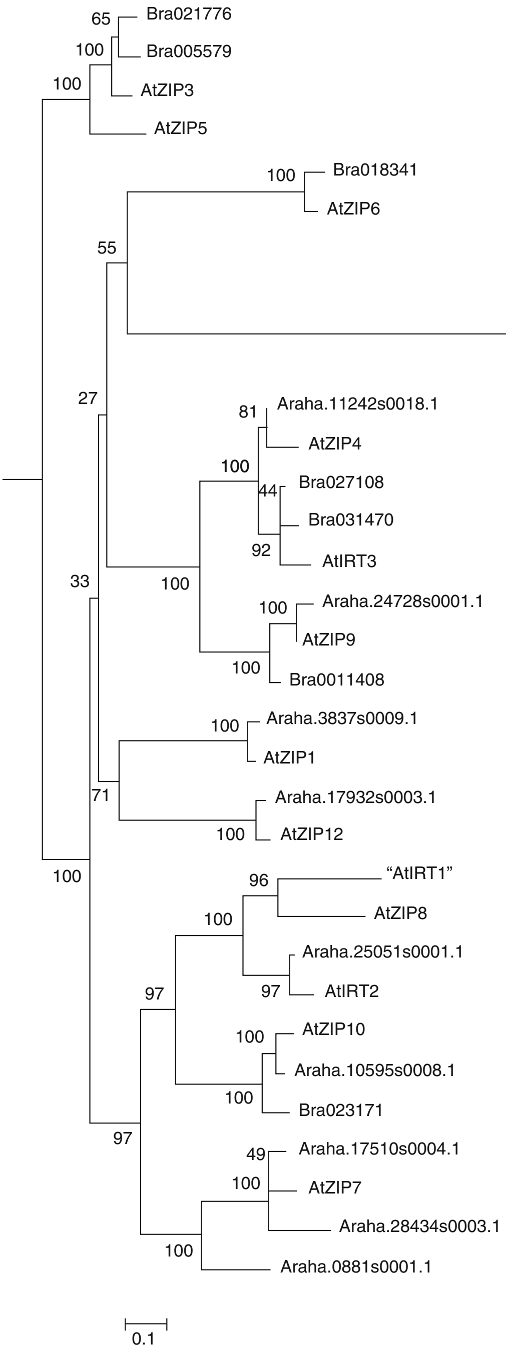


Fig. 16.2 Phylogenetic tree to show relationship among ZIP proteins in *A. thaliana*, *A. halleri*, and *B. rapa*

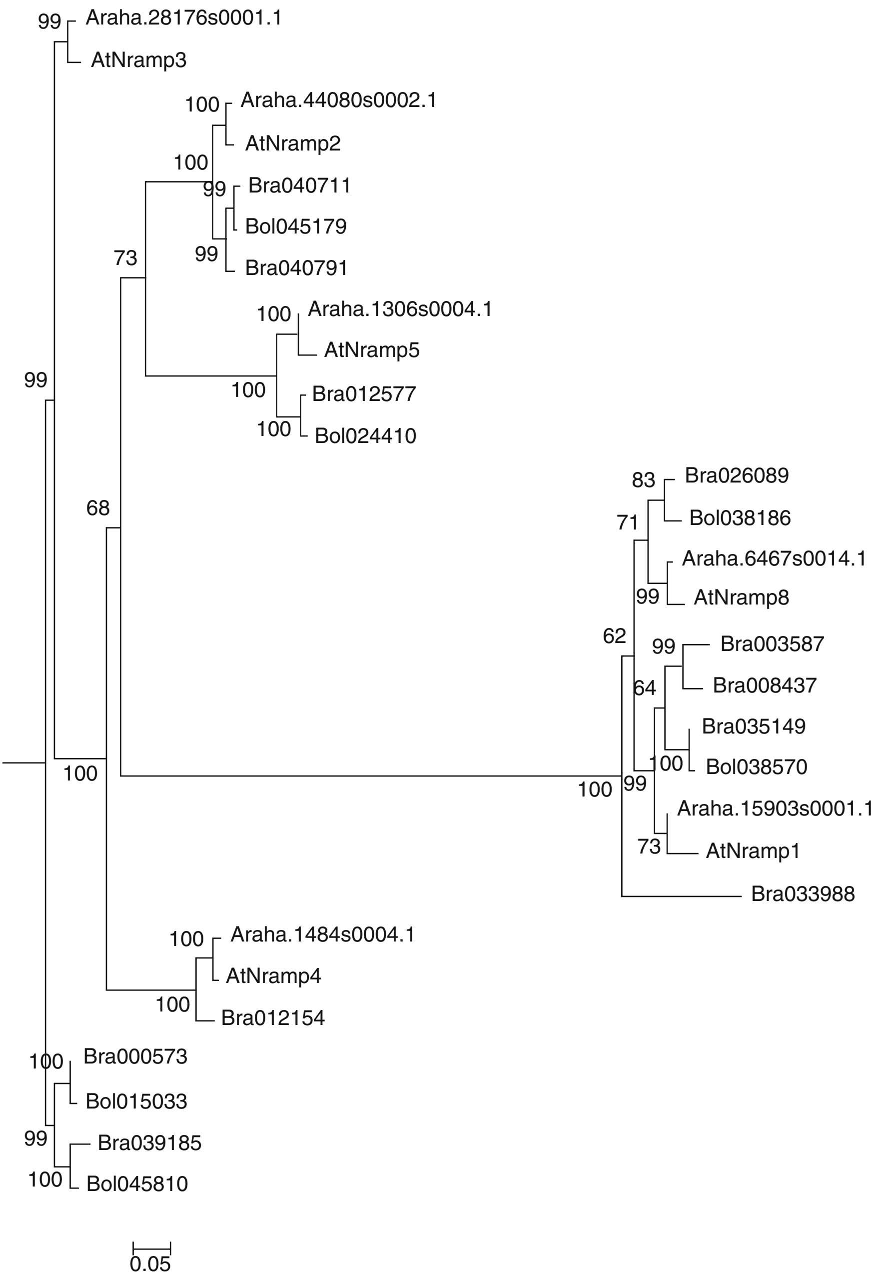


Fig. 16.3 Phylogenetic tree to show relationship among Nramp proteins in *A. thaliana*, *A. halleri*, *B. oleracea*, and *B. rapa*

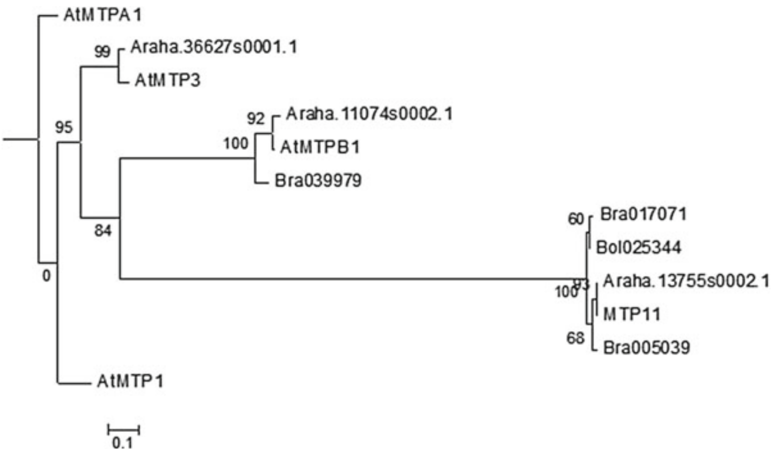


Fig. 16.4 Phylogenetic tree to show relationship among MTP proteins in *A. thaliana*, *A. halleri*, *B. oleracea*, and *B. rapa*

10 changes per 100 amino acids in Fig 16.2, 16.4, and 16.5; 5 changes per 100 amino acids in Fig 16.3 and 16.7; 5 changes per 1000 amino acids in Fig 16.6 and 2 changes per 100 amino acids in Fig 16.8.

16.5 Conclusions and Future Perspectives

The identification of key genes involved in hyperaccumulation mechanism has led to a better understanding of phytoremedial potential of plants. Here, we discussed that the genes responsible for heavy metal transport in *A. thaliana*, which are upregulated in *A. halleri*, have remained conserved in these plants. The rapid advance in genome sequencing of plants is enriching the databases available and adding on to the availability of plants that can be engineered upon, making them available for molecular studies. Techniques such as microarrays and qPCR can be used to study the regulation of these genes in these plants. And further, genes can be upregulated to increase the phytoremedial potential of plants before field application.

Acknowledgments Financial support from Council of Scientific & Industrial Research (S. no. 08/109(0011)/2013-EMR-I), and University Grants Commission [No. F/PDFSS201415SC-UTT8854] is duly acknowledged.

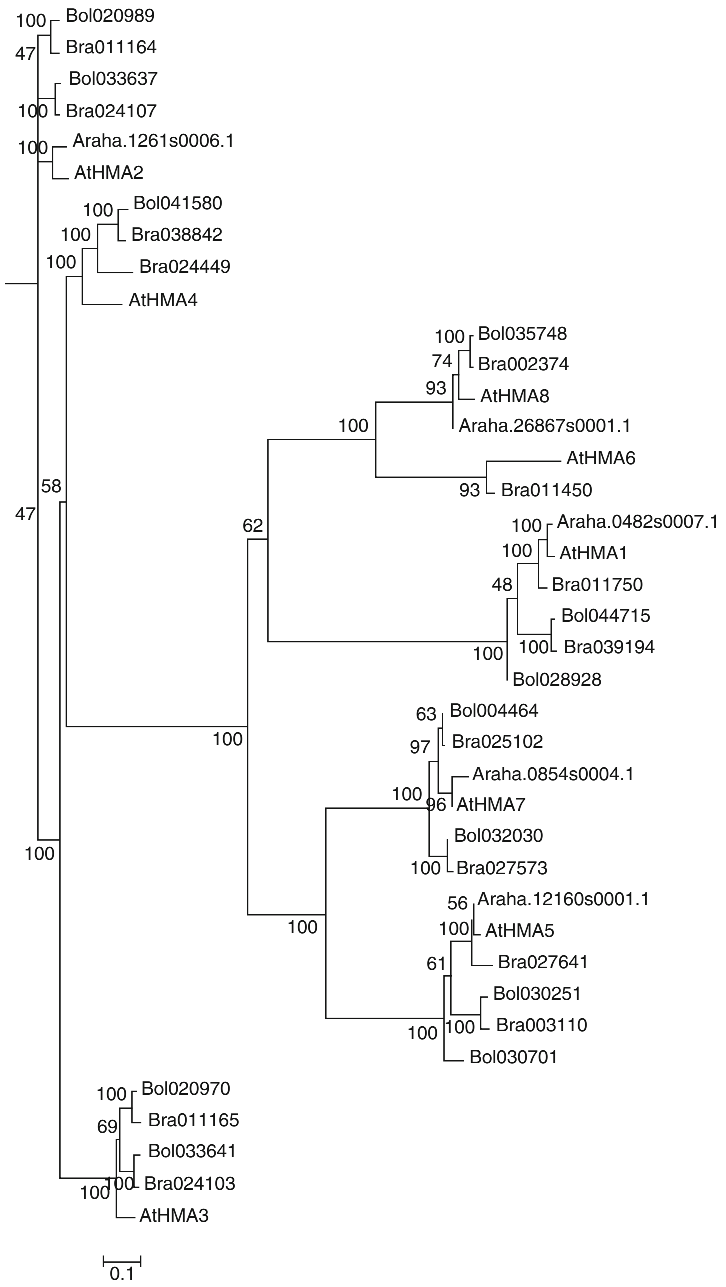


Fig. 16.5 Phylogenetic tree to show relationship among HMA proteins in *A. thaliana*, *A. halleri*, *B. oleracea*, and *B. rapa*

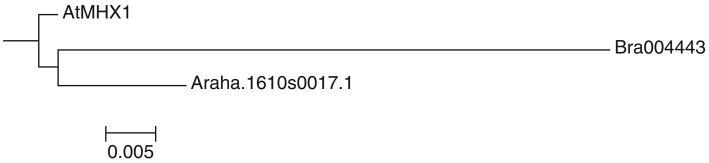


Fig. 16.6 Phylogenetic tree to show relationship among MHX proteins in *A. thaliana*, *A. halleri*, and *B. rapa*

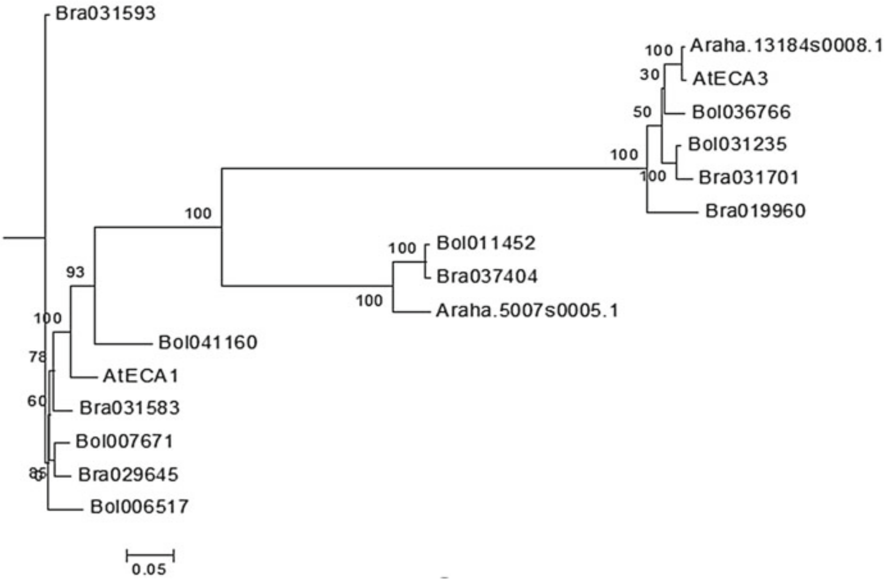


Fig. 16.7 Phylogenetic tree to show relationship among ECA proteins in *A. thaliana*, *A. halleri*, *B. oleracea*, and *B. rapa*

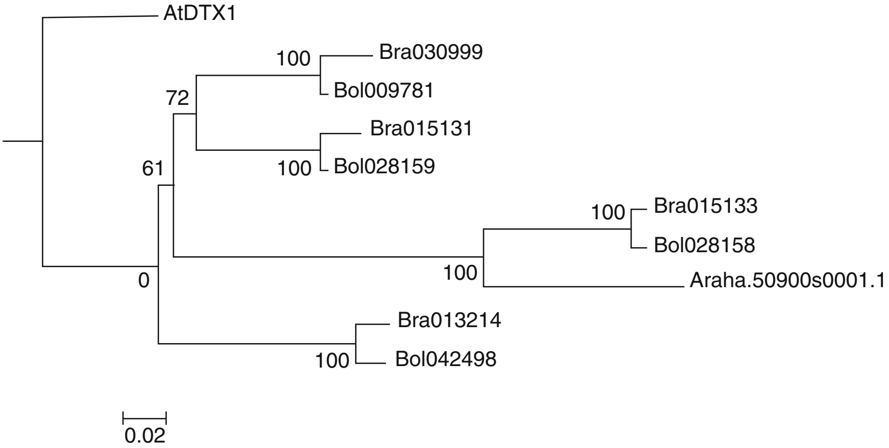


Fig. 16.8 Phylogenetic tree to show relationship among DTX proteins in *A. thaliana*, *A. halleri*, and *B. rapa*

References

1. Burken JG, Schnoor JL (1997) Uptake and metabolism of atrazine by poplar trees. Environ Sci Technol 31(5):1399–1406
2. Salt DE, Smith RD, Raskin I (1998) Phytoremediation. Annu Rev Plant Physiol Plant Mol Biol 49:643–668
3. Vassil AD, Kapulmik Y, Raskin I, Salt DE (1998) The role of EDTA in lead transport and accumulation by Indian mustard. Plant Physiol 117:447–453
4. Baker AJM, McGrath SP, Reeves RD, Smith JAC (2000) Metal hyperaccumulator plants: a review of the ecology and physiology of a biological resource for phytoremediation of metal-polluted soils. In: Terry N, Banuelos G (eds) Phytoremediation of contaminated soil and water. Lewis, Boca Raton, pp 85–108
5. Ma LQ, Komar KM, Tu C (2001) A fern that accumulates arsenic. Nature 409:579
6. Brooks RR (1998) Plants that hyperaccumulate heavy metals. CAB International, Wallingford
7. Reeves RD, Baker AJM (2000) Phytoremediation of toxic metals. Wiley, New York, pp 193–229
8. Schmoger ME, Oven M, Grill E (2000) Detoxification of arsenic by phytochelatins in plants. Plant Physiol 122:793–801
9. Lytle CM, Lytle FW, Yang N, Qian H, Hansen D, Zayed A, Terry N (1998) Reduction of Cr(VI) to Cr(III) by wetland plants: potential for in situ heavy metal detoxification. Environ Sci Technol 32:3087–3093
10. Horne AJ (2000) Phytoremediation by constructed wetlands. In: Terry N, Bañuelos G (eds) Phytoremediation of contaminated soil and water. Lewis, Boca Raton, pp 13–40
11. Raskin I, Smith RD, Salt DE (1997) Phytoremediation of metals: using plants to remove pollutants from the environment. Curr Opin Biotechnol 8:221–126
12. Berti WR, Cunningham SD (2000) Phytostabilization of metals. In: Raskins I, Ensley BD (eds) Phytoremediation of toxic metals using plants to clean up the environment. Wiley, New York, pp 71–88
13. Burken JG, Shanks JV, Thompson PL (2000) Phytoremediation and plant metabolism of explosives and nitroaromatic compounds. In: Spain JC et al (eds) Biodegradation of nitroaromatic compounds and explosives. Lewis, Washington, DC, pp 239–275
14. Kramer U, Chardonnens AN (2001) The use of transgenic plants in the bioremediation of soils contaminated with trace elements. Appl Microbiol Biotechnol 55:661–672
15. Hansen D, Duda PJ, Zayed A, Terry N (1998) Selenium removal by constructed wetlands: role of biological volatilization. Environ Sci Technol 32:591–597
16. Rugh CL, Wilde HD, Stack NM, Thompson DM, Summers AO, Meagher RB (1996) Mercuric ion reduction and resistance in transgenic Arabidopsis thaliana plants expressing a modified bacterial merA gene. Proc Natl Acad Sci U S A 93:3182–3187
17. Van Huysen T, Abdel Ghany S, Hale KL, LeDuc D, Terry N, Pilon Smits EAH (2003) Overexpression of cystathionine synthase enhances selenium volatilization in Brassica juncea. Planta 218:71–78
18. Le Duc DL, Tarun AS, Montes-Bayon M, Meija J, Malit MF (2004) Overexpression of selenocysteine methyltransferase in Arabidopsis and Indian mustard increases selenium tolerance and accumulation. Plant Physiol 135:377–383
19. Environmental Protection Agency (EPA) (2001) Office of sold-waste and emergency response. Brownfields technology primer: selecting and using phytoremediation for site cleanup. EPA 542-R-01-006. Environ Sci Technol 31:182–186
20. Raskin I (1995) Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants. Biotechnology 13:468–474
21. Watanabe ME (1997) Phytoremediation on the brink of commercialisation. Environ Sci Technol 31:182–186
22. Varun M, D'souza R, Pratas J, Paul MS (2012) Metal contamination of soils and plants associated with the glass industry in North Central India: prospects of phytoremediation. Environ Sci Pollut Res 19:269–281

23. D'souza R, Varun M, Pratas J, Paul MS (2013) Spatial distribution of heavy metals in soil and flora associated with the glass industry in North central India: implications for Phytoremediation. Soil Sediment Contam 22:1–20
24. Gaitán-Solis E, Taylor NJ, Siritunga D, Stevens W, Schachtman DP (2015) Overexpression of the transporters At*ZIP1*and At*MTP1* in cassava changes zinc accumulation and partitioning. Front Plant Sci 6:492
25. Chiang HC, Lo JC, Yeh KC (2006) Genes associated with heavy metal tolerance and accumulation in Zn/Cd hyperaccumulator arabidopsis halleri: a genomic survey with cDNA microarray. Environ Sci Technol 40(21):6792–6798
26. Talke IN, Hanikenne M, Kramer U (2006) Zinc-dependent global transcriptional control, transcriptional deregulation, and higher gene copy number for genes in metal homeostasis of the hyperaccumulator *Arabidopsis halleri*. Plant Physiol 142:148–167
27. Assunção AG, Herrero E, Lin YF, Huettel B, Talukdar S, Smaczniak C, Immink RG, van Eldik M, Fiers M, Schat H, Aarts MG (2010) *Arabidopsis thaliana* transcription factors bZIP19 and bZIP23 regulate the adaptation to zinc deficiency. Proc Natl Acad Sci USA 107:10296–10301
28. Milner MJ, Seamon J, Craft E, Kochian LV (2013) Transport properties of members of the ZIP family in plants and their role in Zn and Mn homeostasis. J Exp Bot 64:369–381
29. Ricachenevsky FK, Menguer PK, Sperotto RA, Williams LE, Fett JP (2013) Roles of plant metal tolerance proteins (MTP) in metal storage and potential use in biofortification strategies. Front Plant Sci 4:144
30. Hermand V, Julio E, deBorne FD, Punshon T, Ricacheneysky FK, Arnaud B, Gosti F, Berthomieu P (2014) Inactivation of two newly identified tobacco heavy metal ATPases leads to reduced Zn and Cd accumulation in shoots and reduced pollen germination. Metallomics 6:1427–1440
31. Kramer U, Hanikenne M, Kroymann J, Talke I, Haydon M, Lanz C, Motte P, Weigel D (2009) *Arabidopsis halleri* as a model organism to study the extreme complex trait of metal hyperaccumulation. In: 20th international conference on *Arabidopsis* research
32. Becher M, Talke IN, Krall L, Kramer U (2004) Cross-species microarray transcript profiling reveals high constitutive expression of metal homeostasis genes in shoots of the zinc hyperaccumulator *Arabidopsis halleri*. Plant J 37:251–268
33. Ueno D, Milner MJ, Yamaji N, Yokosho K, Koyama E, Clemencia Zambrano M, Kaskie M, Ebbs S, Kochian LV, Ma JF (2011) Elevated expression of TcHMA3 plays a key role in the extreme Cd tolerance in a Cd- hyperaccumulating ecotype of *Thlaspi caerulescens*. Plant J 66:852–862
34. Verbruggen N, Hermans C, Schat H (2009) Molecular mechanisms of metal hyperaccumulation in plants. New Phytol 181:759–776
35. Brown SL, Chaney RL, Angle JS, Baker AJM (1995) Zinc and cadmium uptake by hyperaccumulator *Thlaspi caerulescens* grown in nutrient solution. Soil Sci Soc Am J59:125–133
36. Shen ZG, Zhao FJ, McGrath SP (1997) Uptake and transport of zinc in the hyperaccumulator *Thlaspi caerulescens* and the non-hyperaccumulator *Thlaspi ochroleucum*. Plant Cell Environ 20:898–906
37. Kupper H, Lombi E, Zhao FJ, Mcgrath SP (2000) Cellular compartmentation of cadmium and zinc in relation to other elements in the hyperaccumulator *Arabidopsis halleri*. Planta 212:75–84
38. Zhao F, Lombi E, Breedon T, Mcgrath S (2000) Zinc hyperaccumulation and cellular distribution in *Arabidopsis halleri*. Plant Cell Environ 23:507–514
39. Bert V, Bonnin I, Saumitou-Laprade P, DeLaguerie P, Petit D (2002) Do *Arabidopsis halleri* from non metallicolous populations accumulate zinc and cadmium more effectively than those from metallicolous populations? New Phytol 155:47–57
40. Cosio C, Martinoia E, Keller C (2004) Hyperaccumulation of cadmium and zinc in *Thlaspi caerulescens* and *Arabidopsis halleri* at the leaf cellular level. Plant Physiol 134:716–725
41. Shanmugam V, Lo JC, Yeh KC (2013) Control of Zn uptake in *Arabidopsis halleri*: a balance between Zn and Fe. Front Plant Sci 4:281

Chapter 17
Phytoremediation of Shooting Range Soils

Tharanga Bandara and Meththika Vithanage

17.1 Shooting Ranges: A Troublemaker to the Environment

Ecosystem contamination by various anthropogenic activities, such as mining, shooting practise, municipal and domestic waste discharge, industrial waste, and wastewater discharge, has become a worldwide predicament, due to its destructive effects on human health as well as flora and fauna. Although many studies have assessed the environmental risks from the other anthropogenic activities, the risks from the shooting ranges have not been focused as such, may be due to the access difficulties, located apart, etc. Shooting ranges, which are also known as firing ranges, are designed to use firearms for the purposes of military training, sportive shooting, or to create private security professionals [1, 2]. Any country's defense requires shooting ranges to train and maintain the capabilities of soldiers in marksmanship [3]. The total number of shooting ranges are in Finland, Switzerland, the USA, and Korea has been reported in 2000–2500, 2170, 12,000, 1400, respectively [4–7]. Most of the military shooting ranges are located in isolated areas to avoid noise pollution and to reduce the risk to the public community. However, shooting ranges in urban areas are designed as indoor ranges [1]. Some shooting ranges are considered as small arms firing ranges; including military small arms training ranges, law enforcement training ranges, and recreational rifle, pistol, trap, skeet, and sporting clay ranges [8].

The extent of the shooting range depends on the type of weapons that are trained in the range. Compared to civilian shooting ranges, at military sites the number of contaminants is higher involving energetic compounds (i.e., TNT), that is, compounds with high amount of stored chemical energy [9]. Generally, the typical shooting range consists with firing line, range floor, target and impact berm [1].

T. Bandara • M. Vithanage (✉)
Chemical and Environmental Systems Modeling Research Group,
National Institute of Fundamental Studies, Kandy 20000, Sri Lanka
e-mail: meththikavithanage@gmail.com

A.A. Ansari et al. (eds.), *Phytoremediation*, DOI 10.1007/978-3-319-40148-5_17

- Firing line
  Firing line is the location that the trainers are positioned. It is normally open or constructed house.
- Range floor
  Range floor is the distance between the target and the firing line. The distance of the range floor depends on the type of weapons practice in the range.
- Target
  Generally, movable or fixed targets are made by paper, plastic, or metals.
- Impact berm
  Impact berm is located behind the target. Traditionally consist of earthen berms and between 4.5 and 8 m high. More recently, earthen berms have been replaced with sand traps, steel traps, and rubber traps. Used bullets and their fragments are contained in impact berm.

Heavy metals from the bullet fragments may contaminate the shooting range soils via dissolution and release into the soil solution, then flow and transport to groundwater and surface water bodies in the surrounding [7]. In a case of releasing a shooting range into the public purposes, the areas must be restored, which is one of the strenuous activities in the world. Although there are many advanced but high cost techniques that can be used for shooting range soil restoration, phytoremediation can be considered as one of the low cost, easy technique without the need of skilled labor [10]. This chapter discusses the possibility of phytoremediation technique, potential plants that can be used, and future research needs for shooting range soils perhaps for restoration or else to protect the surrounding environment from cross-contamination.

17.1.1 Types of Shooting ranges

Shooting ranges are classified into two categories: (a) high velocity shooting ranges, (b) low velocity shooting ranges [11].

(a) High velocity shooting ranges
    High velocity shooting ranges are used for target shooting with pistols and rifles. The typical components of high velocity shooting ranges are a firing line, targets, backstop, and ground and overhead baffles. Length and width vary from range to range and typical lengths for ranges between 22 and 200 m. Generally, widths of ranges depend on the number of fire stations in the shooting range.

(b) Low velocity shooting ranges
    Shotguns are used to shoot clay targets on skeet, trap, and sporting clay ranges in these types of shooting ranges. A typical trap range consists of five shooting positions and one structure, the “trap house,” from which the targets are thrown by a machine called a “trap.” The shooting positions are located 14.6 m from the trap house. The major environmental risk related to shooting ranges is weathering bullets and fragments with time and thereby releasing heavy metals/metalloids and may be TNT into the surrounding ecosystems. Although the soil generally receives the primary contaminant load at shooting ranges, there is

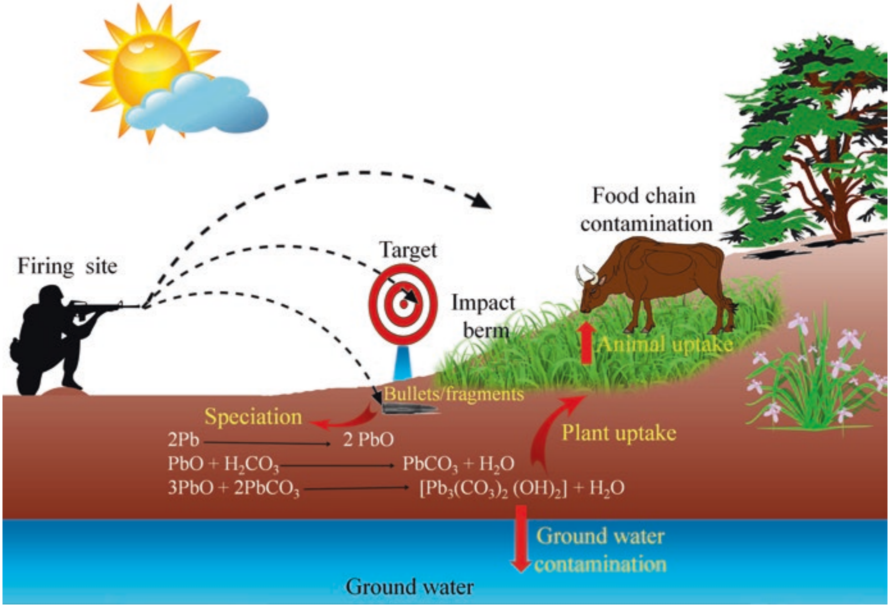


Fig. 17.1 The risk of ecosystem contamination by shooting ranges

also a risk to groundwater and surface water pollution due to contaminant transport from the soil by leaching or runoff (Fig. 17.1). Contamination emerge from long operational times, the type of weapons, soil type, climatic condition as well as insufficient risk management measures.

17.2 Toxic Contaminants in Shooting Ranges and Their Ecotoxicity

Shooting range soils happened to be contaminated with lead (Pb), copper (Cu), antimony (Sb), arsenic (As), nickel (Ni), zinc (Zn), and silver (Ag) from bullets and their fragments [12, 13]. As shooting ranges are generally located in rural area, mostly these heavy metals can contaminate surrounding agricultural lands. Other than that, toxic heavy metals and metalloids may pose a high risk to the plants and other life in the surrounding ecosystems. Despite the size of shooting ranges, at some point the land area is indeed to be restored. When the bullet fragments enter into the soil, metals and metalloids are subsequently transformed into a wide range of species through oxidation and deterioration of the pellets and become bioavailable in soils [13, 14]. The most soluble and stable chemical species can cause serve toxicity to the surrounding environment. Table 17.1 reports the recorded total Pb, Cu, and Sb concentrations in few shooting ranges. Most of these elements and their species present in shooting range soils are classified as potential human

Table 17.1 Total deposition of Pb, Cu, and Sb at shooting ranges

Country	Pb (mg/kg)	Cu (mg/kg)	Sb (mg/kg)	Reference
Korea	17,468	1168	164	[14]
	4626	225	NA	[26]
	3970.65	NA	67.48	[87]
	4626	225	23	[52]
	1100	30	NA	[61]
	69.5–506.0	151.4–313.2	NA	[88]
Italy	212–1898	40–65	0.07–16.3	[13]
Switzerland	466–644	57.1	19.7	[89]
	2100–>500,000	43–5100	1300–17,500	[35]
	44–33,600	17–1250	0.13–831	[90]
	500	66	21	[91]
	530	190	15	[32]
Australia	81,000	350	600	[3]
Pakistan	1331	84.50	NA	[60]
USA	2520–35,868	NA	NA	[16]
	12,710–48,400	NA	NA	[92]
Finland	28,700	NA	NA	[93]

carcinogens. Therefore, identification of animal health hazard and phytotoxicity of plants are exceptionally important.

17.3 Heavy Metals Speciation in Shooting Range Soils

The reactivity, mobility, and bioavailability of heavy metals and metalloids depend on their speciation. Speciation refers to the distribution of the elements among their various physical and chemical forms and possible oxidation states [15]. Speciation induces grave environmental risks by increasing mobility and bioavailability. Specially, due to precipitation, most of the less soluble forms of heavy metals and metalloids are migration through surface runoff and soil erosion.

17.3.1 Lead (Pb) Speciation and Toxicity

Lead is one of the major elements present in the shooting range soil. On an average, new bullets and pellets consist over 90 % of Pb [7]. More than 60,000 tons of Pb is annually deposited in the US shooting ranges [16], and it varies between 200 and 6000 tons in the Netherlands, Denmark, Canada, and England [17]. Lead is present in two oxidation states (Pb^{2+} and Pb^{4+}), but the divalent ion (Pb^{2+}) is dominant in the environment. Soil adjacent to the shooting ranges having Pb levels exceeded the safe threshold for human health, 5 mg L^{-1} [18]. Lead can be transformed into a wide range of Pb species in shooting ranges, including Pb-oxides, Pb-hydroxides, Pb-carbonates, Pb-sulfates, and Pb-carboxylates [14]. A three-step mechanism is involved in weathering of metallic Pb in soil which are oxidation, carbonation, and dissolution [19].

Oxidation:

$$2\mathrm{Pb}(\mathrm{s})+\mathrm{O}_2 \rightarrow 2\mathrm{PbO}(\mathrm{s})$$

Carbonation:

$$\mathrm{PbO}(\mathrm{s})+\mathrm{H}_2\mathrm{CO}_3 \rightarrow \mathrm{PbCO}_3(\mathrm{s})+\mathrm{H}_2\mathrm{O}$$

Dissolution:

$$3\mathrm{PbO}(\mathrm{s})+2\mathrm{H}_2\mathrm{CO}_3 \rightarrow \mathrm{Pb}_3(\mathrm{CO}_3)_2(\mathrm{OH})_2(\mathrm{s})+\mathrm{H}_2\mathrm{O}$$

Bullet material consists of two layers with the inner layer being predominantly massicot (PbO) and the outer layer being predominantly hydrocerussite [$Pb_3(CO_3)_2(OH)_2$]. This suggested that Pb-oxides were being replaced by Pb-carbonates in shooting range soils [19]. The solubility of Pb in soil is strongly pH-dependent. In the case of high soil pH, Pb may precipitate as hydroxide, phosphate, carbonate, and relatively stable Pb-organic complexes, while decreasing soil pH will increase the solubility of Pb [20]. However, after enter to the bloodstream, 99% of Pb is accumulated in erythrocytes, and the remaining 1% of the Pb is left with plasma [21]. In plants, it has been proved that the accumulation of heavy metals may cause many physiological, biochemical, and structural disorders like decline in chlorophyll contents, photosynthetic rate, biomass, and uptake of essential elements. Lead phytotoxicity poses negative influences on seed germination and root and shoot growth [22]. It induces the production of reactive oxygen species (ROS) which can cause harmful effects on vital constituents of plant cells: protein oxidation, lipid peroxidation, enzyme inactivation, or DNA damages [23].

17.3.2 Copper (Cu) Speciation and Toxicity

Copper is an essential micronutrient for plant growth and human and animal health. Copper plays a vital role in humans, and it is necessary for the development of connective tissue, nerve coverings, bone, and also participates in both Fe and energy metabolism [24]. According to the European union standards, safe limits of Cu in the soil is stated as 140 $\mu g\ g^{-1}$ [25]. However, the total concentration of Cu in Korea, Germany, and Australia have been reported 225, 817, and 1250 mg/kg, respectively [26–28].

Copper is the third most abundant trace element in the human body [20]. However, it can be poisonous at excessive levels in bioavailable forms. Naturally, Cu can be exist with various soil minerals such as sulfides, carbonates, and hydroxides [29]. However, these minerals are only stable under the alkaline and anaerobic environment hence, with decreasing soil pH and increasing soil oxidation condition these are dissolved and mobilized. Copper has two main oxidation states (Cu^+ and Cu^{2+}) and among them, Cu^{2+} is most abundant, toxic, and bioavailable form in the soil [29]. However, many other Cu speciation may occur in soil: Cu^+, $CuOH^+$, $Cu(OH)_2^{2+}$, $Cu(CO_3)_2^{2-}$, $Cu(OH)_4^{2-}$, $Cu(OH)_3^-$, CuO_2^{2-}, $HCuO_2^-$ [20]. Moreover, Cu is one of the least mobile trace elements in soil due to higher affinity to adsorbed clay minerals, organic matter, and Fe-Mn oxides.

Copper is considered as a micronutrient for plants and plays important role in CO_2 assimilation and ATP synthesis [25]. While Cu is an essential micronutrient, exposure to excess Cu has an adverse effect on plant growth. At molecular level, Cu ions generate reactive oxygen species such as O_2^-, H_2O_2, and ^-OH. These reactive radicals cause oxidative damage to lipids, proteins, and nucleic acids. Excessive Cu leads to plant growth retardation and leaf chlorosis [25], causes degradation of chlorophyll, and impedes photo system activity [30], and the effect of Cu toxicity is largely on root growth and morphology [31].

17.3.3 Antimony (Sb) Speciation and Toxicity

Antimony is a potentially toxic metalloid and highly toxic with unknown biological functions that may produce adverse effects in humans and the ecosystem. The total Sb concentration of shooting ranges in Germany, Switzerland, Australia, and Korea have been reported 437, 5067, 325, and 23 mg/kg, respectively [27, 32–34]. The oxidation state has a critical influence on the mobility and bioavailability of Sb in soils. The species of Sb vary with the oxidative or reduction condition of soil. Under oxidative condition, the most abundant Sb species is $Sb(OH)_6^-$ [35]. Other than soil oxidative condition, pH of the soil solution influences the mobility and bioavailability of the species. Under low pH, $Sb(OH)_6^-$ is adsorbed to Fe oxides by reducing mobility of the species however, when $pH > 2.5$ it becomes mobile. Under reducing conditions of the soil, the most stable complex is $Sb(OH)_3^0$ [35]. Compared to $Sb(OH)_6^-$, Fe oxide-$Sb(OH)_3^0$ is immobile for a large range of pH, even in the neutral condition. Therefore, the mobility of Sb in shooting range soil is mainly controlled by $Fe[OH]_2$ and $Fe[OH]_3$ and secondary minerals phases $Ca[Sb(OH)_6]_2$ and $Pb[Sb(OH)_6]_2$ [1].

Micro amounts of Sb exposure via inhalation will stimulate respiratory tract, mucous membrane of alimentary canal and skin, even result in pulmonary edema, inflammation of the lungs, chronic bronchitis, and chronic emphysema; oral exposure to Sb in humans resulted in gastrointestinal effects [36]. In plants, Antimony inhibits photosynthesis and decreases utilization of essential elements [37]. Antimony exists as both inorganic and organic forms in tissues; however, inorganic Sb is the dominant species. According to the WHO recommendation, the maximum permissible pollutant concentration for Sb is 36 mg/kg for soils and for drinking water the limit is set to 5 μg L^{-1} [38]. The LD50 for antimony potassium tartrate in rabbit and rats has been calculated as 115 and 600 mg kg^{-1} for mice [39]. High levels of Sb in soils do not necessarily result in significant accumulation of Sb in plant tissues though. The concentration of Sb in plants depends on several factors including the phytoavailability of Sb, the speciation of Sb in the soil and the coexisting ions in soils, such as phosphorus and calcium [40]. Phosphorus may facilitate plant uptake of Sb via release of Sb into the soil pore water through competitive adsorption while soil having more Ca it may reduce the plant uptake of Sb due to co-precipitation [$Ca(Sb(OH)_6)_2$] [40].

17.3.4 Arsenic (As) Speciation and Toxic Effects

Arsenic is the 20th most abundant element in the geosphere and widely exists in soil, groundwater, and surface water throughout the world [41, 42]. On an average, a bullet contains <2 % of As [43]. The total As concentration of shooting ranges in the USA, Australia, and Finland have been reported 13.6, 15.8, and 200 mg/kg, respectively [9, 28, 44]. The inorganic forms of arsenic, arsenite (As^{3+}) and arsenate (As^{5+}), are toxic to human health. Among these two oxidation state As^{3+} considered as more toxic, soluble, and mobile compound compared to the As^{5+}. Humans are exposed to As primarily from air, food, and water. In many areas, As levels in the environment have exceeded the safe threshold for human health, 10 $\mu g\ L^{-1}$ [42]. Soil As concentration in shooting range soil exceed 1057 mg/kg, and therefore it poses a high risk to the surrounding ecosystem exceeding human threshold level [45]. Arsenic speciation and behavior in soil are strongly influenced by redox conditions. As^{3+} is soluble and mobile compared to the As^{5+}. Arsenic solubility in soil mainly control by the Fe-Al oxides and clay minerals. Therefore, in soil and water environments As solubility is controlled by the redox potential but also by the types of sorbent available (Fe^{3+} and Mn^{4+} hydroxides) and the As minerals themselves [38, 41]. Plant growth stimulation has often been observed as a result of low levels of As additions to soils [46]. Compared to the organic form of As (Methyl arsonates), inorganic forms are highly toxic to the plants. However, upward translocation of organic forms are much higher than inorganic forms in plant [47]. Further, inorganic As inhibits enzyme activity and As^{3+} reacts with the sulphydryl groups of proteins affecting many enzymes containing such groups.

17.4 Reclaim Shooting Range Soils

Numerous physicochemical and biological techniques have been adopted for the elimination of heavy metals from the shooting range soil, and these are summarized in Fig. 17.2

Soil washing is one of the major physical techniques that is used to reclaim the shooting range soil [1]. Liquids (water occasionally with solvents) used to enhance the removal ability of heavy metals from soils in this method [1, 48]. Soil washing technique involved several steps : (1) excavation of the contaminated soil; (2) remedial treatment of the contaminated soil; (3) solid/liquid separation of all contaminants;

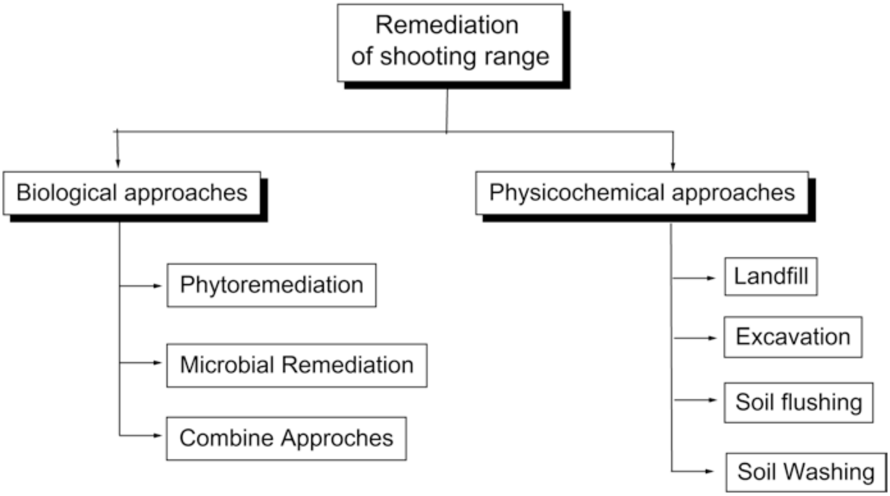


Fig. 17.2 Common methods of elimination of heavy metals from the shooting range

(4) treatment or disposal of all residues; and (5) final soil deposition [49]. However, the effectiveness of this technique mainly depends on the soil type. As an example, if the soil contains high amount of silt and clay, this technique can not be applied [48]. Most importantly, soil physical techniques in remediation and restoration is destructive, costly and need skilled labor. Several soil amendments, including both organic and inorganic materials have been used to stabilize or immobilize metals in shooting range soil (Table 17.2) [14, 16, 50, 51]. These are the primarily used as soil chemical techniques for restoration. Cation exchange, adsorption, precipitation, or surface complexation is the fundamental processes that are involved in metal immobilization through various soil amendments [52].

An alternative approach to remediate Pb-contaminated soils has been developed, which uses P-rich materials, such as P fertilizers, phosphate rock, biosolids, and manures, to immobilize Pb in soils [53]. However, this has no capacity to remediate metalloids present in the soil, in fact the presence of P increases the release of As into the soil due to the competition. Hence, a careful understanding is needed before application of such amendments into restoration activity since the application of an amendment may increase the release of more toxic compound into the system. Anyway, the immobilization of metals with phosphate compounds perform under several mechanisms including direct metal absorption by phosphate compounds and precipitation of metals with phosphate solution as metal phosphates [54]. In addition, especially with Pb, phosphate can transformation to highly insoluble pyromorphite compounds ($Pb_{10}(PO_4)_6X_2$, X = Cl, Br, F, OH) [55].

Liming materials are basically used to ameliorate soil acidity but at the same time it is used to stabilize metals in the soil, hence possibly be effective stabilizers of shooting range contaminants [54, 56]. Most widely use liming materials are cement, lime, and fly ash, and these immobilize contaminants by adsorption, espe-

Table 17.2 Common materials as amendments to remediate shooting range soil

Material	Target metals or metalloids	Reference
Portland cement	Pb	[16]
Quicklime	Pb	[16]
Monocalcium phosphate	Pb	[16]
Slaked lime	Pb, Cu, Ni, Zn, and Sb	[51]
Acidic fertilizer	Pb, Cu, Ni, Zn, and Sb	[51]
Gypsum waste	Pb	[50]
Incinerated poultry waste	Pb	[50]
Biochar	Pb and Cu	[26]
	Pb, Sb, and Cu	[14]
	Pb	[7]
	Pb, Cu and Zn	[94]
Natural iron oxide	Pb, Sb, and Cu	[14]
Gibbsite	Pb, Sb, and Cu	[14]
Silver nanomaterial	Pb, Sb, and Cu	[14]
Eggshell	Pb	[33]
Calcined eggshell	Pb	[33]
Mussel shell	Pb	[12]
Cow bone	Pb	[12]
Oyster shells	Pb and Cu	[52]

cially calcium alumina hydrate or calcium silicate hydrate, or via precipitation as metal hydroxides under alkaline pH associated with cement and lime [16]. Egg shells, oyster shells, and mussel shells are considered as waste materials that having liming characteristics and therefore can be used as soil amendments to remove heavy metals in the shooting range soil [52]. These waste materials mainly consist of calcite ($CaCO_3$), and it effectively reduces bioavailable Pb contents due to the increase in soil pH. In contrast, water soluble Cu increased with increasing application of lime-based waste materials, and it was mainly due to the formation of soluble complexes between Cu and dissolves organic carbon [52]. Therefore, selection of appropriate amendment for immobilizing metals in shooting range soils is very important.

Biochar, a material obtained from pyrolysis of biomass, has recently gained interest as an alternative soil amendment for the remediation of metal-contaminated soils [14, 57, 58]. Biochar having more functional groups on their surfaces and help to form strong bonds with transition metals [14]. Biochar application was effective in Pb, Cu, and Cd immobilization in shooting range soil, thereby reducing the bioavailability and phytotoxicity of heavy metals [59]. In contrast, Sb (or As) mainly exists as oxyanions in the soil solution and repulsive electrostatic interactions between those anions and negatively charged biochar particles may result in desorption from the soil [14]. These findings further indicate that application of an amendment focusing one pollutant is not successful in multi-metals-contaminated soils. Hence, an attention towards focusing multi-metals is exceptionally important.

17.5 Phytoremediation of Shooting Range Soils

Phytoremediation is an inexpensive and sustainable technology that is most useful to remediate contaminants within the root zone of the plants [10]. Some plants in the environments have a potential ability to tolerate high concentrations of trace elements, and some have the capacity to absorb and retain heavy metals/metalloids and transition metals in high concentrations, called hyperaccumulators. Generally, hyperaccumulators are used for phytoremediation. Figure 17.3 showed the common ideal characters of the plant to serve as phytoremediation of shooting range soil.

17.5.1 *Common Plant Species Used for Shooting Range Soil Remediation*

Phytoremediation is an in situ remediation technique having several processes, including phytoextraction, phytodegradation, rhizofiltration, phytostabilization, and phytovolatilization that utilizes the inherent abilities of living plants to remediate contaminated site by eco-friendly manner. However, phytoremediation of shooting range soils are poorly documented. Table 17.3 depicts the common plant species that are used to reclaim shooting range soil. The phytoremediation ability of *Helianthus annuus*, *Zea maize*, *Brassica campestris*, and *Pisum sativum* were tested

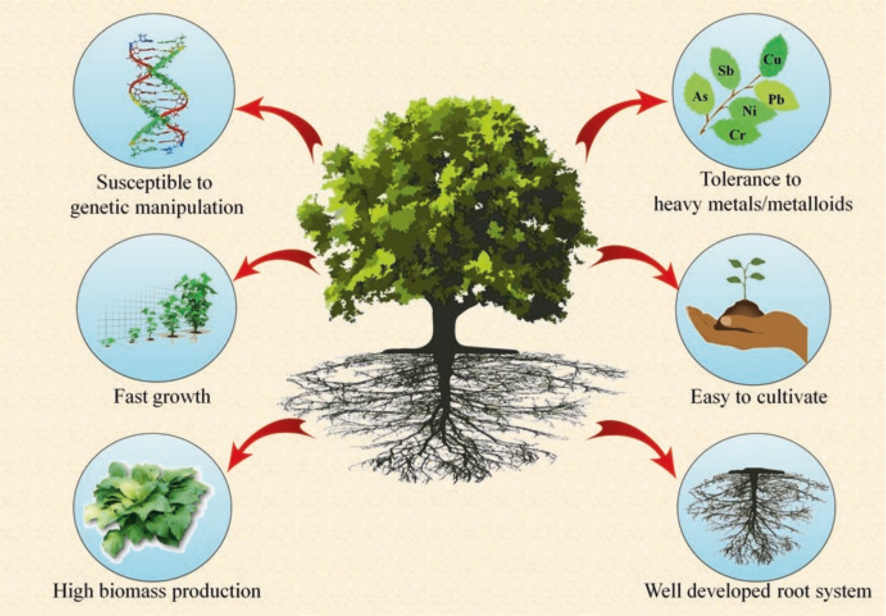


Fig. 17.3 Ideal plant characters for phytoremediation of shooting range soil

Table 17.3 Common plant species used for phytoremediation of shooting range soil

Plant	Metals	Mechanism	Reference
Zea mays L.	Sb	Phytostabilizer	[95]
Vetiveria zizanioides	Pb, Cu, Zn, and Fe	Phytoextraction	[10]
Helianthus annuus	Cd	Phytoextraction	[60]
Zea maize	Pb	Phytoextraction	[60]
Pisum sativum	Pb	Phytoextraction	[60]
Helianthus annuus	Cd and Pb	Phytostabilizer	[61]
	TNT	Phytotransformation	[61]
Abutilon avicennae	Cd and Pb	Phytostabilizer	[61]
	TNT	Phytotransformation	[61]
Aeschynomene indica	Cd and Pb	Phytostabilizer	[61]
	TNT	Phytotransformation	[61]
Echinochloa crusgalli	Cd and Pb	Phytostabilizer	[61]

for the soil of firing range contaminated with several metals such as Cd, Cu, Co, Ni, Cr, and Pb [60]. Among those plants, *Pisum sativum* reveals maximum elimination efficiency (96.23 %) for Pb thus evidencing it to be Pb hyperaccumulator from the soil of shooting ranges [60]. Moreover, results showed that *Zea maize* noticeably reduced the levels of all the selected metals (Cd, Cu, Co, Ni, Cr, and Pb) in the soil, but the highest phytoextraction capacity was shown for Pb (66.36 %), which was enhanced to approximately 74 % of EDTA application [60]. Thus, agricultural lands adjacent to shooting ranges may easily be remediated using *Pisum sativum* and *Zea maize*. Sunflower (*Helianthus annuus*) has grown as a crop for its edible oil and edible fruits as well as used as bird food, as livestock forage. Sunflower removes Cd and it may be good for the removal of Pb and Cu as well [60, 61]. Not just heavy metals, *Helianthus annuus* remediate 2,4,6-trinitrotoluene (TNT) present in the shooting range by the mechanism of phytotransformation.

Chrysopogon zizanioides, commonly known as "Vetiver grass," "wonder grass," "miracle grass," or "magic grass" and grown in several parts of the globe. This plant is capable of soil and water conservation as well as phytoremediation applications because of its vigorous root system, rapid growth, high biomass, and tolerance for adverse environmental conditions [62, 63]. Use of Vetiver grass together with the fertilizer has exhibited a considerable potential for firing ranges, and it has evidently reported a higher plant factor than the translocation factor indicating minimizing Pb mitigation from the soil to plant [10]. Furthermore, Vetiver grass has proven a great ability to remove human carcinogen TNT as well [64]. Vetiver grass grown under hydroponic media with TNT (40 mg L^{-1}) was able to remove TNT completely within 8 days, where a 40 % of TNT was removed during the first 20 h [64]. A complete removal of TNT in urea-treated soil was observed by Vetiver grass at the low initial TNT concentration (40 mg kg^{-1}) [65].

Echinochloa crusgalli, commonly known as cockspur, barnyard millet, Japanese millet, water grass, or common barnyard grass, mainly originating from tropical Asia. Herbaceous *Echinochloa crusgalli* is highly resistant to a broad scope of heavy metal concentrations, and therefore it has greater potential to act as hyperaccumulator [66]. Especially, *Echinochloa crusgalli* is more capable of removing Cd from the shooting range soil via phytostabilizing mechanism [61]. *Aeschynomene indica* is a species of legume family, and it spreads to many parts of the world, including Africa, Asia, Australia, and the southeastern United States. *Aeschynomene indica* plays a significant role in the removal of both Cd and TNT from the contaminated soil [61].

Antimony is generally taken up by terrestrial plants in proportion to the concentration of soluble Sb in soil [67]. Due to the lack of oxygen, waterlogged soils become reductive, leading to the reduction of Sb^{5+} to Sb^{3+} and decrease in its solubility, which may influence the plant uptake of Sb. Waterlogging condition increased shoot Sb concentrations of *Lolium perenne* L. by 10-fold but decreased uptake in *Holcus lanatus* L. by 80 % [68]. The results indicated that the plants up taken of Sb vary with the oxidation state and speciation. As an example *Lolium perenne* L. uptake Sb^{3+} well, whereas *Holcus lanatus* L. exhibits high uptake for Sb^{5+} [68]. However, it is important to use native species for phytoremediation because these plants are often better adapted in terms of survival, growth, and reproduction under environmental stress than plants introduced from other environments.

17.5.2 Possible Other Plants for Phytoremediation of Shooting Range Soil

Currently, a limited number of plant species have been tested to salvage shooting range soils. Many other species which are already been tested for different heavy metals and metalloids may be used to reclaim shooting ranges. Table 17.4 shows the possible other plant species for shooting range soil restoration based on the literature. However, none of the phytoremediation experiments focused remediation of both metals and metalloids at the same time in a shooting range. The work carried out have been focused either on single heavy metal or two metals or single metalloid.

Few different plant species were reported as potential plants to remediate polluted lands by mining activities. Among tested species *Paspalum notatum* (Bahia grass) showed higher removal efficiency for Cd [62]. *Tagetes patula* (French marigold) is another plant that can be used as Cd hyperaccumulator [69]. *Calotropis procera*, *Croton bonplandianum*, *Cyperus rotundus*, *Datura stramonium*, *Euphorbia hirta*, *Parthenium hysterophorus*, *Phyllanthus amarus*, *Sida cordifolia*, *Solanum nigrum*, *Solanum xanthocarpum*, *Spinacia oleracea* and *Tridax procumbens* growing naturally in the India and those weed species are potentially suitable for phytoremediation and restoration of land contaminated toxic metals such as Cr, Cu, Ni, Pb, and Cd; however, this may create ecological problems as well [70]. *Sesbania grandiflora* is considered as one of the food crops that is grown in tropical regions, and it has the ability to phytoremediation of Pb by mechanism of rhizofiltration [71]. Kenaf (*Hibiscus cannabinus*) is another suitable plant for the remediation of Pb contaminated shooting range soil since more than 85 % of Pb was reported to be accumulated in *Hibiscus cannabinus* roots [72].

Table 17.4 Possible other hyperaccumulators for phytoremediation of shooting ranges

Plant	Responsible metal(s) or metalloid(s)	Reference
Paspalum notatum	Pb and Cd	[62]
Sesbania rostrata	Pb, Zn, Cu, and Cd	[63]
Sesbania sesban	Pb, Zn, Cu, and Cd	[63]
Populus alba L.	Cd, Cu, Fe, Pb, and Zn	[96]
Populus nigra L.	Cd, Cu, Fe, Pb, and Zn	[96]
Lantana camara	Pb	[97]
Noaea mucronata	Pb, Zn, Cu, Cd, and Ni	[98]
Amaranthus retroflexus	Fe	[98]
Medicago sativa L.	Cd, Cu, Zn, and Hg	[99]
Lupinus albus L.	Cd, Cu, Ni, and Zn	[100]
Typha domingensis	Cd, Cu, Fe, Pb, and Zn	[101]
Polypogon fugax	Cu	[76]
Epilobium hirsutum	Cu	[76]
Calotropis procera	Cr, Cu, Ni, Pb, and Cd	[70]
Croton bonplandianum	Cr, Cu, Ni, Pb, and Cd	[70]
Cyperus rotundus	Cr, Cu, Ni, Pb, and Cd	[70]
Datura stramonium	Cr, Cu, Ni, Pb, and Cd	[70]
Phalaris arundinacea L.	Co, Pb, and Zn	[102]
Tagetes patula	Cd	[69]
Pteris cretica	As and Sb	[81]
Cytisus scoparius	As and Sb	[82]

Serpentine ecosystem contain elevated amounts of Ni, Cr, Mn, Fe, and Co [73]. The accumulation and distribution of Ni in the leaves and roots of the Mediterranean shrub *Alyssoides utriculata* was assessed for its potential in phytoremediation of Ni-contaminated soils [74]. South African Ni hyperaccumulator plant *Berkheya coddii* has very good potential for phytoremediation of contaminated soils and for phytomining of Ni [75]. Moreover, the identified potential of these species for phytomining reported a yield of 100 kg/ha of Ni. Hence, these plants must be tested to see whether they are metal specific or they have a potential for multi-metal and metalloid remediation. Copper is a major trace element that is present in the shooting range soil, and presently a limited number of plant species are tested as phytoremediation. Phytoremedial plants species in Cu mining sites such as *Polypogon fugax*, *Epilobium hirsutum* may be suitable for the remediation of Cu-contaminated firing ranges [76].

Pteris vittata one of the major phytoremediation plants that can effectively remove both arsenate and arsanite [77, 78]. Hyperaccumulator *Pteris cretica* was identified as another fern that having greater As-accumulation capability [79]. Use of both plant and microbes to remediate As-contaminated soil more effective due to synergistic effect. Silverback fern (*Pityrogramma calomelanos*) with rhizobacteria effectively increases plant biomass and As content [80]. Hyperaccumulating fern, *Pteris cretica* possible to use remediate the As/Sb co-contaminated soils [81]. *Cytisus scoparius* and *Trifolium pratense* are most outstanding herbaceous excluders for the As and Sb, indicating 1 > translocation factors values [82]. However, it is indeed important to understand their behavior at the presence of Pb, Cu like metals together with metalloids.

17.5.3 Transgenic Plants for Phytoremediation of Heavy Metals and Explosive Compounds

Transgenic plants exhibiting new or improved phenotypes are engineered by the over expression and/or introduction of genes from other organisms, such as bacteria or mammals [83]. In the past, transgenic plants for phytoremediation were first developed to improve heavy metal tolerance and tobacco plants (*Nicotiana tabacum*) [83]. Wild type tobacco plant (*Nicotiana glauca*) were successfully engineered by wheat gene encoding phytochelatin synthase (*TaPCS1*), and it showed potentially higher Pb and Cd tolerance compared to the non-transgenic plant [84]. The transgenic Indian mustard (*Brassica juncea* L.) accumulated 1.5–2 fold Cd and Zn concentration compared to the wild type Indian mustard [85]. Bana grass (*Pennisetum glaucum* and *Pennisetum purpureum*) is one of the best examples for hybridized plants having a higher efficiency of removing Cd and Pb in the polluted environment [62].

Most of the herbaceous phytoaccumulators can efficiently absorb toxic heavy metals; however, they are not suited for phytoremediation, due to their slow growth, small biomass, and shallow root system compared to tree species [86]. Poplar tree is one of the excellent candidates for genetic engineering for phytoremediation and yeast cadmium factor 1 (*ScYCF1*) gene introduced poplar trees having excellent phytoremediation ability compared the non-transgenic poplar trees [86]. Therefore, transgenic approaches can be successfully employed to promote phytoremediation of metals and metalloids at the same time from shooting soil by their accumulation in the aboveground biomass involved mainly implementation of metal transporters, improved production of enzymes metabolism and production of metal-detoxifying chelators.

17.6 Remarks

Shooting practice at shooting ranges is a source of contamination that has created seriously polluted sites in many countries due to the presence of excessive concentrations of metals and metalloids. The risks to human health, flora, and fauna arising from environmental contamination in shooting ranges depend on the surrounding land use and future use. Especially, agricultural land in the surrounding area of the shooting ranges is more prolonged to food chain contaminated by toxic metals and metalloids. In addition, where groundwater is used as a source of domestic water, from an aquifer nearby a shooting range may cause a significant risk to human and animal health.

Most of the phytoremediation studies carried out at shooting ranges is particularly limited specifically an incomplete understanding of the reclaiming soils rich in heavy metals and metalloids together. Only a limited number of plant species have been tested up to now such as *Vetiveria zizanioides*, *Helianthus annuus*, *Zea maize*, *Pisum sativum*, and *Helianthus annuus* and the identification of potentially phytoremedial plants are an urgent necessity. Generally, shooting range soil contaminated with heavy metals and metalloids. Therefore, identification or modification of plants for the phytoremediation of both heavy metals and metalloids are needed. At a point, the plant must be tested not only for heavy metals and metalloids but also for TNT and other explosives as well. In this case, transgenic plants may play an important role. Other than that soil microbes play an important role in the remediation of polluted soils involving different mechanisms including improve the stress of heavy metals affecting the remediation of soil or plant growth.

Further, the symbiotic association of soil microbes and hyperaccumulator plants can improve the efficiency of phytoremediation significantly. Therefore, future research needed to be focused on the effect of both plants and microbes combination would be more useful. At the moment, most research has been conducted in the laboratory conditions, and there is a lack of information about the practical use of phytoremediation at shooting range sites. In addition, many of the tested plants were agricultural species, which are not recommended for phytoremediation. Hence, more studies on plants suited for practical application in the field would be better.

References

1. Ahmad M, Lee SS, Moon DH, Yang JE, Ok YS (2012) A review of environmental contamination and remediation strategies for heavy metals at shooting range soils. In: Environmental protection strategies for sustainable development. Springer, pp 437–451
2. Cardim GP, Dias MA, Noguti RH, de Best R, da Silva EA (2014) Mathematical morphology applied to automation of indoor shooting ranges. Int J Appl Math 27(6):549–566
3. Sanderson P, Bolan N, Bowman M, Naidu R, Gilkes R (2010) Distribution and availability of metal contaminants in shooting range soils around Australia. In: Proceedings of the 19th world congress of soil science: soil solutions for a changing world, Brisbane, Australia, 1–6 August 2010. Symposium 3.5. 1 Heavy metal contaminated soils. International Union of Soil Sciences (IUSS), c/o Institut für Bodenforschung, Universität für Bodenkultur, pp 65–67
4. Hockmann K, Tandy S, Lenz M, Reiser R, Conesa HM, Keller M, Studer B, Schulin R (2015) Antimony retention and release from drained and waterlogged shooting range soil under field conditions. Chemosphere 134:536–543
5. Sorvari J, Antikainen R, Pyy O (2006) Environmental contamination at Finnish shooting ranges—the scope of the problem and management options. Sci Total Environ 366(1):21–31

6. Ackermann S, Gieré R, Newville M, Majzlan J (2009) Antimony sinks in the weathering crust of bullets from Swiss shooting ranges. Sci Total Environ 407(5):1669–1682
7. Moon DH, Park J-W, Chang Y-Y, Ok YS, Lee SS, Ahmad M, Koutsospyros A, Park J-H, Baek K (2013) Immobilization of lead in contaminated firing range soil using biochar. Environ Sci Pollut Res 20(12):8464–8471
8. Larson SL, Teeter CL, Medina VF, Martin WA (2007) Treatment and management of closed or inactive small arms firing ranges. DTIC Document
9. Sorvari J (2011) Shooting ranges: environmental contamination. Encycl Environ Health 5:41–50
10. Wilde E, Brigmon R, Dunn D, Heitkamp M, Dagnan D (2005) Phytoextraction of lead from firing range soil by Vetiver grass. Chemosphere 61(10):1451–1457
11. USEPA (2003) TRW recommendations for performing human health risk analysis on small armshooting ranges
12. Ahmad M, Lee SS, Yang JE, Ro H-M, Lee YH, Ok YS (2012) Effects of soil dilution and amendments (mussel shell, cow bone, and biochar) on Pb availability and phytotoxicity in military shooting range soil. Ecotoxicol Environ Saf 79:225–231
13. Migliorini M, Pigino G, Bianchi N, Bernini F, Leonzio C (2004) The effects of heavy metal contamination on the soil arthropod community of a shooting range. Environ Pollut 129(2):331–340
14. Rajapaksha AU, Ahmad M, Vithanage M, Kim K-R, Chang JY, Lee SS, Ok YS (2015) The role of biochar, natural iron oxides, and nanomaterials as soil amendments for immobilizing metals in shooting range soil. Environ Geochem Health 37:931–942
15. Dytrtová J, Šestáková I, Jakl M, Száková J, Miholová D, Tlustoš P (2008) The use of differential pulse anodic stripping voltammetry and diffusive gradient in thin films for heavy metals speciation in soil solution. Open Chem 6(1):71–79
16. Cao X, Dermatas D, Xu X, Shen G (2008) Immobilization of lead in shooting range soils by means of cement, quicklime, and phosphate amendments. Environ Sci Pollut Res 15(2):120–127
17. Vantelon D, Lanzirotti A, Scheinost AC, Kretzschmar R (2005) Spatial distribution and speciation of lead around corroding bullets in a shooting range soil studied by micro-X-ray fluorescence and absorption spectroscopy. Environ Sci Technol 39(13):4808–4815
18. Yin X, Saha UK, Ma LQ (2010) Effectiveness of best management practices in reducing Pb-bullet weathering in a shooting range in Florida. J Hazard Mater 179(1):895–900
19. Ma LQ, Hardison DW Jr, Harris WG, Cao X, Zhou Q (2007) Effects of soil property and soil amendment on weathering of abraded metallic Pb in shooting ranges. Water Air Soil Pollut 178(1–4):297–307
20. Hough RL (2010) Copper and lead. In: Trace elements in soils. Blackwell, pp 441–460
21. Shafiq-ur-Rehman (2013) Effect of lead on lipid peroxidation, phospholipids composition, and methylation in erythrocyte of human. Biol Trace Elem Res 154(3):433–439
22. Rodriguez E, da Conceição SM, Azevedo R, Correia C, Moutinho-Pereira J, de Oliveira JMPF, Dias MC (2015) Photosynthesis light-independent reactions are sensitive biomarkers to monitor lead phytotoxicity in a Pb-tolerant Pisum sativum cultivar. Environ Sci Pollut Res 22(1):574–585
23. Pourrut B, Shahid M, Douay F, Dumat C, Pinelli E (2013) Molecular mechanisms involved in lead uptake, toxicity and detoxification in higher plants. In: heavy metal stress in plants. Springer, Berlin, pp 121–147
24. Fraga CG (2005) Relevance, essentiality and toxicity of trace elements in human health. Mol Aspects Med 26(4):235–244
25. Nagajyoti P, Lee K, Sreekanth T (2010) Heavy metals, occurrence and toxicity for plants: a review. Environ Chem Lett 8(3):199–216
26. Lee S-E, Ahmad M, Usman AA, Awad YM, Min S-H, Yang JE, Lee SS, Ok YS (2011) Effects of biochar on soil quality and heavy metal availability in a military shooting range soil in Korea. 한국토양비료학회지 44(1):67–77

27. Spuller C, Weigand H, Marb C (2007) Trace metal stabilisation in a shooting range soil: mobility and phytotoxicity. J Hazard Mater 141(2):378–387
28. Sanderson P, Naidu R, Bolan N, Bowman M, Mclure S (2012) Effect of soil type on distribution and bioaccessibility of metal contaminants in shooting range soils. Sci Total Environ 438:452–462
29. Bakshi S, He ZL, Harris WG (2013) Particulate copper in soils and surface runoff from contaminated sandy soils under citrus production. Environ Sci Pollut Res 20(12):8801–8812
30. Babu TS, Marder JB, Tripuranthakam S, Dixon DG, Greenberg BM (2001) Synergistic effects of a photooxidized polycyclic aromatic hydrocarbon and copper on photosynthesis and plant growth: evidence that in vivo formation of reactive oxygen species is a mechanism of copper toxicity. Environ Toxicol Chem 20(6):1351–1358
31. Sheldon A, Menzies N (2005) The effect of copper toxicity on the growth and root morphology of Rhodes grass (Chloris gayana Knuth.) in resin buffered solution culture. Plant and Soil 278(1–2):341–349
32. Robinson BH, Bischofberger S, Stoll A, Schroer D, Furrer G, Roulier S, Gruenwald A, Attinger W, Schulin R (2008) Plant uptake of trace elements on a Swiss military shooting range: uptake pathways and land management implications. Environ Pollut 153(3):668–676
33. Ahmad M, Hashimoto Y, Moon DH, Lee SS, Ok YS (2012) Immobilization of lead in a Korean military shooting range soil using eggshell waste: an integrated mechanistic approach. J Hazard Mater 209:392–401
34. Sanderson P, Naidu R, Bolan N (2014) Ecotoxicity of chemically stabilised metal (loid) s in shooting range soils. Ecotoxicol Environ Saf 100:201–208
35. Scheinost AC, Rossberg A, Vantelon D, Xifra I, Kretzschmar R, Leuz A-K, Funke H, Johnson CA (2006) Quantitative antimony speciation in shooting-range soils by EXAFS spectroscopy. Geochim Cosmochim Acta 70(13):3299–3312
36. Qi C, Wu F, Deng Q, Liu G, Mo C, Liu B, Zhu J (2011) Distribution and accumulation of antimony in plants in the super-large Sb deposit areas, China. Microchem J 97(1):44–51
37. Tisarum R, Ren JH, Dong X, Chen H, Lessl JT, Ma LQ (2015) A new method for antimony speciation in plant biomass and nutrient media using anion exchange cartridge. Talanta 144:1171–1175
38. Arai Y (2010) Arsenic and antimony. In: Trace elements in soils. Wiley, Chichester, pp 383–408
39. Gad SC (2014) Antimony. In: Wexler P (ed) Encyclopedia of toxicology, 3rd edn. Academic, Oxford, pp 274–276
40. Feng R, Wei C, Tu S, Ding Y, Wang R, Guo J (2013) The uptake and detoxification of antimony by plants: a review. Environ Exp Bot 96:28–34
41. Xu L, Wu X, Wang S, Yuan Z, Xiao F, Ming Y, Jia Y (2015) Speciation change and redistribution of arsenic in soil under anaerobic microbial activities. J Hazard Mater 301:538–546
42. Vithanage M, Dabrowska BB, Mukherjee AB, Sandhi A, Bhattacharya P (2012) Arsenic uptake by plants and possible phytoremediation applications: a brief overview. Environ Chem Lett 10(3):217–224
43. Sneddon J, Clemente R, Riby P, Lepp NW (2009) Source-pathway-receptor investigation of the fate of trace elements derived from shotgun pellets discharged in terrestrial ecosystems managed for game shooting. Environ Pollut 157(10):2663–2669
44. Peddicord RK, LaKind JS (2000) Ecological and human health risks at an outdoor firing range. Environ Toxicol Chem 19(10):2602–2613
45. Dermatas D, Cao X, Tsaneva V, Shen G, Grubb DG (2006) Fate and behavior of metal (loid) contaminants in an organic matter-rich shooting range soil: Implications for remediation. Water Air Soil Pollut Focus 6(1–2):143–155
46. Walsh LM, Sumner ME, Keeney DR (1977) Occurrence and distribution of arsenic in soils and plants. Environ Health Perspect 19:67
47. Patra M, Bhowmik N, Bandopadhyay B, Sharma A (2004) Comparison of mercury, lead and arsenic with respect to genotoxic effects on plant systems and the development of genetic tolerance. Environ Exp Bot 52(3):199–223

48. Khan FI, Husain T, Hejazi R (2004) An overview and analysis of site remediation technologies. J Environ Manage 71(2):95–122
49. Semer R, Reddy KR (1996) Evaluation of soil washing process to remove mixed contaminants from a sandy loam. J Hazard Mater 45(1):45–57
50. Hashimoto Y, Taki T, Sato T (2009) Sorption of dissolved lead from shooting range soils using hydroxyapatite amendments synthesized from industrial byproducts as affected by varying pH conditions. J Environ Manage 90(5):1782–1789
51. Conesa HM, Wieser M, Studer B, González-Alcaraz MN, Schulin R (2012) A critical assessment of soil amendments (slaked lime/acidic fertilizer) for the phytomanagement of moderately contaminated shooting range soils. J Soil Sediment 12(4):565–575
52. Ahmad M, Moon DH, Lim KJ, Shope CL, Lee SS, Usman AR, Kim K-R, Park J-H, Hur S-O, Yang JE (2012) An assessment of the utilization of waste resources for the immobilization of Pb and Cu in the soil from a Korean military shooting range. Environ Earth Sci 67(4):1023–1031
53. Chen M, Ma LQ, Singh SP, Cao RX, Melamed R (2003) Field demonstration of in situ immobilization of soil Pb using P amendments. Adv Environ Res 8(1):93–102
54. Mahar A, Ping W, Ronghua L, Zhang Z (2015) Immobilization of lead and cadmium in contaminated soil using amendments: a review. Pedosphere 25(4):555–568
55. Li L, Xing W, Scheckel KG, Xiang G, Ji H, Li H (2013) Lead retention in a calcareous soil influenced by calcium and phosphate amendments. J Hazard Mater 262:250–255
56. Sanderson P, Naidu R, Bolan N, Bowman M (2011) Critical review on chemical stabilization of metal contaminants in shooting range soils. J Hazard Toxic Radioact Waste 16(3):258–272
57. Herath I, Kumarathilaka P, Navaratne A, Rajakaruna N, Vithanage M (2015) Immobilization and phytotoxicity reduction of heavy metals in serpentine soil using biochar. J Soil Sediment 15(1):126–138
58. Vithanage M, Rajapaksha AU, Tang X, Thiele-Bruhn S, Kim KH, Lee S-E, Ok YS (2014) Sorption and transport of sulfamethazine in agricultural soils amended with invasive-plant-derived biochar. J Environ Manage 141:95–103
59. Park JH, Choppala GK, Bolan NS, Chung JW, Chuasavathi T (2011) Biochar reduces the bioavailability and phytotoxicity of heavy metals. Plant and Soil 348(1–2):439–451
60. Tariq SR, Ashraf A (2013) Comparative evaluation of phytoremediation of metal contaminated soil of firing range by four different plant species. Arab J Chem Available from DOI doi:10.1016/j.arabjc.2013.09.024
61. Lee I, Baek K, Kim H, Kim S, Kim J, Kwon Y, Chang Y, Bae B (2007) Phytoremediation of soil co-contaminated with heavy metals and TNT using four plant species. J Environ Sci Health A 42(13):2039–2045
62. Xia HP (2004) Ecological rehabilitation and phytoremediation with four grasses in oil shale mined land. Chemosphere 54(3):345–353
63. Yang B, Shu WS, Ye ZH, Lan CY, Wong MH (2003) Growth and metal accumulation in vetiver and two Sesbania species on lead/zinc mine tailings. Chemosphere 52(9):1593–1600
64. Makris KC, Shakya KM, Datta R, Sarkar D, Pachanoor D (2007) High uptake of 2,4,6-trinitrotoluene by vetiver grass—potential for phytoremediation? Environ Pollut 146(1):1–4
65. Das P, Datta R, Makris KC, Sarkar D (2010) Vetiver grass is capable of removing TNT from soil in the presence of urea. Environ Pollut 158(5):1980–1983
66. Kim S-H, Park J-S, Lee I-S (2009) Characterization of cadmium-binding ligands from roots of Echinochloa crusgalli var. frumentacea. J Plant Biol 52(2):167–170
67. Tschan M, Robinson BH, Schulin R (2009) Antimony in the soil–plant system—a review. Environ Chem 6(2):106–115
68. Wan X-m, Tandy S, Hockmann K, Schulin R (2013) Changes in Sb speciation with waterlogging of shooting range soils and impacts on plant uptake. Environ Pollut 172:53–60
69. Sun Y, Zhou Q, Xu Y, Wang L, Liang X (2011) Phytoremediation for co-contaminated soils of benzo[a]pyrene (B[a]P) and heavy metals using ornamental plant Tagetes patula. J Hazard Mater 186(2):2075–2082

70. Kumar N, Bauddh K, Kumar S, Dwivedi N, Singh D, Barman S (2013) Accumulation of metals in weed species grown on the soil contaminated with industrial waste and their phytoremediation potential. Ecol Eng 61:491–495
71. Malar S, Manikandan R, Favas PJ, Sahi SV, Venkatachalam P (2014) Effect of lead on phytotoxicity, growth, biochemical alterations and its role on genomic template stability in Sesbania grandiflora: a potential plant for phytoremediation. Ecotoxicol Environ Saf 108:249–257
72. Ho WM, Ang LH, Lee DK (2008) Assessment of Pb uptake, translocation and immobilization in kenaf (*Hibiscus cannabinus* L.) for phytoremediation of sand tailings. J Environ Sci 20(11):1341–1347
73. Vithanage M, Rajapaksha AU, Oze C, Rajakaruna N, Dissanayake C (2014) Metal release from serpentine soils in Sri Lanka. Environ Monit Assess 186(6):3415–3429
74. Roccotiello E, Serrano HC, Mariotti MG, Branquinho C (2015) Nickel phytoremediation potential of the Mediterranean Alyssoides utriculata (L.) Medik. Chemosphere 119:1372–1378
75. Robinson B, Brooks R, Howes A, Kirkman J, Gregg P (1997) The potential of the high-biomass nickel hyperaccumulator Berkheya coddii for phytoremediation and phytomining. J Geochem Explor 60(2):115–126
76. Ghaderian SM, Ravandi AAG (2012) Accumulation of copper and other heavy metals by plants growing on Sarcheshmeh copper mining area, Iran. J Geochem Explor 123:25–32
77. Danh LT, Truong P, Mammucari R, Foster N (2014) A critical review of the arsenic uptake mechanisms and phytoremediation potential of Pteris vittata. Int J Phytoremediation 16(5):429–453
78. Uddin AJ, Manirul M, Mayeda U, Roni M, Mehraj H (2015) Evaluation of *Pteris vittata* as trap plant to mitigate arsenic from arsenic contaminated soil. J Sci Technol Environ Inform 1(2):75–80
79. Singh N, Ma LQ (2007) Assessing plants for phytoremediation of arsenic-contaminated soils. In: Phytoremediation. Springer, Berlin, pp 319–347
80. Jankong P, Visoottiviseth P, Khokiattiwong S (2007) Enhanced phytoremediation of arsenic contaminated land. Chemosphere 68(10):1906–1912
81. Feng R, Wei C, Tu S, Wu F, Yang L (2009) Antimony accumulation and antioxidative responses in four fern plants. Plant and Soil 317(1–2):93–101
82. Jana U, Chassany V, Bertrand G, Castrec-Rouelle M, Aubry E, Boudsocq S, Laffray D, Repellin A (2012) Analysis of arsenic and antimony distribution within plants growing at an old mine site in Ouche (Cantal, France) and identification of species suitable for site revegetation. J Environ Manage 110:188–193
83. Van Aken B (2008) Transgenic plants for phytoremediation: helping nature to clean up environmental pollution. Trends Biotechnol 26(5):225–227
84. Gisbert C, Ros R, De Haro A, Walker DJ, Bernal MP, Serrano R, Navarro-Aviñó J (2003) A plant genetically modified that accumulates Pb is especially promising for phytoremediation. Biochem Biophys Res Commun 303(2):440–445
85. Bennett LE, Burkhead JL, Hale KL, Terry N, Pilon M, Pilon-Smits EAH (2003) Analysis of transgenic Indian mustard plants for phytoremediation of metal-contaminated mine tailings. J Environ Qual 32(2):432–440
86. Shim D, Kim S, Choi Y-I, Song W-Y, Park J, Youk ES, Jeong S-C, Martinoia E, Noh E-W, Lee Y (2013) Transgenic poplar trees expressing yeast cadmium factor 1 exhibit the characteristics necessary for the phytoremediation of mine tailing soil. Chemosphere 90(4):1478–1486
87. Ahmad M, Lee SS, Lim JE, Lee S-E, Cho JS, Moon DH, Hashimoto Y, Ok YS (2014) Speciation and phytoavailability of lead and antimony in a small arms range soil amended with mussel shell, cow bone and biochar: EXAFS spectroscopy and chemical extractions. Chemosphere 95:433–441
88. Kim Y-N, Kim K-H (2010) Sequential fractionation and chemical speciation of Cd, Zn, Cu and Pb in the soils from two shooting ranges in Gyeonggi province, Korea. Pedologist 53(3):118–125

89. Evangelou MW, Hockmann K, Pokharel R, Jakob A, Schulin R (2012) Accumulation of Sb, Pb, Cu, Zn and Cd by various plants species on two different relocated military shooting range soils. J Environ Manage 108:102–107
90. Mozafar A, Ruh R, Klingel P, Gamper H, Egli S, Frossard E (2002) Effect of heavy metal contaminated shooting range soils on mycorrhizal colonization of roots and metal uptake by leek. Environ Monit Assess 79(2):177–191
91. Conesa H, Wieser M, Studer B, Schulin R (2011) Effects of vegetation and fertilizer on metal and Sb plant uptake in a calcareous shooting range soil. Ecol Eng 37(4):654–658
92. Cao X, Ma LQ, Chen M, Hardison DW, Harris WG (2003) Lead transformation and distribution in the soils of shooting ranges in Florida, USA. Sci Total Environ 307(1):179–189
93. Levonmäki M, Hartikainen H (2007) Efficiency of liming in controlling the mobility of lead in shooting range soils as assessed by different experimental approaches. Sci Total Environ 388(1):1–7
94. Uchimiya M, Bannon DI, Wartelle LH (2012) Retention of heavy metals by carboxyl functional groups of biochars in small arms range soil. J Agric Food Chem 60(7):1798–1809
95. Tschan M, Robinson BH, Nodari M, Schulin R (2009) Antimony uptake by different plant species from nutrient solution, agar and soil. Environ Chem 6(2):144–152
96. Baldantoni D, Cicatelli A, Bellino A, Castiglione S (2014) Different behaviours in phytoremediation capacity of two heavy metal tolerant poplar clones in relation to iron and other trace elements. J Environ Manage 146:94–99
97. Alaribe F, Agamuthu P (2015) Assessment of phytoremediation potentials of Lantana camara in Pb impacted soil with organic waste additives. Ecol Eng 83:513–520
98. Chehregani A, Noori M, Yazdi HL (2009) Phytoremediation of heavy-metal-polluted soils: screening for new accumulator plants in Angouran mine (Iran) and evaluation of removal ability. Ecotoxicol Environ Saf 72(5):1349–1353
99. Chen F, Wang S, Mu S, Azimuddin I, Zhang D, Pan X, Al-Misned FA, Mortuza MG (2015) Physiological responses and accumulation of heavy metals and arsenic of Medicago sativa L. growing on acidic copper mine tailings in arid lands. J Geochem Explor 157:27–35
100. Fumagalli P, Comolli R, Ferre C, Ghiani A, Gentili R, Citterio S (2014) The rotation of white lupin (Lupinus albus L.) with metal-accumulating plant crops: a strategy to increase the benefits of soil phytoremediation. J Environ Manage 145:35–42
101. Eid EM, Shaltout KH, El-Sheikh MA, Asaeda T (2012) Seasonal courses of nutrients and heavy metals in water, sediment and above-and below-ground Typha domingensis biomass in Lake Burullus (Egypt): perspectives for phytoremediation. Flora-Morphol Distrib Funct Ecol Plant 207(11):783–794
102. Polechońska L, Klink A (2014) Trace metal bioindication and phytoremediation potentialities of Phalaris arundinacea L. (reed canary grass). J Geochem Explor 146:27–33

Part II

Phytoremediation of Metalloid Contaminants

Chapter 18
Potential Promising Set of Plant–Microbe Interactions for the Revegetation of Open-Pit Mining and Smelting Areas in Brazil

Wesley de Melo Rangel, Jerusa Schneider,
Cláudio Roberto Fonsêca Sousa Soares, Rafael Dutra de Armas,
Paulo Ademar Avelar Ferreira, Admir José Giachini, José Oswaldo Siqueira,
and Fatima Maria de Souza Moreira

18.1 Introduction

World economic development is basically supported by the mining industry, which provides raw materials for various manufacturing industries, including agricultural fertilizers and infrastructure. The activity is strongly linked to social development by generating wealth and job opportunities. On the other hand, mineral exploitation, particularly mining, is a major cause of environmental impacts [1]. Mining, especially of non-ferrous metals, which extraction produces large amounts of wastes, is one of the main anthropogenic causes of environmental pollution, mainly with trace elements (TE) in soils and water bodies. These concentrations affect vegetation cover [2] and the biological properties of the soil [3–5].

In mining areas, not only the natural vegetation is suppressed, but there is also an intense movement of soil in open-pit mines and a generation of large volumes of wastes, that aggravates the disturbance of the surrounding areas [6]. These effects exacerbated by the removal of vegetation cover in contaminated areas accelerate the degradation of the whole environment (Fig. 18.1).

W. de Melo Rangel (✉) • F.M. de Souza Moreira
Federal University of Lavras, Lavras, Minas Gerais, Brazil
e-mail: wesleyrangeu@gmail.com

J. Schneider
University of Campinas, Campinas, São Paulo, Brazil

C.R.F.S. Soares • R.D. de Armas • A.J. Giachini
Federal University of Santa Catarina, Florianópolis, Santa Catarina, Brazil

P.A.A. Ferreira
Federal University of Santa Maria, Santa Maria, Rio Grande do Sul, Brazil

J.O. Siqueira
Vale Institute of Technology Sustainable Development, Belém, Pará, Brazil

A.A. Ansari et al. (eds.), *Phytoremediation*, DOI 10.1007/978-3-319-40148-5_18

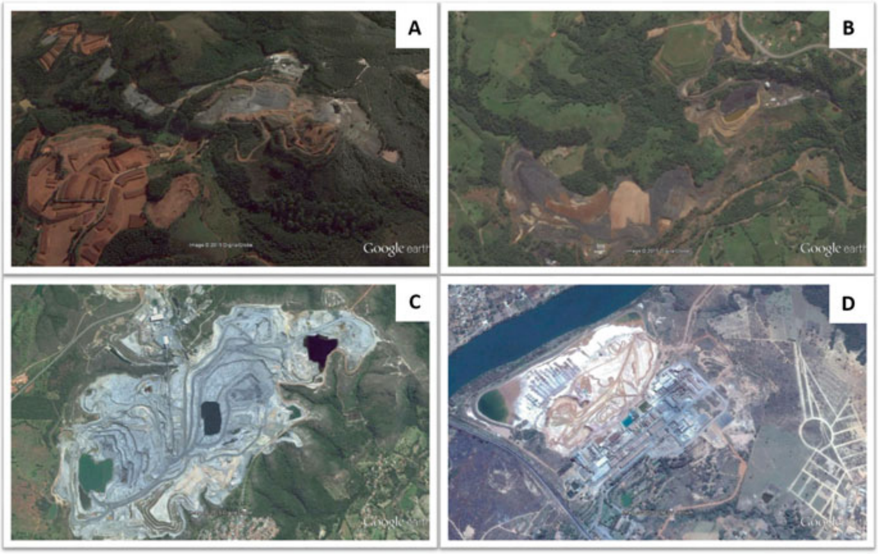


Fig. 18.1 Open-pit mining and smelting ore areas in Brazil. (**a**) Bauxite ore mining area located in Poços de Caldas, Minas Gerais (altitude of viewing point 2.6 km), (**b**) Coal ore mining area located in Criciúma, Santa Catarina (altitude of viewing point 2.0 km), (**c**) Gold mining area located in Paracatu, MG (altitude of viewing point 5.0 km), (**d**) Zinc smelting area located in Três Marias, MG (altitude of viewing point 2.6 km). *Source*: Google Earth Image © 2015 DigitalGlobe. Accessed October 2015

The unprotected land surface is more prone to water and wind erosion, and to the leaching of contaminants into the water table, triggering a ripple effect that spread contaminants to other areas. Additionally, after the removal of the vegetation, a loss of soil organic matter occurs, deteriorating the structure, water availability, and biological activity of the soil, thus affecting the supply of essential nutrients to plants, such as phosphorus (P), sulfur (S), and especially nitrogen (N) [7]. Areas that have suffered such interferences, in most cases, devoid natural biotic factors that facilitate spontaneous regeneration, requiring the aid of human actions to replenish the biotic components to the damaged environment.

A really promising technology for in situ land reclamation is phytoremediation, which is showing satisfactory results for environmental rehabilitation [8, 9]. This low cost technique creates minimal disturbance to the soil when compared to physical and chemical practices [10]. Phytoremediation uses plants and the associated microbiota to remove, immobilize, or render the contaminants harmless to the environment. It has many advantages, including permanent nature, low maintenance costs, protection against wind and water erosion, therefore, improving soil structure, soil fertility, and the aesthetic appearance of contaminated areas [8]. Moreover, it allows the establishment of a biocoenosis and biological succession.

Brazilian mineral resources are huge with about 8,400 mines producing 72 mineral substances in total, highlighting iron ore, bauxite, primary aluminum, zinc

Table 18.1 pH and total trace elements content in mining and smelting areas in Brazil, and guiding values established by the National Environment Council (CONAMA)

	Trace elements (mg dm^{-3})					
Area (mining/smelting)	pH $_{H2O}$	As	Cd	Pb	Zn	Cu
Paracatu-MG						
Gold mining[a]	5.0	396	–	–	0.2	0.4
Três Marias-MG						
Zn smelting	5.2	–	170	612	13,533	865
Criciúma-SC						
Coal mining	3.8	8.6	17.3	125	422	–
Guiding values by CONAMA						
Natural land	3.5	<0.5	17	60	35	
Industrial	150	160	4,400	10,000	10,000	

[a] Content found at B1 site (subsurface layer after removing topsoil)

concentrate, copper, niobium alloy, nickel, and gold, of which the production has overcome in 2014 [11, 12]. Although the mining industry is linked to both social and economic development, open-pit mining is environmentally very unfriendly due mainly to the large-scale removal of vegetation, soil, and overburden, besides being one of the major sources for TE entering soils. Phytoremediation success, for land reclamation, is linked to beneficial microorganisms, which benefit plant growth by performing essential biological processes, either in the rhizosphere or inside the plant [9, 13]. For that reason, native rhizobia and arbuscular mycorrhizal fungi are being studied in soils from mining and smelting areas contaminated with TE in Brazil. These microorganisms can provide important information on well-adapted genotypes holding a huge potential for biotechnological applications, especially concerning the plant growth promotion aspect, which is important for the phytoremediation approach.

Therefore, this chapter focuses on studies developed in Brazil in soils exploited by bauxite, coal, gold, and zinc mining and processing of mining ores (Table 18.1).

18.2 Promising Set of Plant–Microbe Interactions for the Revegetation of Open-Pit Mining and Smelting Areas in Brazil

18.2.1 Bauxite Open-Pit Mining in the State of Minas Gerais (MG), Southeast Brazil

Aluminum (Al) is the most abundant among metals in the earth's crust and the third among general elements. Obtaining Al in metallic forms starts usually at a bauxite ore mining. Besides using it for obtaining Al, bauxite is also used in chemical abrasives and cement industries. The world's bauxite reserves total 25.6 billion tons. Brazil is the fifth largest holder, with 3.8 billion tons of metallurgical bauxite. Domestic production occupies the third place in the international ranking with 32.8 million tons (Mt), data from 2013, behind Australia (77 Mt) and China (47 Mt). Within the country, the state of Pará is the largest producer (85 %), followed by Minas Gerais (14 %). The states of Maranhão, Amapá, Santa Catarina, São Paulo, Rio de Janeiro, Amazonas, and Goiás sum 1 % altogether.

Bauxite extracted at Poços de Caldas (MG) has one of the highest aluminum contents in the country. Extraction in this site is done in open-pit mines, with prior removal of soil organic layer (topsoil). The topsoil layer is the main source for plant propagules, organic matter, and microorganisms. Establishing vegetation, usually the first step towards remediation, becomes a challenge in these areas, since the soil usually lies with impaired fertility, low organic matter, and lack of essential nutrients, which are, in turn, fundamental to the development and establishment of plants and microorganisms, which are the revegetation's "drivers" [14]. Therefore, human intervention in the revegetation of these areas is essential.

18.2.1.1 Research Activities on Bauxite Open-Pit Mining Areas

Revegetation of open-pit mining areas has improved in recent years. There are significant advances in the processes allied to soil reconstitution (borrowed soil), the selection of plants species suitable for revegetation, development of methods of propagation, aside from techniques that allow the return of the fauna. However, the reestablishment of a sustainable biological system to create and maintain the flow of energy and nutrients in the exploited areas is still a major challenge. This usually happens because the surface layer of the original soil is replaced by another soil, which can have been stored for a long period elsewhere, reducing the number of plant propagules, as well as the density and diversity of microorganisms that are essential for nutrient cycling [14].

The essentiality of microorganisms to nutrient cycling dates back to the early days, participating in the precipitation of metals such as Fe^{2+} (Archaic 4.0–2.5 Ga) and the consequent creation and establishment of a conducive atmosphere to the development of new forms of life [15–17], consequently helping the colonization of the terrestrial environment by plants [18]. The colonization of plants by arbuscular mycorrhizal fungi (AMF) in the Triassic period, with similar morphology to AMF forms found today, highlights the importance and participation of this group of microorganisms in the evolution of nutrient cycling processes [19]. Most plants found growing in TE-contaminated soils are colonized by AMF, indicating that the fungi probably confer several benefits to the plants, such as increasing the resistance to TE or the absorption and supply of nutrients under these conditions [20–25].

Melloni et al. [21] evaluated the occurrence, diversity, and efficiency of AMF in soil from different areas of bauxite ore extraction in Poços de Caldas, MG, under different vegetation types or stages of revegetation. The revegetation was initiated by seeding grasses and herbaceous legumes, in addition to planting seedlings of native tree species and *Eucalyptus saligna*. The introduction of plant species such as molasses grass, rye grass, *Brachiaria*, pigeon pea, and bracatinga (*Mimosa scabrella*), raised the inoculum potential and diversity of AMF. Among the most prominent fungi, *Gigaspora margarita*, *Gigaspora* sp., *Paraglomus occultum*, *Glomus* sp., *Entrophospora colombiana* (renamed to *Acaulospora colombiana*), and *Acaulospora scrobiculata*. The occurrence of these species was favored by the presence of grasses and bracatinga. When compared to the reference area, the revegetation also recovered the total fungal mycelium in the soil.

On the other hand, the planting of *E. saligna* was not a good choice to boost the AMF. However, in some of the areas planted with eucalypts (16 years old), the density of AMF was higher due to the formation of a diverse understory composed of grasses and shrubs. With this study, the authors were able to show that the recovery of an AMF population in mined areas is dependent on the introduced vegetation, regardless of time of revegetation. The AMF are obligate biotrophic microorganisms with morphology and metabolism well developed to mobilize, assimilate, and carry soil nutrients to the host plant. In general, the nutritional benefit provided by the AMF is greater in poor soils, which is usually the case for soils explored by mining [26]. Therefore, the revegetation of these soils must prioritize plants able to establish symbiotic associations with microorganisms to allow for their proliferation and soil occupancy.

Among nutrients, nitrogen is the most limiting factor for the growth and development of plants and other organisms. Therefore, introducing or increasing the levels of the microbial population with potentiality for N_2 fixation is important in most soils. In that respect, Melloni et al. [27, 28] evaluated the phenotypic diversity of N_2-fixing endophytic and nodulating bacteria in the same areas of bauxite ore mining in Poços de Caldas, MG. The highest densities of endophytic bacteria were observed in revegetated soils with grasses, which are considered common hosts for this group of bacteria [29, 30]. Melloni et al. [27] observed that N_2-fixing endophytic bacteria did not occur nor had low densities in the reference areas (without anthropogenic interference). The highest density of these bacteria was found in mined areas under revegetation. These studies demonstrate the importance that N_2-fixing endophytic and nodulating bacteria have for early stages of revegetation. Once the ecosystem is stable or in climax (e.g., reference areas), the stimulus and energy spent on the biological N_2-fixation is reduced due to a more efficient nutrient cycling [31]. Similar to AMF, the phenotypic diversity of N_2-fixing endophytic and nodulating bacteria are also influenced by plant species, regardless of rehabilitation time.

18.2.2 Coal Open-Pit Mining in the States of Rio Grande do Sul (RS) and Santa Catarina (SC), Southern Brazil

Coal is considered the most abundant fossil fuel on the planet, whose reserves total 847.5 billion tons, and it is of major importance for production of electricity in several countries [32]. In Brazil, coal mining began in the late nineteenth century and intended to initially supply fuel to the railways. Currently, coal is of great importance for the Brazilian economy, with much of that aimed at generating electricity and for the manufacturing of glass and ceramics. Coal reserves in Brazil reach 32.6 billion tons, corresponding to 50 % of the nonrenewable energy resources of the country. The largest reserves are concentrated in the southern states, especially Rio Grande do Sul (RS) and Santa Catarina (SC), with approximately 28 and 3 billion tons, respectively [33, 34]. However, the coal coming from this region is of poor quality and has a high content of contaminants. After the processing, more than 50 % of the extracted volume is waste, which has long been deposited on the soil surface without any environmental care, furthermore burying the most fertile layer of the soil in the process [35]. Thus, despite the important role of coal in the Brazilian economy, the environmental impact of its operation is also significant. The coal-mined area is a national critical hotspot for pollution controls purposes [34].

Coal extraction in southern Brazil involves exploitation in underground and open-pit mines, whose methods entail serious environmental problems due to the large volume of ash generated and the improper disposal of waste. As a result, there are profound physical, chemical, and biological changes in the ecosystems associated with mining areas, endangering the soil, the biota, and the water resources [36]. Open-pit mining was one of the activities that caused the biggest changes in the affected ecosystems due to deforestation, soil removal, and topographical changes over relatively large areas. In addition to direct effects, indirectly it triggers others, such as increased susceptibility to erosion, pollution of water bodies, migration of fauna, and loss of scenic value [37, 38].

Several physical impacts are caused when mines and pits are open to grant access to the coal layer. Loss of soil structure is caused by the removal of surface vegetation. However, from a chemical point of view, acid mine drainage (AMD) is one of the biggest environmental problems of coal mining [39]. AMD is generated by the oxidation of pyrite, and it is primarily catalyzed by the bacterial species *Acidithiobacillus ferrooxidans* and *Thiobacillus denitrificans*. The outcome is the formation of sulfuric acid, sulfate, and ferrous and ferric ions [40, 41]. The result of this oxidative process is the marked reduction in soil pH that reach values lower than 4.0. In such acidic conditions, the majority of the compounds containing TE are solubilized, being subject to leaching and contamination of water sources, besides impairing the establishment of vegetation on the contaminated site [42]. In the state of Santa Catarina alone, it is estimated that there is about 6,700 ha degraded by mining, and that 2/3 of the waterways are contaminated by AMD [43]. Because of mining in these areas, soil fertility is also compromised. Therefore, human intervention is essential for the recovery of these degraded areas [44, 45].

18.2.2.1 Research on Coal Open-Pit Mining Areas

One strategy to rehabilitate these areas is to reestablish vegetation, which is usually difficult. One major problem faced is the selection of plant species that fit in the rehabilitating environment. Additionally, the low nutrient content and poor soil physical conditions represent another drawback. The key aspect for a successful rehabilitation is to find and to establish a “facilitation environment”, which may include microorganisms. Inoculation with plant growth-promoting microorganisms (PGPM) is a promising alternative [46]. Nitrogen fixing bacteria stand out as PGPM because the symbiotic process convert atmospheric nitrogen (N_2), unassimilable in its basic form, into ammonia (NH_3) that can be used by plants [47]. In addition to N_2 fixation, rhizobia can promote plant growth by other mechanisms, such as the solubilization of phosphate, production of phytohormones (e.g., auxins), siderophores, and important enzymes such as ACC deaminase [48–52]. The ability of legume species to establish symbiosis with rhizobia varies from highly specific to interactions considered promiscuous, giving different degrees of efficiency among symbionts [53].

In light of the raised points, it is believed that better results can be obtained when plants are inoculated with rhizobia from the coal mining areas. Therefore, there are leading efforts aiming at isolating rhizobia from soil samples collected in the coal mining areas in RS and SC states, in southern Brazil. The soils from the mining areas have been chemically characterized as extremely acid and with a high degree of TE contamination, highlighting the importance of selecting native rhizobia adapted to these stress conditions. Native rhizobia were captured using *Macroptilium atropurpureum*, *Vigna sativa*, and *Vigna unguiculata*, recognized as trap-plants for their low specificity for species of rhizobia, using a substrate composed of sand and vermiculite. Native rhizobia were isolated from two coal-mining areas located in RS and SC states.

In total 115 isolates were obtained from RS and 19 from SC soils. Isolates were selected (18 strains from RS and 16 strains from SC) based on features of interest, such as rapid growth, which increases the chances of establishment in the field; capacity of growing in different pH ranges (4.0 to 9.0), which allows the inoculum lastingness throughout the recovery process as the soil pH tends to increase overtime; and resistance to TE, since high levels of TE are present in the coal mining areas. Bacterial strains were identified by partial sequencing the 16S rRNA gene. The strains from RS belonged to *Bradyrhizobium* (12 strains), *Rhizobium* (4 strains), and *Burkholderia* (2 strains) genera, and those from SC belonged *Rhizobium* (11 strains), *Gluconobacter* (3 strains), and *Burkholderia* (2 strains) genera. These results confirm the widespread occurrence of different rhizobia genera in the coal mining areas, and validate the choice for *Rhizobium* and *Bradyrhizobium*. It is worth mentioning the occurrence of *Gluconobacter* in SC, which represents a typical endophytic species known to promote plant growth [54, 55]. Nevertheless, there are no reports in the literature on the ability of individuals from this genus to promote nodulation in legume species. Further studies will search for *nod* genes in the genome of *Gluconobacter* strains.

The plant growth promoting potential of those selected strains was evaluated in arboreal and herbaceous legume species, generally used for the recovery of coal degraded areas in RS and SC: *M. atropurpureum* and *V. sativa* (both herbaceous) for RS and *Calopogonium mucunoides*, *V. sativa* (herbaceous), *Mimosa bimucronata*, *M. scabrella*, and *Parapiptadenia rigida* (arboreal) for SC. Plants were grown in a greenhouse, in the same conditions as described above for the capturing of rhizobia.

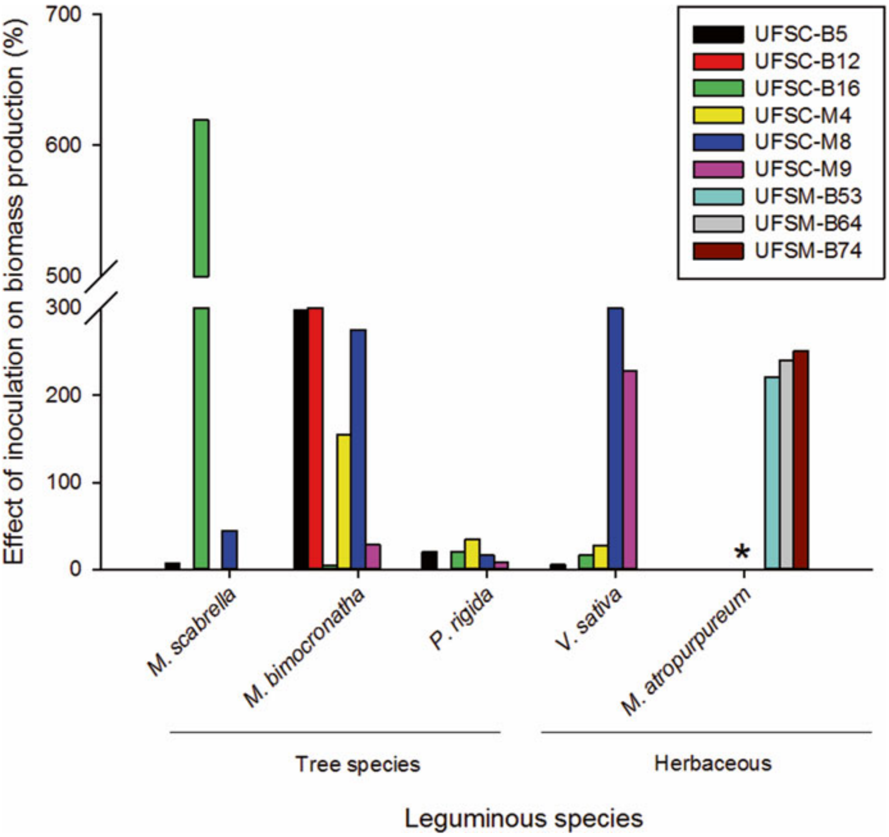


Fig. 18.2 Inoculation effect of native rhizobia from coal ore mining areas (SC and RS) on dry biomass production by arboreal and herbaceous legume species *Only rhizobia from RS were inoculated in *M. atropurpureum*

Although rhizobia did not promote the growth of *V. sativa* in RS neither of *C. mucunoides* in SC soils, three strains (UFSM-B53, UFSM-B64, and UFSM-B74) from RS were highly efficient and increased *M. atropurpureum* shoot dry matter (SDM) by 236 % when compared to the control without inoculation. For SC, two of the 16 selected strains (UFSC-M8 and UFSC-M9) efficiently promoted the growth of *V. sativa*, with an average improvement of 300 % in SDM (Fig. 18.2). Regarding arboreal species, the growth of *M. bimucronata* was improved by more than 200 % by UFSC-M8, UFSC-B12, and UFSC-B5 strains, while UFSC-B16 improved SDM in *M. scabrella* by more than 600 %. These results highlight the high potential of native rhizobia, from coal mining soils, to promote plant growth in such areas, improving nutrient cycling and the revegetation process.

Native rhizobia from contaminated areas might perform a more efficient N_2 fixation process under that stressful condition, with consequent improvements in soil fertility, a factor that is useful in phytoremediation. Besides the importance of efficient native rhizobia, it is also important that the plant is adapted to the adverse conditions commonly encountered in coal mining areas. For that reason, studies are under development to test the production of seedlings using degraded soils from the coal mining areas. This will relate the symbiotic efficiency of selected rhizobia with plant establishment and soil quality in these impaired areas. Besides the promising potential of rhizobia to promote plant growth, another group of microorganisms, composed by arbuscular mycorrhizal fungi, is able to improve plant growth and protection on contaminated soil. AMF increases the absorption of nutrients and water by plants [56, 57]. Mycorrhizal fungi may contribute with up to 80 % of the P, 60 % of Cu, 25 % of N, 25 % of Zn, and 10 % of the K absorbed by plants. Noteworthy is the fact that the largest contribution is for nutrients that have reduced diffusion in the soil [58].

Among the different types of mycorrhizae, arbuscular mycorrhizal fungi (AMF) have an outstanding role in the revegetation of degraded soils [59]. It has been shown that AMF favor the growth of plants in contaminated environments due to direct mechanisms, such as enhanced TE holding capacity [60], or indirect ones, such as stimulating plant biomass production and the concomitant increase in TE phytoextraction [61]. Therefore, in order to evaluate the phytoextracting ability of AMF, five fungal species isolated from mined soils contaminated with TE in SC were tested: *Acaulospora colombiana*, *A. morrowiae*, *Dentiscutata heterogama*, *G. margarita*, and *Rhizophagus clarus*. The fungal isolates were inoculated in leguminous arboreal species typically used in revegetation programs, such as *M. scabrella*, *M. bimucronata*, and *P. rigida*. Apart from the arboreal representatives, the herbaceous grassy species *Chrysopogon zizanioides* (vetiver) was also employed. Besides having a fasciculate and deep root system, a feature that helps to provide great physical and chemical stability for the soil, vetiver is also recognized as tolerant to adverse climatic and edaphic conditions, in addition from being highly tolerant to the presence of elevated concentrations of toxic elements [62]. Assays were conducted in a greenhouse utilizing a sterilized substrate (to eliminate potential native AMF) containing tailings from the coal mining areas of SC.

All treatments with the arboreal leguminous species inoculated with AMF increased the accumulation of TE in the shoots of plants (Fig. 18.3). For *M. scabrella*, average accumulations for Cr, Zn, and Cu were 4.0, 4.7, and 5.5 times higher than the non-inoculated treatment, respectively. For *M. bimucronata*, average accumulations for the same elements were 2.7, 3.5, and 8.5 times higher than the control, respectively. For *P. rigida*, the inoculation with *R. clarus* and *A. morrowiae* promoted increments in the Cu and Zn accumulated levels of 4.8 and 6.2 times, respectively, and Cr accumulations were in average 4.3 times higher for both isolates.

When inoculated to *C. zizanioides*, *G. margarita* stood out among all fungal species for promoting significant increases in the accumulation of all TE evaluated. This is probably directly related to the larger biomass produced by the plant when inoculated with that fungal species. When compared to the control, *G. margarita* promoted increases of 26, 28, and 250 % in the levels of accumulated Cr, Cu, and Zn, respectively. Other AMF, such as *R. clarus* and *A. colombiana*, have also contributed, in a lesser extent, towards the accumulation of Zn, and, therefore, are promising for the phytoextraction of TE in multi-contaminated environments.

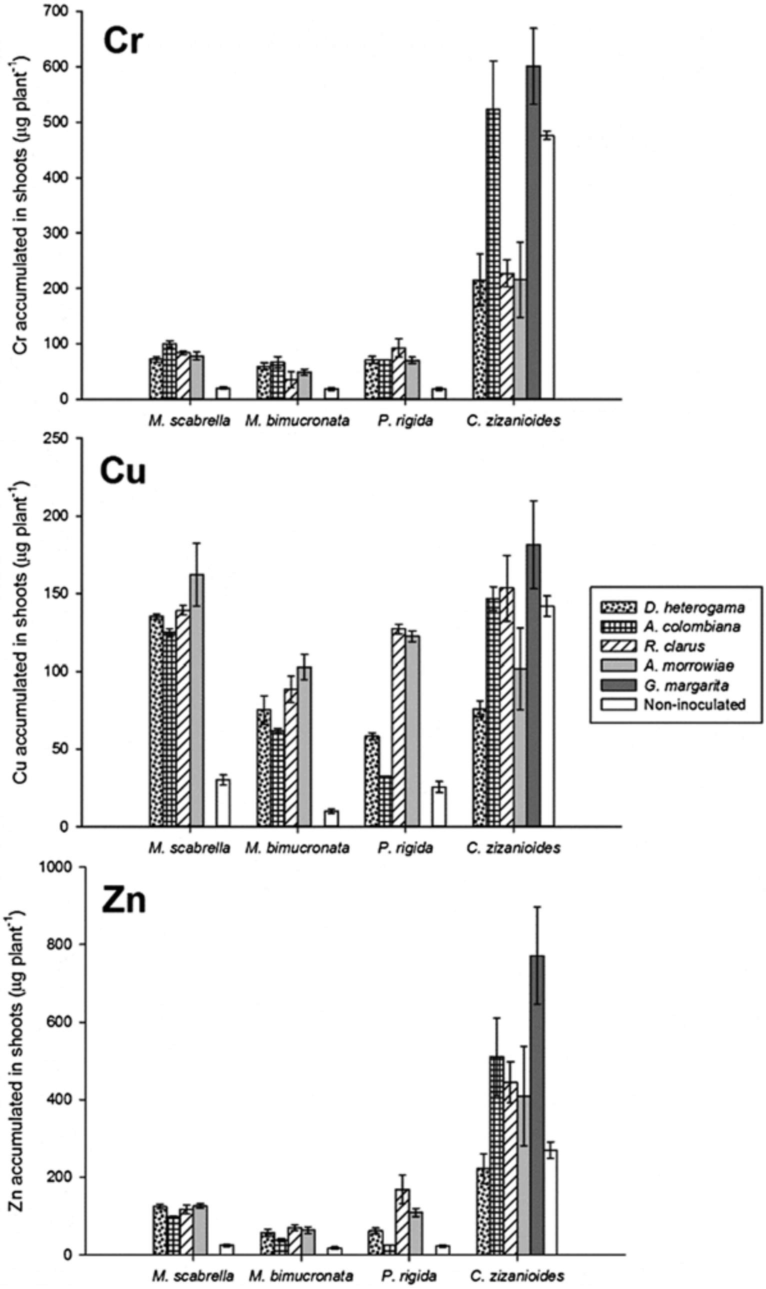


Fig. 18.3 Cr, Cu and Zn accumulation by arboreal legume species grown on a substrate composed by coal tailing + vermiculite + sand (7:3:1) and by grassy species grown on a substrate composed just by coal tailing from a coal mining area in SC

AMF provided increments in the cumulative amounts of several elements, depicting its high potential for the phytoaccumulation of TE from coal mining tailings. Vodnik et al. [63] demonstrated that AMF reduce the concentration of TE in the soil and improve the nutritional status of the plants, with consequent increase in the production of chlorophyll. Other benefits include the ability to produce glycoproteins, such as glomalins, which have an important role in aggregate stability and carbon storage [64], while reducing TE bioavailability by sequestering the phytotoxic elements into particular compartments in the plant's cells [63, 65].

Furthermore, AMF also have significant impact on the structuring of the soil [66] due to the large amount of produced hyphae [67] and the exudation of hydrophobic glycoproteins, called glomalins [68]. Besides acting in the formation of aggregates, glomalins contribute to the increase of C in the soil [69], which, in turn, is important for enhancing soil water contents and holding capacity, root penetration, as well as reducing the erosive potential of soils. These improvements are of great importance for physical reconstruction of soils in mining areas, since soil horizons have been generally inverted as a result of ore extraction.

18.2.3 Gold Open-Pit Mining in the State of Minas Gerais (MG), Southeast Brazil

Domestic production of gold in 2013 highlighted Brazil as the 11th largest producer in the world. The state of Minas Gerais accounts for nearly half of Brazil's production (45.6 %), followed by Goiás (12.3 %), Mato Grosso (11 %), Pará (11 %), Amapá (7.6 %), Bahia (7.4 %), and Maranhão (3.6 %) [11]. In the city of Paracatu, located in the northwestern region of Minas Gerais, gold ores are rich in sulfide compounds. Therefore, the extraction of gold is directly associated with the production of solid wastes rich in As [70, 71]. These wastes are deposited in the form of "sterile" and "waste" piles. The dissolution of As minerals, such as arsenopyrite, arranged in these cells, represents a continuing source of contamination in those environments [72, 73]. Apart from the tailings deposited in the piles, during the processing or treatment of ores, involving physical and chemical separations, there is also the production of mineral residues, which are launched into containment dams in the form of pulp, comprising mineral residues and liquids [74].

The mining activity in areas that concentrate minerals in the form of sulfides can cause intense oxidation of sulfur compounds into sulfate, consequently acidifying the surrounding medium. With the lowering of the pH to values near 2.5–3.5, a large proportion of the compounds containing TE are solubilized and, therefore, subject to leaching and contamination of water supplies. Acid drainage in these areas is controlled by the addition of large amounts of limestone that promotes re-precipitation of heavy cations in the form of sulfates or carbonates. This practice, however, is very expensive and often difficult to operate. Furthermore, it significantly changes the environmental conditions, especially by interfering in the osmotic equilibrium via salt deposition and concentration, and by altering the soil microbiota composition and stability, through changes in the trophic settings [73].

The chemical mechanisms that govern the redox processes and their impact on the environment in areas rich in sulfides are still not well known. A major problem arises when the oxidation of sulfides release toxic amounts of As into the hydrosphere, pedosphere and, by extension, to the entire food chain [75]. Another problem is associated with the construction of tailings dams. During the process, there is need to remove soil surface layers from adjacent areas (loan material) to be used as physical containment of the structure thereof. This compromises soil quality of the adjacent areas, which are then characterized by high degree of compaction and high levels of erosion (hydric and eolic alike), factors that hinder the establishment of vegetation and directly affects the biological activity of the soil [3–5]. A general overview of the study area and the visual environmental impacts associated with gold mining in the city of Paracatu (MG) are presented in Fig. 18.4.

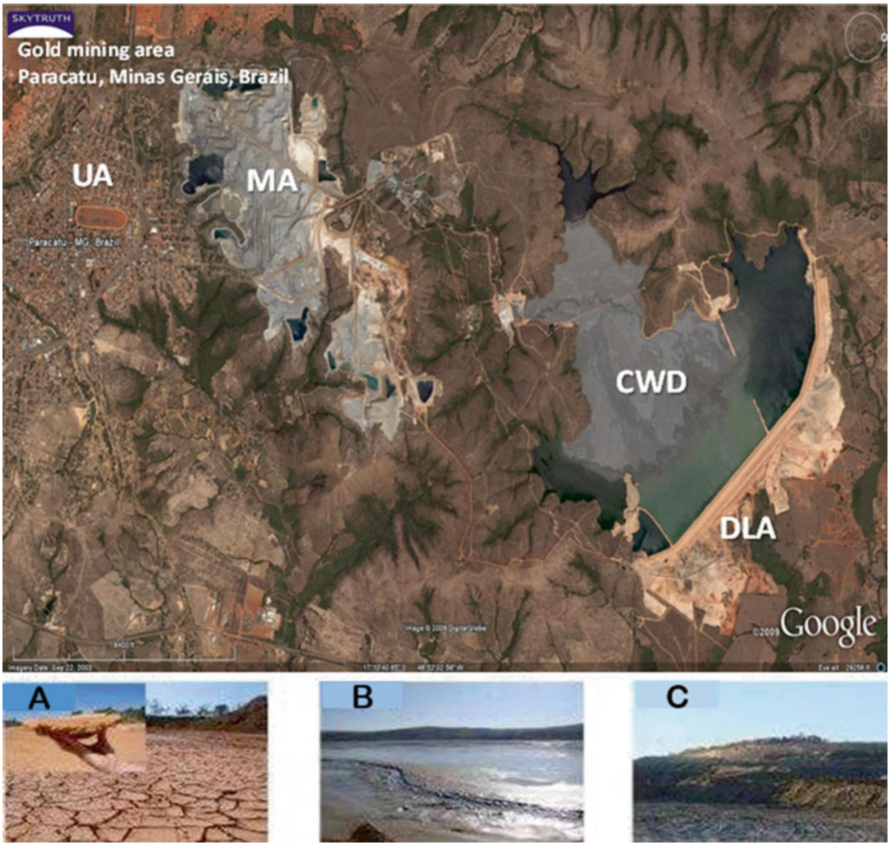


Fig. 18.4 Satellite imagery of the gold mining area located in Paracatu, Minas Gerias, Brazil. *DLA* degraded loan area, *CWD* contaminated waste dam, *MA* mining area, *UA* urban area. (*Source*: Google Earth™). Accessed in 2009. (**a**) Picture taken at degraded loan area, (**b**) picture taken at contaminated waste dam area, and (**c**) picture taken at mining area (open-pit mining zone)

Native microbial populations in soils with high levels of contaminants are likely to possess mechanisms for adaptation into this kind of stress. Therefore, studies on microbial diversity can provide important information about the most suitable genotypes to be used in revegetation programs of these contaminated environments [76, 77].

18.2.3.1 Research on Gold Open-Pit Mining Areas

The ubiquity of AMF, found even in contaminated environments, allied to their biofertilizer properties and plant growth, and biocontrol promoting capacities, highlight the potential of these microorganisms for the revegetation of TE-contaminated soils [78, 79]. Just to illustrate, most plants adapted to As-contaminated soils are generally associated with AMF [20, 22, 25, 80]. In 2007, Chen et al. [81] proposed that AMF reduce the phytotoxicity of As by stimulating and increasing the acquisition of P by the mycorrhizal plants. In that way, the occurrence of AMF in a gold mining area located in Paracatu, MG, were studied by Schneider et al. [25]. The AMF occurrence and species richness [82] are shown in Table 18.2.

Twenty-three species were detected, belonging to *Acaulospora* (10), *Scutellospora* (4), *Racocetra* (3), *Glomus* (4), *Gigaspora* (1), and *Paraglomus* (1). The species *Acaulospora mellea*, *A. spinosa*, *A. paulinae*, *A. scrobiculata*, *Glomus fascicu-*

Table 18.2 Arbuscular mycorrhizal fungal genera and species richness found in different sites during rainy and dry season in a gold mine area in Paracatu, MG-Brazil

	Rainy season				Species	Dry season				Species
AMF species	RPPN	B1	BM	MW	richness	RPPN	B1	BM	MW	richness
Family Acaulosporaceae										
Acaulospora	–	1	–	–	2	5	2	5	–	6
Family Glomeraceae										
Glomus	3	1	–	–	3	3	2	1	–	4
Family Gigasporaceae										
Gigaspora	–	–	–	–	0	1	–	–	–	1
Family Scutellosporaceae										
Scutellospora	1	–	–	–	1	3	3	1	–	5
Family Paraglomeraceae										
Paraglomus	1	1	-	-	1	1	1	1	-	1
Family Racocetraceae										
Racocetra	–	–	–	–	0	2	–	–	–	2
Total species richness	5	2	0	0	7	15	8	8	0	19

absence of AMF species. Number of samples where the fungus species was observed (n=24). RPPN- Private Reserve of Natural Heritage (Reference Area); B1- soil subsurface layer present in areas for Au extraction; BM- not economically usable material, due to its low Au content; MW-area with materials from Au processing (waste retention pond). Modified from Schneider et al. 2013 [83] *latum*, *G. minutum*, *Gigaspora margarita*, *Racocetra castanea*, *R. persicae*, *Scutellospora fulgida*, *S. pelucida*, and *S. biornata* were found only in the reference area. On the other hand, *A. colombiana*, *A. excavata*, *Acaulospora* sp. #1, *Acaulospora* sp. #2, *A. tubercullata*, and *Scutellospora* sp. #1, although in low incidences, inhabited sites with the highest levels of As. *A. morrowiae*, *Glomus clarum* (renamed to *Rhizophagus clarus*), and *P. occultum* were detected in all sites. These results show that contaminated areas may store AMF species adapted to such environmental pressure. It shows that there is need for evaluating AMF potential to promote plant growth on contaminated soils.

Keeping this in mind, Schneider et al. [83] evaluated the potential of some AMF species to promote and protect the growth of *Leucaena leucocephala* in a Quartzipsamment soil (RQ), contaminated with $Na_2HAsO_4{\cdot}7H_2O$ after adding 0, 35, and 75 mg As dm^{-3} of soil. The authors assessed AMF colonization and the production of propagules (spores and extramatrical mycelium). Inoculation treatments included three promising phytoprotective species *A. morrowiae*, *Gigaspora albida*, and *Glomus clarum* (renamed to *Rhizophagus clarus*) for multi-contaminated soils [61, 84], a mix of the three AMF species cited above, and a "Paracatu" mix, composed by *A. morrowiae*, *G. clarum* (renamed to *Rhizophagus clarus*) and *P. occultum*, all isolated from a mining area in Paracatu (MG). Inoculation with *A. morrowiae*, *G. clarum* (renamed to *Rhizophagus clarus*), and the AMF mix promoted shoot dry matter increments of 209, 184, and 170 %, respectively (Fig. 18.5).

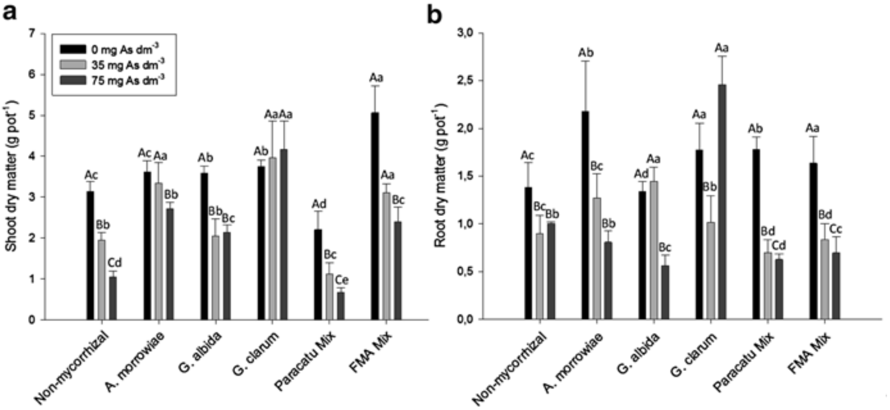


Fig. 18.5 Dry matter production by *Leucaena leucocephala* inoculated with different arbuscular mycorrhizal fungi in As-contaminated soil. (**a**) Shoot dry matter. (**b**) Root dry matter. Means followed by the same letter are not statistically different by Scott-Knott test, $P<0.05$. Upper case letters compare between As doses treatments; lower case letters compare between different AMF inoculation treatments isolates; Vertical bars represent the standard deviation ($n=3$). Modified from Schneider J, Labory CRG, Rangel WM, Alves E, Guilherme LRG (2013b) Anatomy and ultrastructure alterations of *Leucena leucocephala* (Lam.) inoculated with mycorrhizal fungi in response to arsenic-contaminated soil. J Hazard Mater 262: 1245–1258, with permission from Elsevier

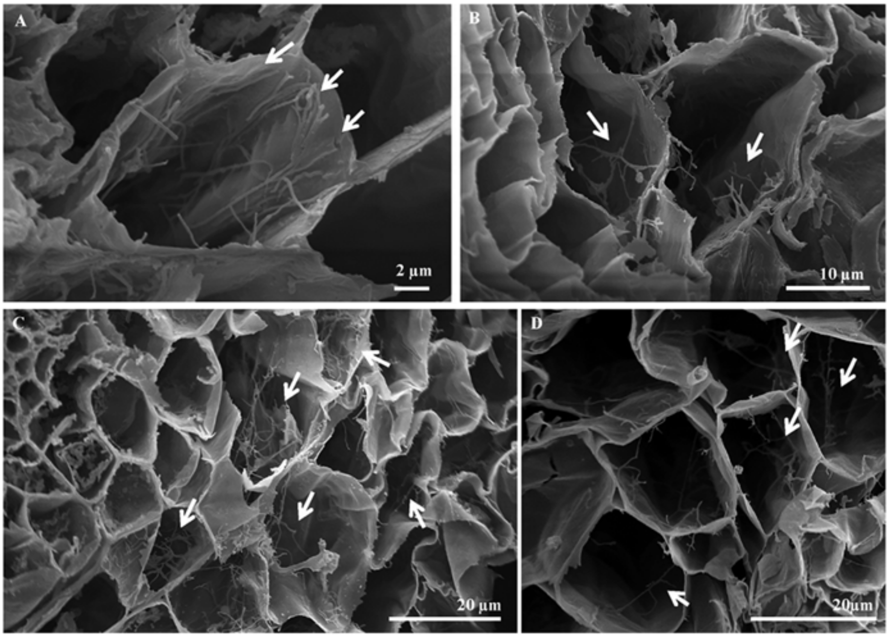


Fig. 18.6 Scanning electron micrographs of *L. leucocephala* roots. (**a**) Mycorrhizal hyphae (*arrows*) in the As-uncontaminated treatment inoculated with *G. clarum*; (**b**) Mycorrhizal hyphae (*arrows*) in the As-uncontaminated Mix treatment inoculated with AMF; (**c**) Mycorrhizal hyphae (*arrows*) in the 35 mg dm^{-3} As treatment inoculated with *G. clarum*; (**d**) Mycorrhizal hyphae (*arrows*) in the 75 mg dm^{-3} As treatment inoculated with *G. clarum*. Reprinted from Schneider J, Labory CRG, Rangel WM, Alves E, Guilherme LRG (2013b) Anatomy and ultrastructure alterations of *Leucena leucocephala* (Lam.) inoculated with mycorrhizal fungi in response to arsenic-contaminated soil. J Hazard Mater 262: 1245–1258, with permission from Elsevier

Overall, inoculated plants had higher growth than non-inoculated plants. However, the mycorrhizal colonization rate was significantly reduced with the addition of As (ranging from 43 to 11 % for treatments without As addition and 75 mg As kg^{-1}, respectively), mainly in the treatment with the highest levels of As. Even though the mycorrhizal colonization rate was greatly reduced in the treatment that received 75 mg As kg^{-1} soil (39 % of root colonization), there are AMF species (e.g., *G. clarum* renamed to *Rhizophagus clarus*) capable of establishing symbiosis even under these high levels of As (Fig. 18.6).

Mycorrhizal association stimulated increases in the concentration of important nutrients in the plants, such as P. This nutritional P enhancement and the improved plant growth is one of the beneficial effects observed in mycorrhizal plants exposed to TE stress [81, 85]. Besides the advantages of a better nutritional status, different physiological behaviors have been reported in mycorrhizal plants in response to metal exposure [86–89]. As it can be seen in Fig. 18.7, mycorrhizal plants retained the highest levels of As in the roots, demonstrating the phytoprotective effect of AMF for plant development in contaminated sites [80, 90–93].

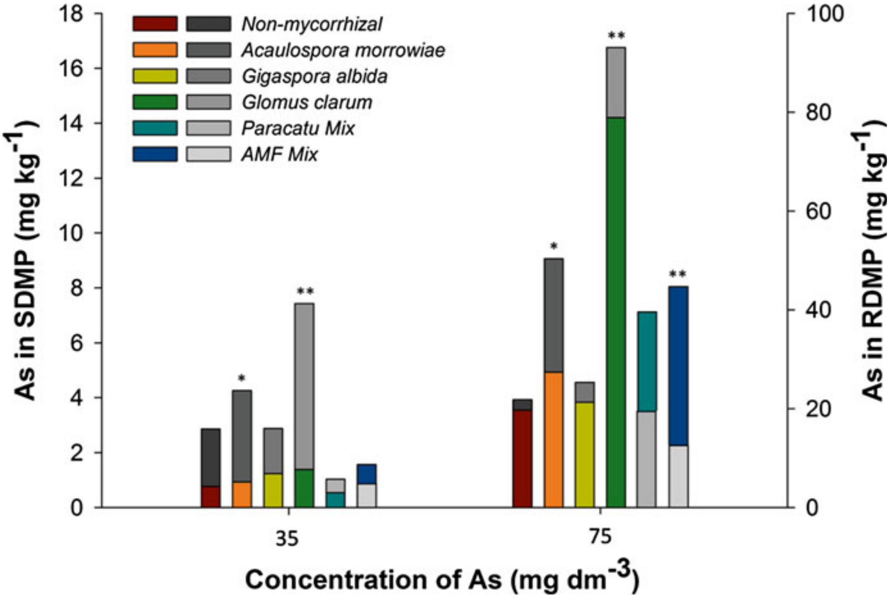


Fig. 18.7 Concentration of arsenic (mg dm^{-3}) in the shoot and root of *L. leucocephala* growing in soil contaminated with As. **P* ≤ 0.05; ***P* ≤ 0.01; *NS*, not significant; treatments with concentrations of 0 mg dm^{-3} the values were lower than the detection limit of the method (~0.060 mg dm^{-3}). Modified from Schneider J, Labory CRG, Rangel WM, Alves E, Guilherme LRG (2013b) Anatomy and ultrastructure alterations of *Leucena leucocephala* (Lam.) inoculated with mycorrhizal fungi in response to arsenic-contaminated soil. J Hazard Mater 262: 1245–1258, with permission from Elsevier

This phytoprotective effect promoted by AMF was further evaluated in *Acacia mangium*, *Crotalaria juncea*, *Enterolobium contortisiliquum*, and *Stizolobium aterrimum* development in As-contaminated soil from the gold mining area in Paracatu (MG) by Rangel et al. [80], whom concluded that AMF have an important role in As-tolerance and uptake. The phytoprotective mechanism is mediated by P uptake, as pointed by Chen et al. [81]. Even though the results varied according to plant and AMF species, the phytoprotective effect of AMF delayed the onset of As toxicity symptoms in the shoots of the plants. The activity of Ascorbate Peroxidase was a good enzymatic indicator of this phytoprotective effect in the shoots. It has been suggested that the protection provided by enzymes against oxidative stresses induced by metals can be one of the main benefits promoted by mycorrhizal fungi, conferring tolerance to plants in contaminated soils [80, 94]. However, it is important to keep in mind that the nonenzymatic antioxidant system may also be involved in these responses, and therefore should be considered in future studies [95, 96]. *C. juncea* and *S. aterrimum* accumulated more As in the roots when inoculated with AMF. For the latter, this effect was seen regardless of AMF species inoculated, while for the former the effect was more pronounced when inoculation was done with *Acaulospora* sp. Besides demonstrating the phytoprotective effect promoted by AMF, these results show the potential of the symbiosis for As phytostabilization, as high arsenic concentrations were immobilized in the roots, and increases in the P/As ratio in the plants were responsible for greater production of shoot biomass.

In addition to AMF, native rhizobia populations are also well adapted to soils with high levels of TE. Therefore, the study of rhizobia diversity can provide important information on genotypes that could potentially be employed in phytoremediation programs [76, 97]. Legume inoculation with beneficial rhizobia strains, well adapted to these highly stressed conditions, might be of interest both from an ecological and economic point of view [98–101]. In that respect, bacteria have been isolated from *S. aterrimum* and *Crotalaria spectabilis* nodules collected from the same gold mining area contaminated with As in Paracatu, MG. All strains isolated from the nodules were characterized in culture medium, identified by 16S rRNA gene partial sequencing, assayed for tolerance against As, and tested for several plant growth-promoting traits. Moreover, strain authentication (i.e., ability to induce nodule formation) and symbiotic efficiency have been checked in a greenhouse experiment. From the isolated native rhizobia, the strain UFLA 05-16 (*Rhizobium tropici*) was identified as highly tolerant to As and able to stimulate several plant growth-promoting traits, besides presenting high N_2 fixing efficiency when in symbiosis with *S. aterrimum*. Therefore, it represents a potential model to be used for the phytoremediation of As-contaminated soils (Rangel et al.—unpublished data).

Recently, Santos et al. [5] evaluated the process of revegetation in the gold mining area of Paracatu (MG), focusing primarily on the biological attributes of the soil. The authors noticed that aside from the removal of vegetation and contamination by As, the soils also had low microbial biomass and thus activity, emphasizing that only genotypes adapted to stressful conditions were still present in the soil. The study highlighted the contribution of a mix of legume species (*Albizia lebbeck*, *Acacia crassicarpa*, *A. holosericea*, *A. mangium*, *Pseudosamanea guachapele*, and *Sesbania virgata*) associated with an uncontaminated soil cover layer for the establishment of a microbial community composed by bacteria, fungi and actinobacteria assessed by microbial biomass carbon determination, substrate-induced respiration, and total density (Fig. 18.8). Although the study has focused just on general soil biological attributes, it is interesting to mention that the revegetated environment has grouped with a reserve of natural heritage by Principal Component Analysis. It shows the re-establishment of the total densities of bacteria, fungi and phosphate solubilizing fungi.

It is well known that plant-associated bacteria and fungi are crucial and able to enhance plant survival and growth in contaminated soils through several mechanisms [102–104]. Therefore, using the microbial potential to support sustainable technologies, such as phytotechnologies for the revegetation of mining areas, might be of high interest. The challenge for the future is to further unravel the complex interactions between plants and microbes, but also to understand at a deeper level their interactions with the different kinds of TE present in the soil.

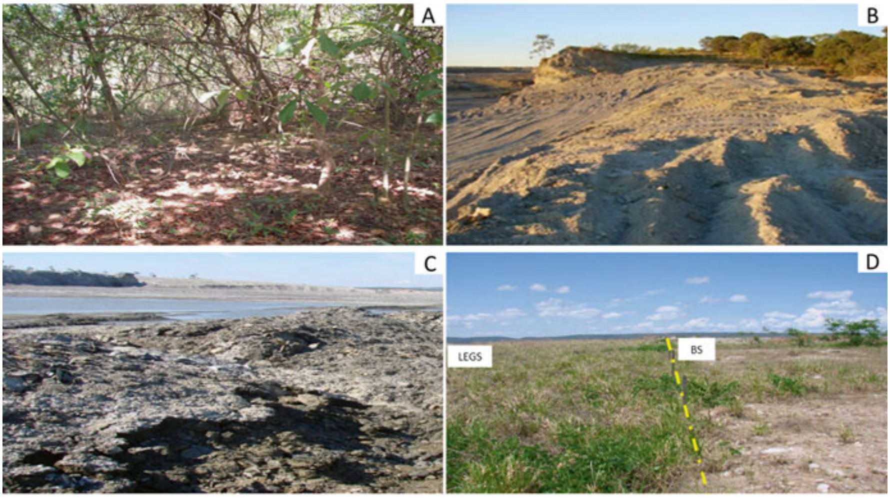


Fig. 18.8 Photos of the different studied sites in the gold mining area in Paracatu, MG. (**a**) Private reserve of natural heritage (RPPN) site, (**b**) B1 site—rather weathered sulfide substrate without revegetation, (**c**) barren material (BM) site—economically unusable material due to low Au grade and (**d**) growth of different leguminous species (LEGS) on the left, and *Brachiaria* sp. and *Stizolobium* sp. invasion (BS) on the right. *Source*: Santos JV, Rangel WM, Guimarães AA, Jaramillo PMD, Rufini M, Marra LM, López MV, Silva MAP, Soares CRFS, Moreira FMS. Soil biological attributes in arsenic-contaminated gold minig sites after revegetation. Ecotoxicol 22: 1526–1537, Copyright 2013 Springer Science+Business Media

18.2.4 Zinc Open-Pit Mining and Smelting Activities in the State of Minas Gerais (MG), Southeast Brazil

The state of Minas Gerais is responsible for the entire production of Zn of Brazil [11]. Mines located in the cities of Vazante and Paracatu supply the Zn rich feedstock (silicate and sulfide minerals) to the metallurgical plants located in Três Marias and Juiz de Fora. At the unit of Três Marias, the deposition of tailings contaminated with various TE spread to an area of about 18 ha. In areas adjacent to the Zn processing industry, the contamination came from the deposition of wastes, which were originally contained, and eventually spread by means of physical processes like conveying by the erosive action of rain, or by deliberate movements of soil. Although with a non-homogeneous horizontal and vertical distribution, total concentration of metals in the surface layers showed average values of 13,533 mg Zn kg^{-1}, 170 mg Cd kg^{-1}, 865 mg Cu kg^{-1}, and 612 mg Pb kg^{-1} (extracted by aqua regia). The exchangeable portion ($MgCl_2$) had values ranging from 231 to 1,407 mg Zn kg^{-1}, 14 to 390 mg Cd kg^{-1}, and 11 to 33 mg Pb kg^{-1}. Copper was hardly present in the exchangeable form due to the large amount of carbonated material present in the area [105].

In industrial waste deposition areas, there is a predominance of exchangeable forms of Zn and Cd, which represent great environmental risk. Copper and Pb, on the other hand, are generally present in the residual medium. Therefore, high levels of Zn and Cd are generally responsible for the advanced state of degradation observed in some areas, and are among the most notorious impediments for the establishment of spontaneous revegetation. These conditions can lead to side effects, such as loss of soil structure, consequently leading to increased soil erosion, promoting the transportation of the contaminants to surrounding water sources, compromising the quality of the soil, water, and human health [106].

18.2.4.1 Research on Zn Open-Pit Mining and Smelting Areas

Although phytoremediation is a rather recent phytotechnology (being in its "twenties"), it is already known that the interaction with soil microorganisms plays an important role in its success [103]. Several chemical and physical processes have been used for recovering TE-contaminated soils. However, phytoremediation is a really promising technology for in situ land reclamation [2, 107], showing satisfactory results for either organic or inorganic contamination [9, 108]. AMF are important partners in phytoremediation, increasing the ability of plants to absorb water and nutrients and to protect plant against TE stresses [109–113].

Klauberg-Filho et al. [23] investigated the adaptation of native AMF to a contaminated soil from the Zn-smelting area located in Três Marias, MG. The authors have studied four sites within the same polluted area. Site characteristics are as follow: (1) *Brachiaria mutica* with reduced phytotoxicity symptoms, (2) *Andropogon* sp., (3) *B. mutica* with aggravated phytotoxicity symptoms, and (4) *B. decumbens*. Interestingly, although the contamination of the area was high, diversity of AMF was also high (Table 18.3). The authors have identified 21 species belonging to *Acaulospora* (7), *Scutellospora* (6), *Glomus* (5), *Gigaspora* (2), and *Entrophospora*

Table 18.3 Mycorrhizal colonization (MC) rate, spore density (SD), diversity indexes, and Zn, Cu, Cd, and Pb contents in different sites from the Zn-smelting area located in Três Marias, MG (field samples)

			Diversity indexes[a]			Total metal content (mg dm^{-3})			
Site	MC (%)	SD (n°/50 g)	SR	*H'*	D_s	Zn	Cu	Cd	Pb
1	27	335a	3.8a	0.30ab	0.61ab	4,146	265	18	102
2	12	50ab	4.0a	0.42a	0.44b	6,055	455	90	52
3	14	35bc	3.0a	0.36a	0.48b	13,309	812	90	125
4	18	8c	1.2b	0.10b	0.82a	16,080	586	39	125

[a] *SR* species richness, *H'* Shannon-Wiener index, D_S Simpson index. Means followed by different letters, in the same column, are statistically different by Tukey ($P \leq 0.05$). ($n = 6$). Modified from Klauberg-Filho O, Siqueira JO, Moreira FMS (2002) Vesicular arbuscular mycorrhizae fungi in soils polluted with heavy metals. R Bras Ci Solo 26: 125–134, licensed under a Creative Commons Attribution License

(1). Using the culture-trap method with *B. decumbens* as trap, the study showed that *Paraglomus occultum*, *Acaulospora morrowiae*, *A. mellea*, *Glomus intraradices*, *G. clarum* (renamed to *Rhizophagus clarus*), and *Scutellospora pellucida*, in this order, were the predominant species in the contaminated area. Field data showed the occurrence of *A. mellea* and *G. clarum* (renamed to *Rhizophagus clarus*) in all sampled sites, depicting the adaptation potential of those species to contaminated soils. These results show the inoculum potential of AMF for using in revegetation processes.

This high species number is not in agreement with the results of other authors around the world. In a soil contaminated with Zn in the Netherlands, Griffiöen et al. [110] identified only one AMF species (*Scutellospora dipurpurescens*). Ietswaart et al. [114] and Noyd et al. [115] have each identified only two AMF species in studies carried in soils contaminated with TE in the Netherlands and the USA, respectively. In other related studies, Pawlowska et al. [116] isolated six and Sambandan et al. [117] 14 species of AMF in soils contaminated with TE in Poland and in India, respectively. In all the cited studies, AMF were identified directly from the contaminated soils, which may have underestimated the actual number of species present in the sites. Therefore, the use of both methods, assessing the diversity of AMF directly from the contaminated soil and the diversity of AMF using trap cultures, is a more reliable approach to access the "real" diversity of those AMF present in the soil, as suggested by Leal et al. (submitted data).

In an in vitro assay, Cabral et al. [118] demonstrated the differentiated retention capacity of Cd, Cu, Pb, and Zn in the tissues of AMF. This behavior has been attributed to the production of glycoproteins (glomalins) by the fungi. By acting as protecting agents for plants, and by favoring the extraction of soil TE [61], these proteins can play an important role in the plant's tolerance against TE. Moreover, glomalins may take part in soil physical improvement [119]. This feature is of extreme interest for phytoremediation approaches, especially considering that most land plants form mycorrhizal symbioses even in highly contaminated conditions [21, 23, 25, 80, 83].

Silva et al. [61] explored the ability of 14 AMF species to promote plant growth and improve Cd, Zn, Pb, and Cu extraction competences by *B. decumbens*. The authors revealed the promising effects of *A. morrowiae*, *A. spinosa*, and *G. gigantea* for the extraction of all TE investigated. As shown previously by Klauberg-Filho et al. [23], *A. morrowiae* was the second most predominant AMF species in a soil from the Zn-smelting area situated at Três Marias (MG). Therefore, it shows how promising this species is for the phytoremediation of Cd, Zn, Pb, and Cu contaminated soils.

Using Três Marias (MG) soil as source of contamination, Carneiro et al. [120] evaluated the establishment of various plants and AMF species. Treatments had 0, 7.5, 15, 30, and 45 % (v/v) of contaminated soil. Final volumes were obtained by adding a typical non-contaminated Acric Red Latosol (Oxisol). The contaminated soil had 12,000 mg Zn kg^{-1}, 120 mg Cd kg^{-1}, 800 mg Pb kg^{-1}, and 1,900 mg Cu kg^{-1}. Four AMF species *A. scrobiculata*, *G. occultum* (renamed to *Paraglomus occultum*), *G. etunicatum*, and *G. margarita* were inoculated to nine herbaceous plant species *Andropogon gayanus*, *B. brizantha*, *Brassica* sp., *Chloris gayana*, *Melinis minutiflora*, *Pennisetum americanum*, *Rhynchelytrum repens*, *Setaria anceps*, and *Sorghun sudanensis*. Inoculated plants have accumulated higher contents of Zn, Cd, and Pb, and reduced the translocation of Cd and Pb to the shoots, showing that AMF can improve phytostabilization. Moreover, the phytoextraction of Zn and Cd by *Brassica* sp. was improved around 9 % due AMF inoculation. This phytoextraction mechanism is an important indirect effect provided by the AMF to the plants [61], as it is a promising feature for phytoremediation processes [107, 121].

Besides AMF, mutualistic symbioses between legumes and rhizobia also play an important role in the recovery of contaminated areas [28, 98]. Several benefits may come from these combinations. Among them, larger biomass yields, and therefore increased soil protection, soil N-enrichment, aside from positively aiding in land reclamation. Other benefits include prompting the microbial, plant, and wildlife diversity of the area [7]. In addition to reducing costs, N_2 fixation avoid the damaging effects of ammoniacal fertilizations, which acidifies the soil, and by consequence increases the bioavailability of many cationic TE. Studies seeking information on the effects of rhizobia in TE-contaminated soils have been developed by the Biology, Microbiology, and Soil Biology Processes laboratory at the Federal University of Lavras (MG), since the late 1990s. Mostasso et al. [122] conducted experiments to investigate the nodulation and the plant growth-promoting potential by different rhizobia inoculated to different leguminous species. The experiment was performed using the Três Marias (MG) soil, Zn-smelting site contaminated with Cd, Cu, Pb, and Zn. Accidental leakages of metallurgical wastes in this site brought the TE soil contents to sky-high levels (mg dm^{-3}): 18,600 for Zn, 600 for Pb, 596 for Cu, and 135 for Cd.

In soils with 0, 15, 30, 45, and 60 % TE contamination, Mostasso et al. [122] tested the attenuating potential of a few plants species inoculated with strains of rhizobia. The different symbioses set were composed by *Enterolobium contortisiliquum* inoculated with *Bradyrhizobium japonicum* (strain BR 4406), *Sesbania virgata* inoculated with *Azorhizobium doebereinerae* (strain BR 5401^T), *Leucaena* sp. inoculated with *Sinorhizobium* sp. (strain BR 827), and *Macroptilium atropurpureum* inoculated with *B. elkanii* (strain INPA 173A). Under these conditions, the symbiosis between *E. contortisiliquum* and *B. japonicum* showed the best tolerance levels, while *M. atropurpureum* was the most affected plant. The authors attributed the best tolerance of *E. contortisiliquum* to the fact that this plant species is arboreal and has a slower growth rate compared to others. This fact may have provided the species an extra contact time with the contaminants, providing better adaptation to the toxic effects of TE. On the other hand, *M. atropurpureum* as an herbaceous fast-growing species had its adaptation to the adverse conditions compromised. Regarding nodulation, the authors concluded that *B. japonicum* (BR 4406) was the most tolerant strain on increasing levels of contaminants, with a greater number of nodules compared to other tested rhizobia. This condition was later confirmed by Trannin et al. [77], who found even higher and better tolerance levels for the *E. contortisiliquum-B. japonicum* (BR 4406) symbiosis growing in contaminated soil, than when in symbiosis with *Bradyrhizobium* sp. (strains UFLA 01-457 and INPA 398). These results has demonstrated that both symbionts (plant and bacterium) might contain individual mechanisms which provides tolerance to the metal-induced stresses.

In vitro experiments carried by Trannin et al. [77] showed that rhizobia strains from contaminated soils are more tolerant to Cd, Cu, and Zn, than rhizobia isolated from non-contaminated environments. The study showed also that *Bradyrhizobium* genus is more tolerant to TE than *Azorhizobium*. This behavior was also detected by Matsuda et al. [123], who evaluated the in vitro tolerance by different rhizobia species to Cd, Cu, and Zn, and found that the most metal-tolerant rhizobia, considering the maximum TE level, were *Bradyrhizobium* > *Rhizobium* = *Mesorhizobium* = *Sino rhizobium* > *Azorhizobium*. Matsuda et al. [124] also assessed the number of bacterial viable cells after 28 days of incubation in soil with different levels of contamination, confirming the higher tolerance of the *Bradyrhizobium* strains.

In addition to rhizobia, which belongs to the Alpha-proteobacteria subclass, order Rhizobiales, there are other bacteria, such as *Burkholderia* and *Cupriavidus*, which belong to the Beta-proteobacteria subclass, order Burkholderiales, capable of fixing N_2 and inducing nodule formation on legume roots [125, 126]. A strain of *Cupriavidus necator* isolated by Florentino et al. [127] was evaluated for its symbiotic efficiency [128] and ability to induce nodule formation in roots of different legume species [129]. Ferreira et al. [130] evaluated the *in vitro* tolerance by 35 strains of *C. necator* to Cd, Cu, Pb, and Zn and assessed the symbiotic efficiency of the most tolerant strains in association with *A. mangium*, *E. contortisiliquum*, *L. leucocephala*, *M. caesalpiniifolia*, *M. pudica*, *M. pigra*, and *M. acutistipula*. From the 35 strains tested, 32 (91 %) were able to grow in concentrations of up to 2.5 mmol L^{-1} of all TE tested. Five strains were able to grow at 10 mmol L^{-1} of Zn and Cu, and one at 7.5 mmol Pb L^{-1}. No strain was able to grow in concentrations above 2.5 mmol Cd L^{-1}. The ranking of TE toxicity followed the order Cd > Pb > Cu = Zn.

Concerning the efficiency of the most tolerant bacterial species, in vitro assays [130] showed that strains of *C. necator* are symbiotically efficient with tree legume species in contaminated soil. Strains UFLA 02-71 effective in symbiosis with *M. caesalpiniifolia*, and UFLA 01-659 effective in symbiosis with both *M. pudica* and *L. leucocephala*, are good examples of highly competitive microorganisms that withstand adverse conditions imposed by TE contamination. These TE-resistant, plant growth-promoting bacteria can play an essential role for the revegetation of open-pit mining and smelting areas.

18.3 Conclusions and Perspectives

Plant–microbe interactions, especially arbuscular mycorrhizal and legume-rhizobia symbioses have real potential for the revegetation of open-pit mining and smelting contaminated soils. In addition to the nutritional role to the mycotrophic plants, AMF also act as a buffering agent, reducing excessive absorption of trace elements, consequently favoring the host plant establishment in stressed environments. However, to increase the success of revegetation, it is necessary to look for efficient fungal isolate for each situation, taking into account the host plant, the fungal species, the contaminants, and the soil, besides the climate conditions to which the symbiosis will be submitted. Using native AMF species from contaminated soils seems to be a factor that increases the chances for phytoprotection success conferred by the fungi to the host plants. Although this mechanism of phytoprotection appear to be TE dependent, the most clear phytoprotective effect is the attenuation of the toxicity. Besides AMF, the intrinsic plant growth-promoting ability hold by rhizobia, plus their multi-element resistance, increases the horizons for exploiting the symbiosis with different legume plants on different conditions of contamination.

Rhizobia and legume-rhizobia symbiosis have the potential to enhance revegetation on phytoremediation concern, through biological N_2 fixation and other plant growth-promoting traits. These effects depend on bacterial species and their origin, besides legume species and TE. The intrinsic plant growth-promoting ability plus the multi-TE resistance hold by rhizobia increase the horizons for exploiting the symbiosis, with different legume species, in the revegetation of open-pit mining and smelting contaminated soils. Moreover, most rhizobia are not pathogenic, which is another crucial point for exploiting the potential of those bacteria to improve plant growth in the framework of phytoremediation purposes.

In Brazil, studies on the role of both mycorrhizal and legume-rhizobia symbioses on the plant growth promotion and attenuation of toxicity caused by TE are still in the initial phase. However, promising results providing valuable information has already been raised to assist the next step that is to apply this biotechnology in field conditions. Despite the promising results already achieved from controlled conditions, more work is needed to better understand the mechanisms, as the expression/suppression of plant and microorganism genes linked to the symbiosis and phytoprotection under the excess of TE.

References

1. Hertwich E, van der Voet E, Suh S, Tukker A, Huijbregts M, Kazmierczyk P, Lenzen M, McNeely J, Moriguchi Y (2010) Assessing the environmental impacts of consumption and production: priority products and materials. United Nations Environment Programme. Available via UNEP. http://www.unep.org/resourcepanel/Portals/24102/PDFs/PriorityProductsAndMaterials_Report.pdf. Accessed 2 Oct 2015
2. Baker AJM, McGrath SP, Sidoli CMD, Reeves RD (1994) The possibility of in situ heavy metal decontamination of polluted soils using crops of metal-accumulating plants. Resour Conserv Recycl 11:41–49
3. Vangronsveld J, Colpaert JV, van Tichelen KK (1996) Reclamation of a bare industrial area contaminated by non-ferrous metals: physicochemical and biological evaluation of the durability of soil treatment and revegetation. Environ Pollut 94:131–140
4. Dias-Júnior HE, Moreira FMS, Siqueira JO, Silva R (1998) Metais Pesados, Densidade e Atividade Microbiana em Solo Contaminado por Rejeitos de Indústria de Zinco. R Bras Ci Solo 22:631–640
5. Santos JV, Rangel WM, Guimarães AA, Jaramillo PMD, Rufini M, Marra LM, López MV, Silva MAP, Soares CRFS, Moreira FMS (2013) Soil biological attributes in arsenic-contaminated gold minig sites after revegetation. Ecotoxicology 22:1526–1537

6. Souza FA, Silva EMR (1996) Micorrizas arbusculares na revegetação de áreas degradadas. In: Siqueira JO (ed) Avanços em fundamentos e aplicação de micorrizas. Universidade Federal de Lavras, Lavras, 290 p
7. Siqueira JO, Soares CRFS, Silva CA (2008) Matéria orgânica em solos de áreas degradadas. In: Santos GA, Silva LS, Canellas LP, Camargo FAO (eds) Fundamentos da Matéria Orgânica do Solo: ecossistemas tropicais e subtropicais, 2nd edn. Metrópole, Porto Alegre, 654 p
8. Vangronsveld J, Herzig R, Weyens N, Boulet J, Adriaensen K, Ruttens A, Thewys T, Vassilev A, Meers E, Nehnevajova E, van der Lelie D, Mench M (2009) Phytoremediation of contaminated soils and groundwater: lessons from the field. Environ Sci Pollut Res 16:765–794
9. Weyens N, Beckers B, Schellingen K, Ceulemans R, Croes S, Janssen J, Haenen S, Witters N, Vangronsveld J (2013) Plant-associated bacteria and their role in the success or failure of metal phytoextraction projects: first observations of a field-related experiment. Microbiol Biotechnol 6:288–299
10. Henry JR (2000) An overview of the phytoremediation of lead and mercury. Available via USEPA. http://www.clu-in.org. Accessed 20 Mar 2010
11. Departamento Nacional de Produção Mineral (DNPM) (2014) Informe Mineral Julho-Dezembro 2014. ISSN 0101-2053. Accessed 17 Aug 2015
12. Instituto Brasileiro de Mineiração (IBRAM) (2015) Informações sobre a economia mineral brasileira 2015. Available via IBRAM. http://www.ibram.org.br/. Accessed 5 Oct 2015
13. Croes S, Weyens N, Janssen J, Vercampt H, Colpaert JV, Carleer R, Vangronsveld J (2013) Bacterial communities associated with Brassica napus L. grown on trace element-contaminated and non-contaminated fields: a genotypic and phenotypic comparison. Microbiol Biotechnol 6:371–384
14. Koch JM (2007) Alcoa's mining and restoration process in South Western Australia. Restor Ecol 15:11–16
15. Ohmoto H (2001) The evolution of O_2 and CO_2 in the atmosphere. In: General Meeting of the NASA Astrobiology Institute, pp 231–232
16. Holland HD (2003) The geologic history of seawater. Treatise on geochemistry, vol 6. Harvard University, Cambridge, pp. 583–625
17. Frimmel HE (2005) Archean atmospheric evolution: evidence from the Witwatersrand gold fields, South Africa. Earth Sci Rev 70:1–46
18. Brundrett MC (2002) Coevolution of roots and mycorrhizas of land plants. New Phytol 154:275–304
19. Phipps CJ, Taylor TN (1996) Mixed arbuscular mycorrhizae from the Triassic of Antarctica. Mycologia 88:707–714
20. Wang J, Zhao F, Meharg AA, Raab A, Feldmann J, McGrath SP (2002) Mechanisms of arsenic hyperaccumulation in *Pteris vittata*. Uptake kinetics, interactions with phosphate and arsenic speciation. Plant Physiol 130:1552–1561
21. Melloni R, Siqueira JO, Moreira FMS (2003) Arbuscular mycorrhizal fungi in soils of bauxite mining area under rehabilitation. Pesqui Agropecu Bras 38:267–276
22. Agely A, Sylvia DM, Ma LQ (2005) Mycorrhizae increase arsenic uptake by the hyperaccumulator Chinese Brake Fern (*Pteris vittata* L.). J Environ Qual 34:2181–2186
23. Klauberg-Filho O, Siqueira JO, Moreira FMS (2002) Vesicular arbuscular mycorrhizae fungi in soils polluted with heavy metals. R Bras Ci Solo 26:125–134
24. Schneider J, Oliveira LM, Guilherme LRG, Stürmer SL, Soares CRFS (2012) Espécies tropicais de pteridófitas em associação com fungos micorrízicos arbusculares em solo contaminado com arsênio. Quim Nova 35:709–714
25. Schneider J, Stürmer SL, Guilherme LRG, Guilherme LRG, Moreira FMS, Soares CRFS (2013) Arbuscular mycorrhizal fungi in arsenic-contaminated areas in Brazil. J Hazard Mater 262:1105–1115
26. Meharg AA, Cairney JWG (2000) Co-evolution of mycorrhizal symbionts and their hosts to metal-contaminated environments. Adv Ecol Res 30:69–112
27. Melloni R, Nóbrega RSA, Moreira FMS, Siqueira JO (2004) Density and phenotypic diversity of endophytic nitrogen fixing bacteria in soils under rehabilitation after bauxite mining. R Bras Ci Solo 28:85–93

28. Melloni R, Moreira FMS, Nóbrega RSA, Siqueira JO (2006) Efficiency and phenotypic diversity among nitrogen-fixing bacteria that nodulate cowpea [*Vigna unguiculata* (L.) WALP] and common bean (*Phaseolus vulgaris* L.) in bauxite-mined soils under rehabilitation. R Bras Ci Solo 30:235–246
29. Magalhães FMM, Döbereiner J (1984) Ocorrência de *Azospirillum amazonense* em alguns ecossistemas da Amazônia. Rev Microbiol 15:246–252
30. Baldani JI, Azevedo MS, Reis VM, Teixeira KRS, Olivares FL, Goi SR, Baldani VLD, Döbereiner J (1999) Fixação biológica de nitrogênio em gramíneas: avanços e aplicações. In: Siqueira JO, Moreira FMS, Lopes AS, Guilherme LRG, Faquin V, Furtini Neto AE, Carvalho JG (eds) Inter-relação fertilidade, biologia do solo e nutrição de plantas. SBCS/UFLA/DCS, Viçosa, pp 621–666
31. Moreira FMS (1994) Fixação Biológica do nitrogênio em espécies arbóreas. In: Hungria M, Araújo RS (eds) Microrganismos de importância agrícola. Empresa Brasileira de Pesquisa Agropecuária, Brasília, pp 121–149
32. World Coal Institute-WCI (2005) The coal resource: a comprehensive overview of coal. WCI, London. Available via WCI. http://www.worldcoal.org. Accessed 2 Aug 2010
33. Companhia de Pesquisa de Recursos Minerais (CPRM)—Serviço Geológico do Brasil. Available via Ministério de Minas e Energia. http://www.cprm.gov.br/publique/media/capXI_b.pdf. Accessed 8 Sept 2010
34. SEMC. Available via SEMC. http://www.semc.rs.gov.br/. Accessed 15 May 2011
35. Silva LFO, Vallejuelo SFO, Martinez-Arkarazo I, Castro K, Oliveira MLS, Sampaio CH, Brum IAS, Leão FB, Taffarel SR, Madariaga JM (2013) Study of environmental pollution and mineralogical characterization of sediment rivers from Brazilian coal mining acid drainage. Sci Total Environ 447:169–178
36. Soares PSM, Santos MDC, Possa MV (2008) Carvão Brasileiro: Tecnologia e Meio Ambiente. CETEM, Rio de Janeiro, 289 p
37. Barth RC (1989) Avaliação da recuperação de áreas mineradas no Brasil. UFV, Viçosa. (Boletim técnico SIF, 1), 41 p
38. Sanchez JCD, Formoso MLL (1990) Utilização do carvão e meio ambiente. Fundação de Ciência e Tecnologia do Rio Grande do Sul, Porto Alegre (Boletim Técnico, 20), 34 p
39. Silveira NA, Silva RDR, Rubio J (2007) Técnicas para tratamento e aproveitamento de águas ácidas residuais da mineração de carvão. In: III Workshop Gestão e Reuso de Água na Indústria. Florianópolis, pp 1–6
40. Rubio J (1998) Aspectos ambientais no setor minero-metalúrgico. In: Luz AB, Possa MV, Almeida SL (eds) Tratamento de Minérios. CETEM/CNPq-MCT, pp 537–570
41. Rubio J (1998) Environmental applications of the flotation process. In: Castro SH, Vergara F, Sanchez M (eds) Effluent treatment in the mining industry. University of Concepción, Chile, pp 335–364
42. Sharpley AN, Weld JL, Beegle DB, Kleiman PJA, Gburek WJ, Moore JPA Jr, Mullins G (2003) Development of phosphorus indices for nutrient management planning strategies in the United States. J Soil Water Conserv 58:137–52
43. Japan International Cooperation Agency-JICA (1997) Interim report for the feasibility study on recuperation of mined out areas in south region of Santa Catarina in the Federative Republic Brazil. Fundação do Meio Ambiente, Criciúma, 248 p
44. Stahl PD, Perryman BL, Sharmasarkar S, Munn LC (2002) Topsoil stockpiling versus exposure to traffic: a case study on in situ uranium wellfields. Restor Ecol 10:129–137
45. Gonçalves FC (2008) Efeito de plantas de cobertura sobre os atributos físicos de um solo construído na área de mineração de carvão de Candiota-RS após três anos (Dissertação de Mestrado) Universidade Federal de Pelotas, Pelotas 91 p
46. Pereira BA, Castro-Silva MA (2010) Rizobactérias formadoras de endósporos associadas a *Tibouchina urvilleana* de áreas impactadas por rejeitos da mineração de carvão. R Bras Ci Solo 34:563–567
47. Glick BR (2003) Phytoremediation: synergistic use of plants and bacteria to clean up the environment. Biotechnol Adv 21:383–393

48. Marra LM, Oliveira SM, Soares CRFS, Moreira FMS (2011) Solubilisation of inorganic phosphates by inoculant strains from tropical legumes. Sci Agric 68:603–609
49. Marra LM, Soares CRFS, Oliveira SM, Ferreira PAA, Soares BL, Carvalho RF, Lima JM, Moreira FMS (2012) Biological nitrogen fixation and phosphate solubilization by bacteria isolated from tropical soils. Plant Soil 357:289–307
50. Silva K, Cassetari AS, Lima AS, Brandt E, Pinnock E, Vandamme P, Moreira FMS (2012) Diazotrophic *Burkholderia* species isolated from the Amazon region exhibit phenotypical, functional and genetic diversity. Syst Appl Microbiol 35:253–262
51. Munees A, Mulugeta K (2014) Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. J King Saud Univ Sci 26:1–20
52. Oliveira-Longatti SM, Marra LM, Soares BL, Bomfeti CA, Silva K, Ferreira PAA, Moreira FMS (2014) Bacteria isolated from soils of the western Amazon and from rehabilitated bauxite-mining areas have potential as plant growth promoters. World J Microbiol Biotechnol 30:1239–1250
53. Carvalho FG, Selbach PA, Silva AJN (2008) Especificidade hospedeira de variantes *Bradyrhizobium* spp. em soja (cvs peking e clark), caupi e guandu. R Bras Ci Solo 32:2701–2708. doi:10.1590/S0100-06832008000700013
54. Costacurta A, Vanderleyden J (1995) Synthesis of phytohormones by plant-associated bacteria. Crit Rev Microbiol 21:1–18
55. Patten C, Glick BR (1996) Bacterial biosynthesis of indole-3-acetic acid. Can J Microbiol 42:207–220
56. Newsham KK, Fitter AH, Watkinson AR (1995) Multifuncionality and biodiversity in arbuscular mycorrhizae. Trends Ecol Evol 10:407–411
57. Smith SE, Read DJ (2008) Mycorrhizal symbiosis. 3rd edn. Academic, New York, 800 p
58. Marschner H, Dell B (1994) Nutrient uptake in mycorrhizal symbiosis. Plant Soil 159:89–102
59. Soares CRFS, Carneiro MAC (2010) Micorrizas arbusculares na recuperação de áreas degradadas. In: Siqueira JO, Souza FA, Cardoso EJBN, Tsai SM (eds) Micorrizas: 30 anos de Pesquisa no Brasil. UFLA, Lavras, 716 p
60. Nogueira MA, Soares CRFS (2010) Micorrizas Arbusculares e Elementos-Traço. In: Siqueira JO, Souza FA, Cardoso EJBN, Tsai SM (eds) Micorrizas: 30 anos de Pesquisas no Brasil. Lavras, UFLA, pp 475–501
61. Silva S, Soares CRFS, Siqueira JO (2006) Fungos micorrízicos no crescimento e extração de metais pesados pela *Brachiaria decumbens* Stapf. em solo multicontaminado. Pesqui Agropecu Bras 41:1749–1757
62. Truong PNV (2000) The global impact of vetiver grass technology on the environment. In: In: Proceedings of the 2nd International Vetiver Conference. Annals ICV2. Available via VETIVER. http://www.vetiver.org/TVN_IVC2/ICV2_index.htm. Accessed Nov 2015
63. Vodnik D, Grcman H, Macek I, van Elteren JT, Kovacevic M (2008) The contribution of glomalin related soil protein to Pb and Zn sequestration in polluted soil. Sci Total Environ 392:130–136
64. Rillig MC, Caldwell BA, Wösten HAB, Sollins P (2007) Role of proteins in soil carbon and nitrogen storage: controls on persistence. Biogeochemistry 85:25–44
65. González M, Carrillo R, Wright S, Nichols K (2004) The role of glomalin, a protein produced by arbuscular mycorrhizal fungi, in sequestering potentially toxic elements. Environ Pollut 130:317–323
66. Bedini S, Pellegrino E, Avio L, Pellegrini S, Bazzoffi P, Argese E, Giovannetti M (2009) Changes in soil aggregation and glomalin-related soil protein content as affected by the arbuscular mycorrhizal fungal species *Glomus mosseae* and *Glomus intraradices*. Soil Biol Biochem 41:1491–1496
67. Jastrow JD, Miller RM, Lussenhop J (1998) Contributions of interacting biological mechanisms to soil aggregate stabilization in restored prairie. Soil Biol Biochem 30:905–916

68. Leake JR, Johnson D, Donnelly DP, Muckle GE, Boddy L, Read DJ (2004) Networks of power and influence: the role of mycorrhizal mycelium in controlling plant communities and agro ecosystem functioning. Can J Bot Rev 82:1016–1045
69. Lovelock CE, Wright SF, Nichols KA (2004) Using glomalin as an indicator for arbuscular mycorrhizal hyphal growth: an example from a tropical rain forest soil. Soil Biol Biochem 36:1009–1012
70. Ono FB, Guilherme LRG, Penido ES, Carvalho GS, Hale B, Toujaguez R, Bundschuh J (2012) Arsenic bioaccessibility in a gold mining area: a health risk assessment for children. Environ Geochem Health 34:457–465
71. Guilherme LRG, Ono FB, Cantoni M, Abreu CA, Coscione AR, Tappero R, Sparks D (2014) Bioaccessibility of arsenic in a gold mine area in Brazil: why is it so low? In: Litter MI, Nicolli HB, Meichtry M, Quici N, Bundschuh J, Bhattacharya P, Naidu R. One century of the discovery of Arsenicosis in Latin America (1914–2014) As 2014, pp 349–353
72. Ladeira ACQ, Ciminelli VST, Nepomuceno AL (2002) Seleção de solos para a imobilização de arsênio. Rev Esc Minas 55:215–221
73. Silva SR, Procópio SO, Queiroz TFN, Dias LE (2004) Caracterização de rejeito de mineração de ouro para avaliação de solubilização de metais pesados e arsênio e revegetação local. R Bras Ci Solo 28:189–196
74. Matchullat J, Borba RP, Deschamps E, Figueiredo BF, Gabrio T, Schwenk M (2000) Human and environmental contamination in the Iron Quadrangle, Brazil. Appl Geochem 15:181–190
75. Borch T, Kretzschmar R, Kappler A, van Cappellen P, Ginder-Vogelm M, Voegelin A, Campbell K (2010) Biogeochemical redox processes and their impact on contaminant dynamics. Environ Sci Technol 44:15–23
76. Young JPW (1994) Sex and the single cell: the population ecology and genetics of microbes. In: Ritz K, Dighton J, Giller KE (eds) Beyond the biomass. Compositional and functional analysis of soil microbial communities. Wiley, Chichester, pp 101–107
77. Trannin ICB, Moreira FMS, Siqueira JO, Lima A (2001) Tolerance of *Bradyrhizobium* and *Azorhizobium* strains and isolates to copper, cadmium and zinc "in vitro". R Bras Ci Solo 25:305–316
78. Harms H, Schlosser D, Wick LY (2011) Untapped potential: exploiting fungi in bioremediation of hazardous chemicals. Nat Rev Microbiol 9:177–192
79. Cabral L, Soares CRFS, Giachini AJ, Siqueira JO (2015) Arbuscular mycorrhizal fungi in phytoremediation of contaminated areas by trace elements: mechanisms and major benefits of their applications. World J Microbiol Biotechnol 31:1655–1664
80. Rangel WM, Schneider J, Costa ETS, Soares CRFS, Guilherme LRG, Moreira FMS (2014) Phytoprotective effect of arbuscular mycorrhizal fungi species against arsenic toxicity in tropical leguminous species. Int J Phytoremediation 16:840–858
81. Chen B, Xiao X, Zhu Y, Smith FA, Xie ZM, Smith SE (2007) The arbuscular mycorrhizal fungus *Glomus mosseae* gives contradictory effects on phosphorus and arsenic acquisition by *Medicago sativa* Linn. Sci Total Environ 379:226–234
82. Gerdemann JW, Nicolson TH (1963) Spores of mycorrhizal Endogone species extracted from soil wet sieving and decanting. Trans Brit Mycol Soc 46:235–244
83. Schneider J, Labory CRG, Rangel WM, Alves E, Guilherme LRG (2013) Anatomy and ultrastructure alterations of *Leucena leucocephala* (Lam.) inoculated with mycorrhizal fungi in response to arsenic-contaminated soil. J Hazard Mater 262:1245–1258
84. Soares CRFS, Siqueria JO (2008) Mycorrhiza and phosphate protection of tropical grass species against heavy metal toxicity in multi-contaminated soil. Biol Fert Soils 44:833–841
85. Schutzendubel A, Polie A (2002) Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization. J Exp Bot 53:1351–1365
86. Farshian S, Khara J, Malekzadeh P (2007) Effect of arbuscular mycorrhizal (*G. ettunicatum*) fungus on antioxidant enzymes activity under zinc toxicity in Lettuce plants. Pak J Biol Sci 10:1865–1869

87. Andrade SAL, Gratão PL, Azevedo RA, Silveira APD, Schiavinato MA, Mazzafera P (2010) Biochemical and physiological changes in jack bean under mycorrhizal symbiosis growing in soil increasing Cu concentrations. Environ Exp Bot 68:198–207
88. Garg N, Kaur H (2013) Response of antioxidant enzymes, phytochelatins and glutathione production towards Cd and Zn stresses in *Cajanus cajan* (L.) Millsp. Genotypes colonized by arbuscular mycorrhizal fungi. J Agron Crop Sci 199:118–133
89. Estrada B, Aroca R, Barea JM, Ruiz-Lozano JM (2013) Native arbuscular mycorrhizal fungi isolated from a saline habitat improved maize antioxidant systems and plant tolerance to salinity. Plant Sci 201–202:42–51
90. Wong MH (2003) Ecological restoration of mine degraded soils, with emphasis on metal contaminated soils. Chemosphere 50:775–780
91. Gardea-Torresdeya JL, Peralta-Videa JR, Rosa G, Parsons JG (2005) Phytoremediation of heavy metals and study of the metal coordination by X-ray absorption spectroscopy. Coord Chem Rev 249:1797–1810
92. French CJ, Dickinson NM, Putwain PD (2006) Woody biomass phytoremediation of contaminated brownfield land. Environ Pollut 141:387–395
93. Xu P, Christie P, Liu Y, Zhang J, Li X (2008) The arbuscular mycorrhizal fungus Glomus mosseae can enhance arsenic tolerance in Medicago truncatula by increasing plant phosphorus status and restricting arsenate uptake. Environ Pollut 156:215–220
94. Miransari M (2011) Hyperaccumulators, arbuscular mycorrhizal fungi and stress of heavy metals. Biotechnol Adv 29:645–653
95. Mishra S, Srivastava S, Tripathi RD, Kumar R, Seth CS, Gupta DK (2006) Lead detoxification by coontail (*Ceratophyllum demersum* L.) involves induction of phytochelatins and antioxidant system in response to its accumulation. Chemosphere 65:1027–1039
96. Moller IM, Jensen PE, Hansson A (2007) Oxidative modifications to cellular components in plants. Annu Rev Plant Biol 58:459–481
97. Costa PS, Scholte LLS, Reis MP, Chaves AV, Oliveira PL, Itabayana LB, Suhadolnik MLS, Barbosa FAR, Chartone-Souza E, Nascimento AMA (2014) Bacteria and genes involved in arsenic speciation in sediment impacted by long-term gold mining. PLos One 9:e95655. doi:10.1371/journal.pone.0095655
98. Franco AA, Dias LE, Faria SM, Campello EFC, Silva EMR (1995) Use of nodulate and mycorrhizal forest leguminous trees as agents of recovery and maintenance of soil life: a technological model. Oecol Bras 1:459–467
99. Franco AA, Faria SM (1997) The contribution of N_2-fixing tree legumes to land reclamation and sustainability in the tropics. Soil Biol Biochem 29:897–903
100. Stephens JHG, Rask HM (2000) Inoculant production and formulation. Field Crop Res 65:249–258
101. Costa GS, Franco AA, Damasceno RN, Faria SM (2004) Nutrient input through litter in a degraded area revegetated with legume trees. R Bras Ci Solo 28:919–927
102. Khan AG (2006) Mycorrhizoremediation: an enhanced form of phytoremediation. J Zhejiang Univ Sci B 7:503–514
103. Weyens N, van der Lelie D, Taghavi S, Newman L, Vangronsveld J (2009) Exploiting plant-microbe partnerships to improve biomass production and remediation. Trends Biotechnol 27:591–598
104. Moreira FMS, Ferreira PAA, Vilela LAF, Carneiro MAC (2015) Symbioses of plants with rhizobia and mycorrhizal fungi in heavy metal-contaminated tropical soils. In: Sherameti I, Varma A (eds) Soil biology, 1st edn. Springer International Publishing, Berlin, pp 215–243
105. Ribeiro-Filho MR, Curi N, Siqueira JO, Motta PEF (1999) Heavy metals in soils from a waste area in a zinc processing industry. R Bras Ci Solo 23:453–464
106. Soares CRFS, Accioly AMA, Siqueira JO, Moreira FMS (2002) Diagnóstico e reabilitação de área degradada pela contaminação por metais pesados. In: Simpósio Nacional sobre recuperação de áreas degradadas, 5, Belo Horizonte. Anais… DCF/UFLA/SOBRADE, Lavras, pp 56–82

107. Vangronsveld J, Cunningham SD (1998) Introduction to the concepts. In: Vangronsveld J, Cunningham SD (eds) Metal-contaminated soils. Georgetown, pp 1–15
108. Cunningham SD, Shann JR, Crowley DE, Anderson TA (1997) Phytoremediation of contaminated water and soil. In: Kruger EL, Anderson TA, Coats JL (eds) Phytoremediation of soil and water contaminants, vol 664. American Chemical Society, Washington, pp 2–17
109. Turnau K, Kottke I, Oberwinkler F (1993) Element localization in mycorrhizal roots of *Pteridium aquilinum* (L.) Kuhn collected from experimental plots treated with cadmium dust. New Phytol 123:313–324
110. Griffiöen WAJ, Ietswaart JH, Ernerst WHO (1994) Mycorrhizal infection of an *Agrostis capillaris* population on copper contaminated soil. Plant Soil 158:83–89
111. Nogueira AV (1996) As micorrizas e o excesso de metais. In: Siqueira JO (ed) Avanços em fundamentos e aplicação de micorrizas. Universidade Federal de Lavras, Lavras, pp 135–174
112. Leyval C, Turnau K, Haselwandter K (1997) Effect of heavy metal pollution on mycorrhizal colonization and function: physiological, ecological and applied aspects. Mycorrhiza 7:139–153
113. Siqueira JO, Pereira MAM, Simão JBP, Moreira FMS (1999) Efeito da formononetina (7 Hidroxi, 4′ metoxi Isoflavona) na colonização micorrízica e crescimento do milho em solo contendo excesso de metais pesados. R Bras Ci Solo 23:561–568
114. Ietswaart JH, Griffiöen WAJ, Ernst WHO (1992) Seasonality of VAM infection in three populations of *Agrostis capillaris* (Graminae) on soil with or without heavy metal enrichment. Plant Soil 139:67–73
115. Noyd RK, Pfleger FL, Russelle MP (1995) Interactions between native prairie grasses and indigenous arbuscular mycorrhizal fungi: implications for reclamation of taconite iron ore tailing. New Phytol 129:651–660
116. Pawlowska TE, Blaszkowski J, Rühling A (1996) The mycorrhizal status of plants colonizing a calamine spoil mound in southern Poland. Mycorrhiza 6:499–505
117. Sambandan K, Kannan K, Raman N (1992) Distribution of vesicular-arbuscular mycorrhizal fungi in heavy metal polluted soils of Tamil Nadu, India. J Environ Biol 13:159–167
118. Cabral L, Siqueira JO, Soares CRFS, Brasil JEPP (2010) Retenção de metais pesados em micélio de fungos micorrízicos arbusculares. Quim Nova 33:25–29
119. Carrizo ME, Alesso CA, Cosentino D, Imhoff S (2015) Aggregation agents and structural stability in soils with different texture and organic carbon contents. Sci Agric 72:75–82
120. Carneiro MAC, Siqueira JO, Moreira FMS (2001) Establishment of herbaceous plant in heavy metal contaminated soils inoculated with arbuscular mycorrhizal fungi. Pesqui Agropecu Bras 36:1443–1452
121. Accioly AMA, Siqueira JO (2000) Contaminação química e biorremediação do solo. In: Novais RF, Alvarez VHV, Schaefer CEGR (eds) Tópicos em Ciência do solo. Sociedade Brasileira de Ciência do Solo, Viçosa, MG, pp 299–352
122. Mostasso FL (1997) Crescimento e nodulação de leguminosas em solo contaminado com metais pesados (Dissertação de Mestrado). Universidade Federal de Lavras, Lavras, 50 p
123. Matsuda A, Moreira FMS, Siqueira JO (2002) Tolerance of rhizobia genera from different origins to zinc, copper and cadmium. Pesqui Agropecu Bras 37:343–355
124. Matsuda A, Moreira FMS, Siqueira JO (2002) Survival of Bradyrhizobium and Azorhizobium in heavy metal contaminated soil. R Bras Ci Solo 26:249–256
125. Chen WM, Laevens S, Lee TM, Coenye T, De Vos P, Mergeay M, Vandamme P (2001) *Ralstonia taiwanensis* sp. nov. isolated from root nodules of *Mimosa* species and sputum of a cystic fibrosis patient. Int J Syst Evol Microbiol 51:1729–1735
126. Moulin L, Munive A, Dreyfus B, Masson CB (2001) Nodulation of legumes by members of the β-subclass of Proteobacteria. Nature 411:948–950
127. Florentino LA, Moreira FMS (2009) Características simbióticas e fenotípicas de *Azorhizobium doebereinerae*, microissimbiote de *Sesbania virgata*. Rev Árvore 33:215–226

128. Florentino LA, Jaramillo PMD, Silva KB, Silva JS, Oliveira SM, Moreira FMS (2012) Physiological and symbiotic diversity of *Cupriavidus necator* strains isolated from nodules of Leguminosae species. Sci Agric 69:247–258
129. Silva K, Florentino LA, Silva KB, Evie B, Vandamme P, Moreira FMS (2012) *Cupriavidus necator* isolates are able to fix nitrogen in symbiosis with different legume species. Syst Appl Microbiol 35:175–182
130. Ferreira PAA, Bomfeti CA, Júnior RS, Soares BL, Soares CRFS, Moreira FMS (2012) Eficiência simbiótica de estirpes de *Cupriavidus necator* tolerantes a zinco, cádmio, cobre e chumbo. Pesqui Agropecu Bras 47:85–95

Chapter 19
Phytoremediation of Arsenic-Contaminated Soils Using Arsenic Hyperaccumulating Ferns

Nabeel Khan Niazi, Safdar Bashir, Irshad Bibi, Behzad Murtaza, Muhammad Shahid, Muhammad Tariq Javed, Muhammad Bilal Shakoor, Zulfiqar Ahmad Saqib, Muhammad Farrakh Nawaz, Zubair Aslam, Hailong Wang, and Ghulam Murtaza

19.1 Introduction

Arsenic (As) is highly toxic and carcinogenic metalloid extensively dispersed in the environment. It is usually associated with metal ores of copper (Cu), lead (Pb), and gold (Au). Arsenate (As(V)) and arsenite (As(III)) are the most common inorganic forms of As in the soil environment (Fig. 19.1). Therefore, As contamination of soils,

---

N.K. Niazi (✉) • I. Bibi
Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad,
Faisalabad, Punjab 38040, Pakistan

Southern Cross GeoScience, Southern Cross University, Lismore, NSW 2480, Australia

MARUM and Department of Geosciences, University of Bremen, Bremen D-28359, Germany
e-mail: nabeelkniazi@gmail.com; nabeel.niazi@uaf.edu.pk

S. Bashir • M.B. Shakoor • Z.A. Saqib • G. Murtaza
Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad,
Faisalabad, Punjab 38040, Pakistan

B. Murtaza • M. Shahid
Department of Environmental Sciences, COMSATS Institute of Information Technology,
Vehari, Pakistan

M.T. Javed
Department of Botany, Government College University, Allama Iqbal Road, Faisalabad, Pakistan

M.F. Nawaz
Department of Forestry and Range Management, Faculty of Agriculture,
University of Agriculture Faisalabad, Faisalabad, Punjab 38040, Pakistan

Z. Aslam
Department of Agronomy, University of Agriculture Faisalabad,
Faisalabad, Punjab 38040, Pakistan

H. Wang
Key Laboratory of Soil Contamination Bioremediation of Zhejiang Province,
Zhejiang A & F University, Lin'an, Hangzhou, Zhejiang 311300, China

A.A. Ansari et al. (eds.), *Phytoremediation*, DOI 10.1007/978-3-319-40148-5_19

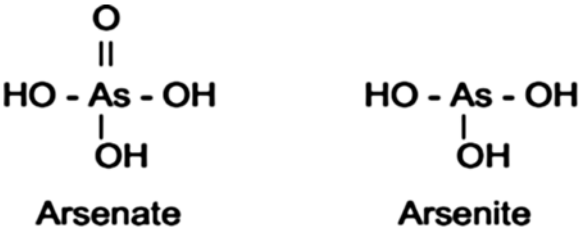


Fig. 19.1 Molecular structure of the two inorganic arsenic (As) species (arsenate, As(V) and arsenite, As(III))

sediments, and groundwater is a global environmental and health concern due to the toxic and carcinogenic nature of As compounds. Natural processes such as weathering of parent material, vulcanization as well as many anthropogenic activities (e.g., metal smelting, coal combustion, mine tailings, leather tanning operations, dyes, chemical weapons, and As-based pesticides) have contributed to elevated As concentrations (up to 3000 mg kg^{-1}) in soils and sediments [1–3]. Several countries have been affected with As in soil and groundwater including Australia, the USA, UK, China, Bangladesh, India, Nepal, Myanmar, Pakistan, Vietnam, Cambodia, and Indonesia [4–7]. Carcinogenic effects of As are well known and it is causing mainly animal and human skin, lung, and bladder cancers [8].

It has been reported that the possible cause of the death of some historians such as Napoleon and the American president, Zachary Taylor, was due to As poisoning [9]. Both As(V) and As(III) are toxic and inorganic As species and, as such, have been declared as a major environmental contaminant by the US EPA [10], although their behavior in interrupting the biological functions in plants is different. Arsenite binds to proteins with sulfhydryl groups and interferes with protein functioning [11]; it hinders respiration by binding to vicinal thiols in pyruvate dehydrogenase and 2-oxo-glutarate dehydrogenase. Arsenite does not act directly as a mutagen but induces intrachromosomal homologous recombination and generates reactive oxygen species [12]. Arsenate, on the other hand, interferes with oxidative phosphorylation and ATP synthesis pathway [11] and is a chemical analogue of phosphate.

Arsenic concentrations in soil exceeding the critical level are considered as potential threat to humans, animals, plants, and microbes. Various remedial measures are available for the remediation of As-contaminated soils. The conventional methods of As removal from the contaminated soils involve physical and chemical methods. Physical methods include capping, soil excavation, and burial of contaminated soil, whereas in chemical methods strong acids, alkalies, or chelating agents are used for soil washing to remove the contaminant (i.e., As) from soil. These conventional approaches are expansive, hazardous in nature, and rather unfeasible to implement, particularly where the volume and area of the contaminated land to-be-treated are large. Nevertheless, use of chemicals can permanently alter the nature of various soil properties and (mineral/organic) constituents kill the beneficial soil microbiota, thereby leaving soil degraded and a futile means for growing plants [13]. The microbial remediation can also be one of the other sustainable and cheap options. The microbial remediation of As involves the reduction and oxidation of As and hence is not an effective detoxification mechanism. Here certain bacteria such as *Pseudomonas arsenitoxidans* L. can gain energy in the presence of As(III) by oxidizing As(III) to As(V) while *Sulfurospirillum arsenophilum* and *Sulfurospirillum barnesii* can reduce As(V) to As(III) [14].

Phytoremediation is a green remediation technology using plants, which is cost-effective, eco-friendly, and an aesthetically pleasing strategy. Given to its high aesthetic value and the extent of contaminated areas, phytoremediation methods have already gained a great scientific and commercial attention. For the phytoremediation of toxic metals from soils and wetlands, two approaches have been proposed which include natural and induced phytoremediation. Recently, genetically modified plants (GMPs) have been proposed to use in phytoremediation technology; however, this approach is being hindered by ideology-driven restrictive legislation over the use of GMPs [15].

Several reports suggest that phytoremediation of As-contaminated soils using As-hyperaccumulating fern species has emerged as a cost-effective, environmental friendly in situ remediation technology. Of the phytoremediation strategies, phytoextraction of As using As-hyperaccumulating ferns is the most studied aspect for remediation of As-contaminated soils. *Pteris vittata* L. (Chinese brake fern) is the well-known As-hyperaccumulator fern species which has been reported to accumulate high concentrations of As (>3000 mg kg^{-1} dry weight (DW)) in the aboveground biomass (fronds) [16]. After the discovery of *P. vittata*, up to 20 other fern species has been identified which can extract As from contaminated soil [17]. The main focus of most of the earlier studies was on pot-based phytoextraction experiments under glasshouse conditions. However, very limited research has been devoted to explore the phytoremediation potential of As-hyperaccumulating ferns under the field environments. For example, Kertulis-Tartar et al. [18] evaluated the phytoextraction potential of *P. vittata* over a period of 2 years at a CCA-contaminated site in Florida, USA. Recently, Niazi et al. [5] compared the phytoremediation potential of well-recognized *P. vittata* with the lesser known As-hyperaccumulator, *Pityrogramma calomelanos* var. *austroamericana* (gold dust fern), at an As-contaminated disused cattle dip site in NSW, Australia. These authors reported that under field conditions and subtropical environments at the studied site, *P. calomelanos* var. *austroamericana* extracted more than twice As than the *P. vittata*. Hence, exploring the phytoremediation potential of these fern species under the field conditions is imperative to study and it will be a focus of this chapter.

There has been an intensive research conducted to understand the mechanisms of As tolerance and uptake in *P. vittata* in order to induce the phytoremediation efficiency of this fern species, but the jury is still out. However, previous research is limited to investigate the As accumulation potential and mechanisms of some fern species, e.g., *P. calomelanos* var. *austroamericana* and *P. umbrosa*. We will discuss the role of various soil amendments and agronomic practices to enhance the phytoremediation potential of the fern species in order to maximize the As extraction from soil and making the technology highly feasible for field implications. This review will describe As dynamics in soil–plant systems; identify the important mechanisms for absorption, transformation, and storage of As by ferns/plants required for improving the As phytoremediation efficiency; and examine differences in As-hyperaccumulating efficiency of fern species for phytoremediation of As-contaminated soils under pot and field conditions, as well as how phosphate, microbes, and various agronomic practices could significantly increase phytoremediation efficiency of the ferns/plants.

19.2 Availability of As in Soil Environment

Arsenic availability is largely determined by the equilibrium between As in soil solution and the solid phase. Generally, the equilibrium is affected by various reactions including adsorption, ion exchange, complexation with inorganic (and organic) ligands, redox reactions, and precipitation-dissolution [19]. These reactions can potentially affect the free ion concentration of As at the soil–water interface, thereby affecting the solubility and bioavailability of As.

19.3 Factors Controlling Availability of As in Soil

19.3.1 Soil Factors

The plant available fraction is not the same as the total concentration in the soil. Phytoavailable form of As is either in soil solution (weakly absorbed to the solid phase) or specifically sorbed to the solid phase but able to transfer in solution during plant growth. The unavailable or the least available fractions (those rendered immobile or least mobile) are strongly bound within the mineral matrix [4, 20–22]. Chemical properties such as soil acidity, redox potential (E_h), and As speciation may also influence the lability and plant uptake of As [23, 24]. The role of soil pH is well documented in determining As mobilization and availability in soil. Previous studies have indicated that a decline in soil pH increases the adsorption of As (As(V)) in soil and decreases its availability for plant uptake [4, 24].

Similar to soil pH, soil E_h is also a well-known soil parameter that can control the fate and speciation of As in soils [25–27]. Generally, under locally reducing conditions, As availability is considered high [26, 28]. Soluble and mobile As concentration can increase 13 times upon reduction of E_h to −200 mV, as compared to 500 mV. This indicates that chemical speciation of As may affect its availability for plant uptake and should be considered under future investigations to delineate the phytoextraction efficiency of As-hyperaccumulating fern species. Iron oxides are widely distributed in soil and sediments and exist as coatings on the surface of primary and secondary clay minerals [23, 29–31]. They form various types of solid phases under different pH, temperature, and pressure conditions. The processes of sorption/desorption, precipitation, and coprecipitation are responsible for As retention on Fe oxides (e.g., goethite, ferrihydrite) surfaces which account for As availability in soil [32–35].

19.3.2 Other Environmental Factors Affecting As Uptake in Plants/Ferns

Anions such as phosphate ($HPO_4^{2-}/H_2PO_4^-$), chloride (Cl^-), and sulfate (SO_4^{2-}) have a greater influence (mainly phosphate) on the sorption/desorption reactions and availability of As in soil. The anions affect the mobility of As in soil depending on the As species present in soil [23, 36]. Phosphate has been found to be the major anion displacing sorbed As from soils and increasing its phytoavailability in the soil solution. These reactions mainly occur at the surfaces of Fe/Al oxides and/or clay minerals [23, 37–40]. Application of relatively high rates of phosphate fertilizers has been reported to increase the As(V) concentration in soil solution in batch and column studies. The presence of phosphate in the equilibrating solution has been found to decrease the As(V) adsorption while the addition of other anions such as Cl^-, NO_3^-, and SO_4^{2-} showed very minute effect on the adsorption reactions. Both phosphate and As(V) occupy the same adsorption sites on the oxides and mineral surfaces and compete with each other. Studies show that As(V) adsorption has been decreased by SO_4^{2-} on Al oxides surfaces but increasing concentration of the anion did not decrease the As(V) adsorption. This showed that SO_4^{2-} did not occupy the same sites as As(V) [23, 38]. The studies indicated that PO_4 is the only major anion which decreases the As(V) adsorption in soil and increases its mobility and other anions have very little effect.

Organic matter can also have influence on the availability and mobility of As in soil. It has two major portions: fulvic acid (FA) and humic acid (HA) which affect the adsorption of As in soil and water systems [23, 41, 42]. The presence of FA showed a great influence on the adsorption of As(V) on alumina between pH 3 and 7.5. Fulvic acid may be adsorbed on alumina by columbic interaction or directly form complexes with As, which decrease the sorption of As complex. Several studies reveal that HA and FA compete with As on oxides and mineral surfaces and enhance its mobility and availability in soil solution [43–47]. These studies suggest that organic matter addition in soil increases its availability by enhancing desorption of As from soil.

Biotransformations of As create further complexity between the solid and solution phase association of As. These include oxidation, reduction, and methylation reactions. However, toxicity of As is related to its oxidation state [23, 48]. The oxidation of As by bacteria was first identified by Green in 1918 when a bacterium (*Bacillus arsenoxydans*) was isolated from the cattle dipping solution. Several other *Bacillus* or *Pseudomonas* spp. have been characterized to be involved in the oxidation of As(III) to As(V). For example, the bacterium, *Alcaligenes faecalis*, oxidizes As(III) to As(V) using As(III) as s terminal electron acceptor [48, 49]. Oxidized form of As is As(V) and can be transformed to As(III) under reduced soil conditions and finally to AsH_3 gas. Soil microbes are able to convert As(V) and As(III) into many volatile reduced forms such as methyl arsines. Methylphenyl arsenic acid and dimethylphenyl arsine oxide are reduced to dimethylphenylarsine by *Candida humicola*. Seven diverse species of Eubacteria and two species of Crenarchaea have been isolated for the reduction of As(V) to As(III) [49]. In addition to the microbial reduction, chemical reduction also occurs in the soil environment [48].

Biological factors including soil bacterial and fungal rhizosphere associations with fern roots may significantly modify the chemical and physical conditions which determine As-hyperaccumulation by plants (ferns) [23, 50]. For example, Agely et al. [50] reported that arbuscular mycorrhizal (AM) fungi could increase aboveground biomass, As accumulation, translocation, and bioconcentration in *P. vittata*. The uptake of As by fern plants continuously alters the concentration and speciation in contaminated soils through release of root exudates and rhizosphere acidification.

19.4 Arsenic Accumulation in Plants

As mentioned earlier, As is a highly toxic metalloid and exists in soil and water environments mainly in two inorganic forms, As(V) and As(III), the later being 60 times more toxic and mobile than As(V). In plants, accumulation of As is attributed to the uptake capacity, intracellular binding sites, and is complicated by tissue and cell specific differences and intercellular transport mechanisms [23, 51]. Arsenic is detected in low concentrations in plants grown in the uncontaminated soils, with As concentration <1.5 μg kg^{-1} [48]. However, in contaminated soils, As can be accumulated by plants and cause phytotoxicity which may eventually lead to plant death. For example, As(V) uptake in plants inhibits phosphate uptake pathway leading to insufficient levels of phosphorylated compounds and retarded plant growth. Arsenite form of As is known to have twice as much phytotoxicity as As(V) since it rapidly combines with the dithiol functional groups and destroys the functioning of sulfhydryl enzymes, thereby causing membrane degradation and immediate cell death [48, 52].

Terrestrial plants have evolved unique strategies to cope with the heavy metal(loid)s (e.g., Zn, Cu, Pb, and As) stress. Based on his conceptual model of metal(loid) uptake in plants, the terrestrial plants can be characterized as excluders, indicators, and accumulators (Fig. 19.2) [17]. According to Fitz and Wenzel [51], the majority of non-As-hyperaccumulating plants can be termed as excluders. In the model proposed by these authors (Fig. 19.2) the mean bioconcentration coefficient (As concentration in plant shoot to As concentration in soil) for all non-hyperaccumulators was 0.025, the highest was 0.28.

Accumulators can actively accumulate high levels of As in the aboveground biomass without adverse affects on plant growth until soil conditions become toxic and plant growth suppressed. These species are characterized by a leaf: root heavy metal(loid) concentration ratio of >1 [51]. Plants that fall into this category are termed as As-hyperaccumulators and all As-hyperaccumulators are ferns belonging to Pteridales from the genera Pteris and Pityrogramma. Figure 19.3 illustrates a typical structure of a fern plant.

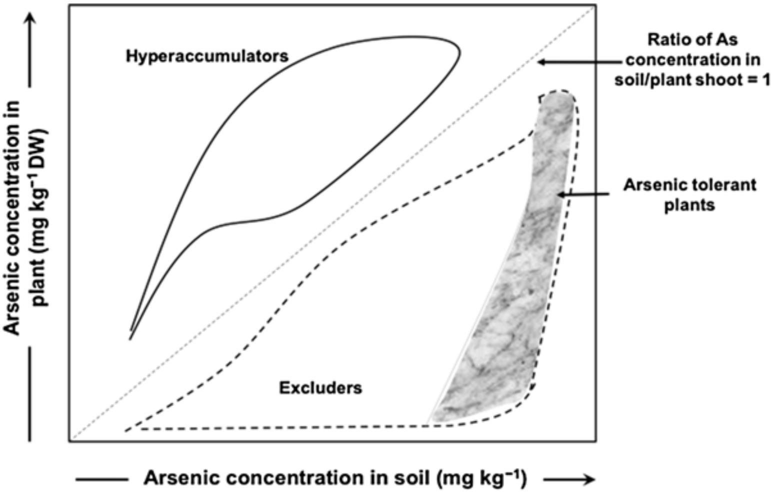


Fig. 19.2 Generalized pattern of As uptake strategies by terrestrial plants. Adapted with permission from Niazi NK (2011) Variability Speciation and Phytoremediation of Soil Arsenic at Cattle Dip Sites in NSW, Australia. PhD thesis, The University of Sydney, Australia

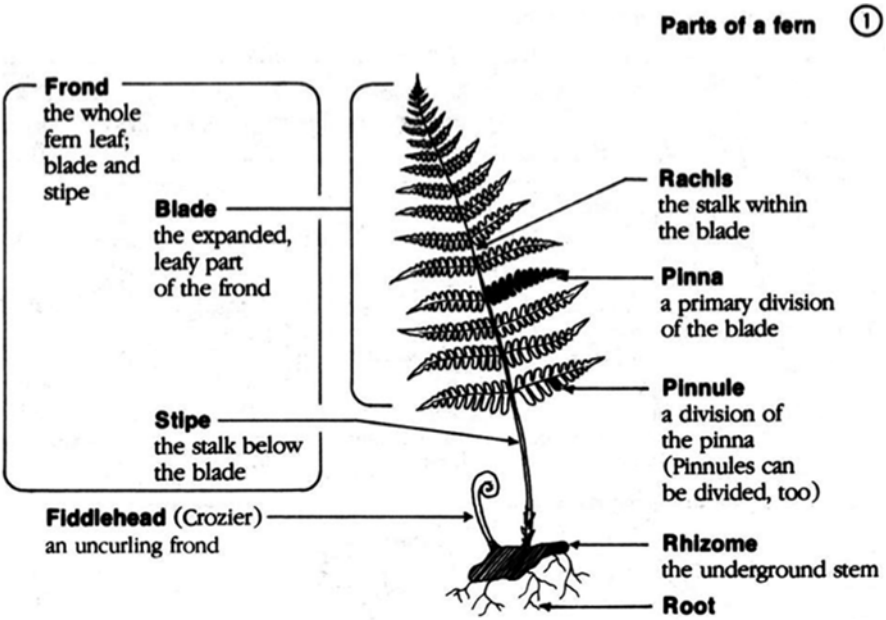


Fig. 19.3 A typical fern plant showing various fern parts

19.5 Arsenic Uptake Mechanisms in Plants/Ferns

Arsenate is chemically similar to phosphate. Both As(V) and phosphate are in the same chemical group and have comparable dissociation constants and solubility products values for their acids and salts, respectively [23, 53]. Plant As uptake is influenced by As source and solubility [23, 54, 55]. Arsenite is thought to be taken up passively by aquaglyceroporins or channels allowing movement of water and neutral solutes in the plant roots including various fern species, such as *P. vittata*. The transport system for As(V) is through the plasma membrane that is the same pathway used by phosphate in ferns and other plant species [51, 56, 57].

Plant exposure to As and heavy metal enriched environments may result in the production of reactive oxygen species, including superoxide anions, hydrogen peroxide (H_2O_2), and hydroxyl radicals which can destroy the cell components [51, 58]. Plants can synthesize enzymatic and nonenzymatic antioxidants in response to the generation of reactive oxygen species. Hence, plants can cope with the detrimental effects of reactive oxygen species by using antioxidant molecules, such as L-ascorbic acid, reduced glutathione (GSH), α-tocopherols, and carotenoids, particularly ascorbic acid [53].

Under no As stress, *P. vittata* was found to have intrinsically higher concentrations of nonenzymatic antioxidants, ascorbate, and glutathione (GSH), in its fronds compared to *P. ensiformis* (a non-As-hyperaccumulator). This suggests that the ascorbate-GTH pool may play a significant role in the ability of *P. vittata* to tolerate and hyperaccumulate As [13, 59]. In previous studies, *P. vittata* has been reported to produce superoxide dismutase (SOD) and catalase (CAT) (antioxidants) when the ferns were exposed to As [59, 60]. Conversely, in the same conditions of the experiment, *P. ensiformis* could not induce the generation of these antioxidants.

19.6 Remediation of As-Contaminated Soils

As discussed above that various anthropogenic activities have led to the contamination of large expanses of land with As. Arsenic-contaminated soils, in many parts of the world (e.g., India, Bangladesh, Australia, the USA, New Zealand), are reported to have impact on the sustainability, productivity, and health of soil environment, leaving large areas of land uninhabitable and unproductive. Increasing growth in the global population has put a demand for remediating the contaminated soils in order to create housing or land to cultivate. Therefore, it is crucial that efforts concentrate on economically and environmentally viable techniques to remediate these contaminated landscapes.

Current physicochemical methods employed to remediate As-contaminated soils are costly and are often restricted to small-scale applications [13]. During the past 10 years, there has been an increasing interest toward the growing of plants (i.e., As-hyperaccumulating ferns) to remove As from contaminated soils. The present

Table 19.1 Various physicochemical methods for the remediation of arsenic-contaminated soils

Method	Description
Capping	An in situ method of remediation in which a hard cover is placed on the surface of the contaminated soil. Capping is also a quite simple method that masks the contaminant exposure. However, it does not remove contaminants from the soil
Solidification and stabilization	This is an in situ method where the contaminated soil is mixed with stabilizers to decrease the mobility of As in soil
Excavation	A commonly used ex situ remediation method which involves the physical removal of the contaminated soil and disposed of it in landfill sites. Although excavation results in fast and quick remediation of the site, however, it is often costly because of the operation, transport, and special landfill requirements
Vitrification	In situ method, where As is chemically bonded inside a glass matrix forming silico-arsenates
Soil washing/acid extraction	Ex situ method of treating the suspension or dissolution of As in a water-based wash solution to concentrate the contaminant
Soil flushing	In situ method that uses water, chemicals, or organics to mobilize As and flush it from the soil

remediation methods for As-contaminated soils include soil removal and washing, physical stabilization, and/or the use of chemical amendments (Table 19.1). All these approaches are thought to be expensive and disruptive, with an average cost of (US dollars) \$404,700 ha^{-1}[13].

Table 19.2 presents a detailed list of various physicochemical and biological methods for removal of metal(loid)s from soils.

19.7 Phytoremediation

Phytoremediation can be defined as a process in which green plants extract, sequester, or stabilize As to render them harmless [61]. This is an emerging remediation technology for the remediation of As-contaminated soils. The ongoing advancement in the field of phytoremediation has been largely driven by the spiralling costs associated with conventional soil remediation methods and the need to use a "green" sustainable process. It has been indicated that in some cases the costs associated with phytoremediation were 15 times less expensive than that of conventional physicochemical remediation strategies [62]; the author suggested that phytoremediation was an economically feasible remediation technology. Moreover, the current physicochemical technologies are aimed mainly for rigorous in situ or ex situ remedy of highly contaminated sites, and thus are not suitable for immensely and extremely variable contaminated areas where contaminants exist at low concentrations and demonstrate high spatial variation [18]. A summary of the advantages and possible disadvantages of phytoremediation is provided in Table 19.3 [17]:

Table 19.2 Various methods and their applicability to remediate arsenic-contaminated soils

Method	Description	Applicability
Oxidation	Using heterotrophic bacteria and chemoautotrophic to oxidize arsenite into a less toxic arsenate	Should be carried out in a controlled environment
Reduction	Reduction of arsenate to arsenite by microorganisms via dissimilatory reduction mechanism	Should be carried out in facultative anaerobe or strictly anaerobe conditions
Methylations	Biomethylations by (As(III) S-adenosylmethionine methyltransferases)	It is reliable biological process of removing arsenic from aquatic mediums
Phytoremediation methods	Using "free-floating plants such as water hyacinth" using aquatic rooted plants such as *Agrostis* sp., *Pteris vittata,* and *Pteris cretica*	Widely accepted in some places
Biological methods	Such as phytoremediation by using aquatic plants or microbial transformation of arsenic	Widely accepted in some places
Physiochemical methods	Filtration or coagulation sedimentation, osmosis or electrodialysis, adsorptions, and chemical precipitations	Widely accepted in some places
Microbial oxidations	Immobilization of arsenic in the solid phase	Required biological activity and microbiological molecular analysis/involved adsorption or coprecipitation with Fe-oxyhydroxides
Engineered bioremediation	Optimizing the environment conditions to promote the proliferation and activity of microorganisms	Favorable method used in highly contaminated area
Intrinsic bioremediation	Degradation of arsenic by naturally occurring microorganisms	More suitable for remediation of soil with a low level of contaminants
Chemical remediation approaches	Stabilization methods using nanosized oxides and Fe(0) (particle size of 1 to 100 nm)	Gained popularity/high success rates but it could be expensive when remediating a large area
	Formation of stable phases, for example, insoluble $FeAsO_4$ (and hydrous species of this compound such as Scorodite $FeAsO_4{\cdot}2H_2O$)	Use of selective stabilizing amendments is a challenging task
	Adsorption by using specific media, immobilization, modified coagulation along with filtration, precipitations, immobilizations, and complexation reaction	Economic but often displayed lower efficiencies (<90 %)

(continued)

Table 19.2 (continued)

Method	Description	Applicability
Physical approaches	Soil flushing using aqueous solutions using surfactants and co-solvents	Applied in the field, efficiency can vary from 0 % to almost 100 %
	Emphasis on stabilization/sodification (S/S)	Treating As containing wastes in water
	Immobilize soluble arsenite using cement	Successfully used to stabilize As-rich sludge
	Washed with sulfuric acid, nitric acid, phosphoric acid, and hydrogen bromide	Chemicals usage/high cost/ usage to smaller scale operations
	Mixing both contaminated and uncontaminated soils operations	High costs/usage to smaller scale

The term phytoremediation includes the following strategies [13], which are illustrated in Fig. 19.4 of this chapter.

19.7.1 Phytoextraction

The use of hyperaccumulating plants is to extract the contaminant from soil and translocate it to the aboveground biomass. For example, hyperaccumulating ferns such as *P. vittata* and *P. calomelanos* var. *austroamericana* to remove As from soil (see Figs. 19.5 and 19.6) [63].

19.7.2 Phytostabilization

The pollutant-tolerant plants are used for mechanical stabilization of contaminated soil in order to prevent bulk erosion, decrease airborne transport, and leaching of contaminants. It is used to provide a cover of vegetation for a moderately to heavily contaminated site, thus preventing wind and water erosion (Fig. 19.4) [64].

19.7.3 Phytoimmobilization

It refers to the use of plants to reduce the mobility and bioavailability of contaminants in soil by formation of precipitates and insoluble compounds, as well as by sorption on roots (Fig. 19.4).

Table 19.3 Advantages and disadvantages of phytoremediation of arsenic-contaminated soil

Advantages	Disadvantages
(a) Cost	(a) Time
1. No requirement for the expensive equipment or highly skilled personnel	1. Many years may be required to remediate a contaminated site
2. Metal and/or metalloid recycling provides further economic gain	2. Several hyperaccumulating plants are slow grower species
(b) Performance	(b) Performance
1. The extent of soil disturbance is minimum compared to conventional methods	1. Remediation is restricted to shallow contamination within rooting zone of remediative plants
2. Adaptable to a range of inorganic and organic compounds	
3. Application (in situ/ex situ) is possible in effluent or soil	2. 100 % reduction may not be achieved
	3. Limited to sites containing low contaminant concentrations
	4. Consumption or utilization of contaminated plant biomass is a cause of concern for secondary pollution
4. In situ applications decrease spread of contaminant via air and water	5. Harvested plant biomass from phytoextraction may be classified as a hazardous waste hence treatment/disposal should be proper
5. Capable of remediating bioavailable fraction	6. Adaptation to the climatic conditions is a growth-limiting factor
(c) Other	(c) Other
1. Publically acceptable; aesthetically pleasant	1. Lack of recognized economic performance data
2. Compatible with risk-based remediation, brownfields	2. Need to displace existing facilities (e.g., wastewater treatment)
	3. Introduction of non-native species may affect biodiversity
3. Can be employed during site investigation or after closure	4. Regulators may not be familiar with the technology and its capabilities
4. In large-scale applications, the potential energy stored can be utilized to generate thermal energy	

19.7.4 Phytovolatilization

The use of plants to volatilize contaminants has been demonstrated for Hg and Se. For Hg, such mechanism was developed by genetic manipulation of plants whereas in the case of Se phytovolatilization naturally occurs in plants. Limited information is available on the As-volatilization in soil indicated that volatile compounds account only for little proportions of total As, in the absence of plant roots (Fig. 19.4).

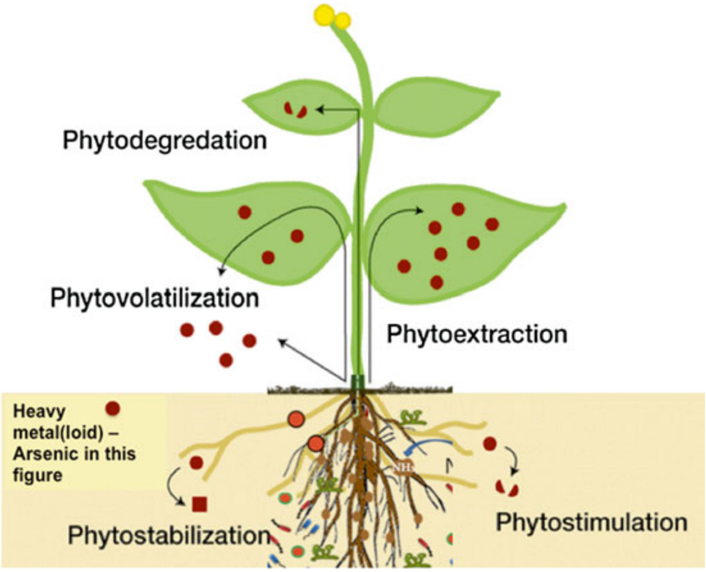


Fig. 19.4 A schematic diagram showing the various phytoremediation strategies for remediation of arsenic-contaminated soils

19.8 Phytoextraction of Soil As Using As-Hyperaccumulating Fern Species

Brooks et al. [65] used the term *hyperaccumulator* for the first time, which they defined as a plant species that could accumulate substantial amounts of a given heavy metal and/or metalloid in aboveground tissue without deleterious effects to the plant (Figs. 19.5 and 19.6). The authors developed this definition particularly for Ni-hyperaccumulating plants; however, several hyperaccumulators for the heavy metal(loid)s, As, Cd, Cu, and Zn, have also been described.

Hyperaccumulating plants are reported to contain >1000 mg kg^{-1} DW or 0.1 % of an element. Generally, hyperaccumulators are reported to have a high rate of accumulation, fast growing behavior, and have a potential to yield larger amount of biomass [13, 65]. Also, bioconcentration factor (BF; ratio of contaminant concentration in plant aboveground biomass to soil) and translocation factor (TF; ratio of contaminant concentration in plant aboveground biomass to plant root) of the hyperaccumulating plants are considered to be >1. However, the soil properties, such as (low) pH and (high) Fe oxides content, can reduce the availability of As in soil and substantially decrease the accumulation rate of a contaminant (e.g., As) by the hyperaccumulating plants. This is particularly important to consider when these plants are grown under the field conditions.

The ladder brake fern, *P. vittata*, is the first known example of a plant that extracts As from soil and can be referred as an As-hyperaccumulator [16]. Ferns are lower

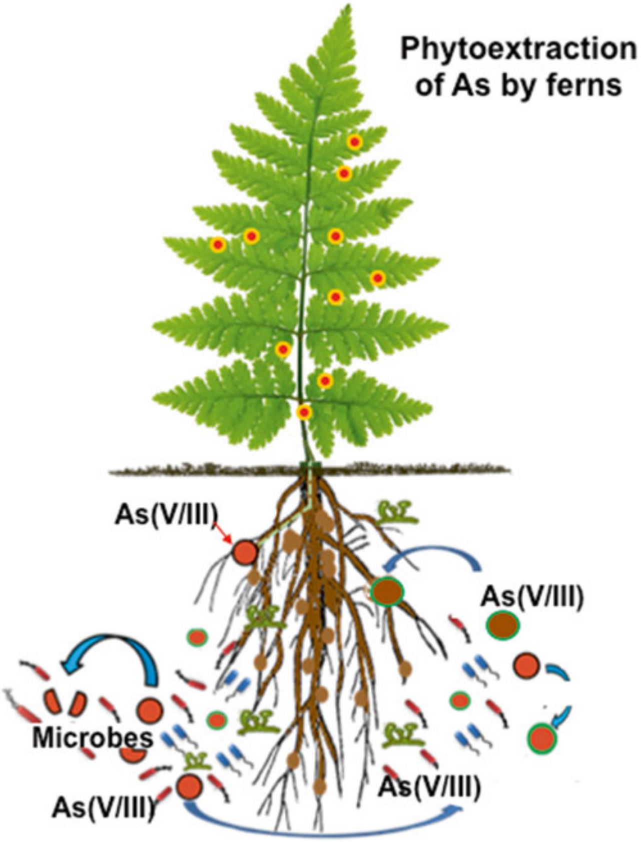


Fig. 19.5 Phytoextraction of arsenic using arsenic-hyperaccumulating fern species—a pictorial view of the process

plants, unlike several of the other identified hyperaccumulating plants, which are dicots or monocots (e.g., plants in mustard family, such as *Thlaspi* spp. and *Brassica* spp.). *Pteris vittata* has long been associated with arsenical mine dumps [66] and Cu/Co-rich substrates [67]. However, its ability to hyperaccumulate As was discovered by Ma et al. [16]. The authors observed that it could accumulate up to 22,630 mg As kg^{-1} DW in the fronds. This fern was highly tolerant to As in soil containing up to 500 mg As kg^{-1}, and soils spiked with 50 mg As kg^{-1} were best for fern growth resulting in biomass production of 3.9 g $plant^{-1}$[68]. Similarly, the highest BF (63) and TF (25) were observed in soils spiked with 50 mg As kg^{-1}.

Since the discovery of *P. vittata*, several other fern species have been identified as potential As-hyperaccumulating species (see Table 19.4 for a list of various identified As-hyperaccumulating fern species) such as the silver fern, *Pityrogramma calomelanos* [69]. Zhao et al. [70] assessed As accumulation in three different accessions of *P. vittata*, two cultivars of *Pteris cretica*, *Pteris longifolia* and *Pteris*

***Pityrogramma calamelonas* var. *austroamericana* (Gold dust fern)**

***Pteris vittata* (Chinese brake fern)**

Fig. 19.6 Photographs showing the two fern species growing on an arsenic-contaminated disused cattle dip site in northern NSW, Australia (Photo by Nabeel K Niazi)

Table 19.4 Review of the confirmed As-hyperaccumulating fern species [updated from Niazi, 17]

Fern species	Family name	Maximum frond As concentration (mg kg^{-1} DW)
Pteris vittata	Pteridaceae	22,630
Pityrogramma calomelanos	Pteridaceae	8350
Pteris cretica var. *albo-lineata*	Pteridaceae	7600
Pteris cretica var. *alexandrae*	Pteridaceae	7600
Pteris longifolia	Pteridaceae	7600
Pteris umbrosa	Pteridaceae	7600
Pteris cretica var. *Nervosa*	Pteridaceae	2594
Pteris cretica var. *Chilsii*	Pteridaceae	1358
Pteris cretica var. *Crista*	Pteridaceae	1506
Pteris cretica var. *Mayii*	Pteridaceae	1239
Pteris cretica var. *Parkerii*	Pteridaceae	2493
Pteris cretica var. *Rowerii*	Pteridaceae	1425
Pityrogramma calomelanos var. *Austroamericana*	Pteridaceae	16,400
Asplenium australasicuma[a]	Aspleniaceae	1240
Asplenium bulbiferuma[a]	Aspleniaceae	2630
Pteris multifida Poir.	Pteridaceae	1145
Pteris oshimensis	Pteridaceae	2142
Pteris biaurita L.	Pteridaceae	3650
Pteris quadriaurita Retz	Pteridaceae	3650
Pteris ryuensis	Pteridaceae	3650
Pteris fauriei	Pteridaceae	1362
Pteris aspericaulis	Pteridaceae	2410

[a] The plants showed As toxicity symptoms when exposed to concentrations >50 mg L^{-1}

umbrosa. Arsenic concentrations among all accessions and species ranged from 6200 to 7600 mg kg^{-1} DW and these authors indicated that As-hyperaccumulation is a constitutive property of the *Pteris* genus. It has been shown, however, that *Pteris* species such as *P. straminea*, *P. tremula* [71], and *P. semipinnata* [72] do not hyperaccumulate As.

Kertulis-Tartar et al. [18] conducted a field experiment for the phytoremediation of an As-contaminated site historically contaminated with copper–chromium–arsenate (CCA) application in Florida, USA. These researchers used *P. vittata* for the phytoextraction of As from soil with As concentration in *P. vittata* fronds more than 2000 mg kg^{-1} DW. They estimated that about 8 years would be needed to decline concentration of acid-extractable As in soil from a mean value of 82–40 mg kg^{-1} As, the limit set by the US EPA. In Australia, Kachenko et al. [73] identified *P. calomelanos* var. *austroamericana*. These authors observed that the fern species has shown a consistent As-hyperaccumulating pattern in the glasshouse conditions and can accumulate As up to 16,400 mg kg^{-1} DW in fronds. Xu et al. [24] compared the phytoremediation potential of *P. calomelanos* var. *austroamericana* and *P. vittata* grown in As-contaminated soils with contrasting soil properties in a glasshouse experiment. They demonstrated that *P. vittata* possessed higher As accumulation and produced greater frond biomass than that of *P. calomelanos* var. *austroamericana*. Recently, Niazi et al. [5] conducted a long-term (27 months) field experiment to compare the phytoremediation efficiency of *P. calomelanos* var. *austroamericana* (gold dust fern) and *P. vittata*, at Wollongbar in NSW, Australia. The authors reported that *P. calomelanos* var. *austroamericana* removed 2.65 times more As than *P.vittata* over 27-month duration of the experimental period. They suggested that *P. calomelanos* var. *austroamericana* was well adapted to the soil and environmental conditions at the experimental site. Further field studies are warranted to evaluate the phytoextraction potential fern species on As-contaminated soils with various soil properties and containing varying As concentrations.

19.8.1 Detoxification and Tolerance of As in Ferns

Arsenic is a nonessential element for plants; however, in As-hyperaccumulators such as *P. vittata*, As is accumulated at high rates and at concentration proportional to As concentrations in soil or growth media [13, 16, 74]. *Pteris vittata* has been reported to survive in soil contaminated with 1500 mg kg^{-1} As and bioconcentrate 2.3 % of As in its aboveground biomass (fronds). This feature of *P. vittata* indicates that the hyperaccumulating ferns possess efficient mechanisms to detoxify As accumulated from soil. Such mechanisms may include chelation, compartmentalization, biotransformation, and cellular repair [13, 75]. For instance, heavy metals are generally transported and deposited in a vacuole as *metal-chelates*. Once free metal(loid) ions in soil solution are taken up by plants into their tissues, they get reduced greatly when chelated by particular high-affinity ligands (such as sulfur-donor, oxygen-donor, or nitrogen-donor ligands). Sulfur-donor ligands including metallothioneins and phytochelatins have the capability to form highly stable complexes with heavy metals, since sulfur is a better electron donor than oxygen. Previous studies show a prominent role of PCs in the detoxification of As in plants [58]. Reina et al. [76] demonstrated that both GSH and PCs were able to complex the majority of As in shoots of lupin plant. However, the function of PCs appears to be negligible in As-hyperaccumulating fern species [13, 77]. In *P. vittata* and *P. calomelanos*, reduction of As(V) to As(III) occurs inside plant cells [16, 78]. This reduction of As(V) inside the plant cells is an intriguing process as As(III) is more toxic than As(V). Additionally, *P. vittata* was shown to have only 4.5 % of its As complexed with PCs, as a GSH-As(III)-PCs complex [13].

In a study by Raab et al. [79], *P. cretica* demonstrated only 1 % of its As complexed with PCs. From these studies, the authors conclude that the PCs may act as a carrier to transport As in a nontoxic form through the cytoplasm and into the vacuoles. However, As complexation with PCs may not be the highly efficient detoxification mechanism in As-hyperaccumulating ferns [13]. Kachenko et al. [80] investigated speciation of As in As-hyperaccumulating fern *P. calomelanos* var. *austroamericana* using X-ray absorption spectroscopy. The authors indicated that As^V absorbed by roots was partially reduced to As(III) prior to transport into aboveground tissues and reported that As(III)–S(2–) compounds might be involved for the biochemical reduction of As(V) to As(III). To our understanding, research is warranted to examine As association with various components during transformation from root to shoot (fronds) in plants (ferns) using techniques such as SIMS or LA-ICP-MS.

19.8.2 Mycorrhizal Association and As Removal

There have been various investigations where mycorrhizae have been reported in plants growing on heavy metal(loid)-contaminated soils [81–83], representing that these fungi have developed a tolerance to heavy metal(loid)s and could play a vital role in the phytoremediation of the contaminated site. However, for arbuscular mycorrhizae (AM) the findings are contradictory. Some reports show high concentrations of heavy metal(loid)s in plants due to AM, even up to toxic levels in plants [84]. Others found reduced plant concentrations of zinc (Zn) and copper (Cu) in mycorrhizal plants [85–87].

Meharg et al. [88] reported that As-tolerant phenotype of *H. lanatus* showed 11 % high phosphorus uptake and infection rate of roots was 34 % higher for AM than control. Wright et al. [89] conducted a field experiment comparing clones of tolerant and non-tolerant *H. lanatus* populations. Almost no difference in AM mycorrhization was detected, but tolerant plants accumulated high concentration of phosphorus in shoots. The role of mycorrhizae in As-hyperaccumulation is yet to be partially resolved and should be explored in future studies. Fitz and Wenzel [90] found that *P. vittata* grown in pots are colonized by AM fungi. In another experiment, Agely et al. [91] investigated the role of mycorrhizal symbiosis in *P. vittata* in plant growth and As and P association. They found that mycorrhizal fungi enhance As transfer as well as plant biomass which contradict the findings of Leyval et al. [92], who reported that AM-limited metal(loid) transfer to the host plant. The prospect of symbionts existing in *P. vittata* has important implications for phytoremediation. Mycorrhizal associations upsurge the absorptive surface area of the plant because of extrametrical fungal hyphae exploring rhizospheres beyond the root hair zone. The defense and greater capacity of increased uptake of minerals result in higher biomass production which is a prerequisite for successful phytoremediation strategy [93].

19.9 Role of Phosphate and Agronomic Practices in Phytoremediation of Arsenic-Contaminated Soils

19.9.1 Phosphate Enhanced Phytoextraction of As

Arsenate is taken up by *P. vittata* and other plant species via the phosphate transport system. Therefore, it is predictable that phosphate will compete with As for plant uptake. Wang et al. [94] found that the maximum net As-influx in phosphate-starved plant increased by 2.5 times compared to the control. While Tu et al. [95] reported that the phosphate addition to a hydroponic system reduced As removal by *P. vittata*. Their results showed that the use of young ferns coupled with feeding of initial low phosphate or split-phosphate application, was more efficient in As removal from water than using older ferns supplied with higher initial phosphate or single phosphate application [96].

Although significant influence of phosphate on the retention and transport of As(V) is well characterized, competitive effect of different phosphate sources on mobilization of As in contaminated soil and further availability to As-hyperaccumulating fern species is lacking in our current understanding. Lessl et al. [3, 97] examined the As removal efficiency of *P. vittata* in three As-contaminated soils (26–126 mg As kg^{-1} soil) for a period of 3.5 years, whereby the soils were amended with soluble phosphate fertilizer or insoluble phosphate rock (PR-soil). *P. vittata* depleted 44 % of soil As from PR-soils compared to 33 % from soluble phosphate amended soils. Their results suggested that the low-soluble P from PR was more effective than soluble phosphate from fertilizer in increasing As uptake by *P. vittata*. It is also reported that the type of phosphate fertilizer source could influence As availability in soil, i.e., diammonium phosphate (DAP) vs. single super phosphates (SSP). There are contradictory reports available with respect to superiority of DAP over SSP [98–101] and vice versa in different crops. Before popularization of phytoremediation of As-contaminated soils, it is extremely vital to establish which phosphate fertilizer source is efficient in enhancing biomass as well as arsenic in As-hyperaccumulating fern species.

19.9.2 Agronomic Practices and Amendment to Enhance Biomass Production and As Phytoextraction

Arsenic phytoremediation by ferns from greenhouse to field conditions clearly requires incorporating agronomical knowledge into the remediation process. The number of field case studies for assessing phytoremediation of As by ferns and other plant species is increasing in the literature. However, the number of successful cases is still limited. More information can be found on plant selection and the comparison of various plant species or cultivars (for their growth and hyperaccumulation) but studies investigating the influence of management options are scarce. Hence there is a need to do research on aspects which are rarely considered, such as soil management practices, crop rotation, intercropping/row cropping, planting methods and plant densities, harvest management, pest and weed control, and irrigation management associated to As phytoremediation. Certain aspects such as tillage practices or plowing methods are impossible to evaluate at a bench scale. Other aspects, such as intercropping, show promise in terms of promoting plant biomass. It is clear from the literature that further field studies are greatly needed in which these practical aspects can be developed and optimized [102]. Applying suitable agronomic measures will be a crucial factor for enhancing the success of phytoremediation of arsenic.

19.10 Cost–Benefit Calculation of Phytoremediation Technology

Wan et al. [103] conducted a 2-year phytoremediation project for soil contaminated with As, cadmium (Cd), and Pb to determine the essential parameters for soil remediation. Their results showed highly efficient heavy metal(loid) removal. The total cost of phytoremediation was calculated to be US \$37.7/m^3, with initial capital and operational costs accounting for 46.02 % and 53.98 %, respectively. The cost of infrastructure (i.e., roads, bridges, and culverts) and fertilizer were the highest, mainly because of slow economic development and serious contamination. The cost of phytoremediation was lower than the reported values of other remediation technologies. Improving the mechanization level of phytoremediation and accurately predicting or preventing unforeseen situations were suggested for further cost reduction. Considering the loss caused by environmental contamination, the authors predicted that the benefits of phytoremediation would offset the project costs in less than 7 years.

19.11 Conclusions and Future Research

Phytoextraction is a promising technology and is very dependent on plant and soil factors, such as soil suitability for plant growth, depth of the contamination, depth of the plant root system, level of contamination, and urgency in cleaning up.

Furthermore, there is a need for a full understanding of the physiology, biochemistry, uptake, etc., of the plants employed, such as hyperaccumulating fern species for phytoextraction of As. A relatively small group of plants, that is fern species, are capable of sequestering As in their shoot (fronds) tissues at high concentrations with the majority of them belonging to the Pteridophytes, which are lower plants. These As-hyperaccumulating fern species offer the challenge of efficient reproduction for large-scale phytoremediation projects. Cultivation of As-hyperaccumulating ferns needs to be optimized in order to provide an effective alternative to common engineering-based As remediation technologies for contaminated soils. In recent years, major scientific progress has been made in understanding the physiological mechanisms of As uptake and transport in these fern species. Although partially understood, very little is known about the molecular basis of As accumulation/hyperaccumulation in plants/ferns. Therefore, the future research should focus on the molecular-genetic technologies and possibly transgenic plants with increased resistance and uptake for As from soil.

Discovering the molecular mechanisms underlying As tolerance and accumulation in the As-hyperaccumulators (ferns) could be facilitated by identifying genes that are both necessary and sufficient for these properties. Endophytes are microorganisms that live within plants and can provide multiple benefits to the plant including increased nutrient acquisition and growth, and improved stress tolerance. Recently, an As-tolerant endophyte has been isolated that reduced the phytotoxic effects of As in inoculated Arabidopsis seedlings. Endophyte-assisted phytoremediation might be an important technological advance enabling this green technology to be utilized on a broader spectrum of As-contaminated areas in combination with As-hyperaccumulating ferns.

Acknowledgements The authors are very grateful to the Grand Challenges Canada–Stars in Global Health (GCC Grant No. S5 0433-01) and International Foundation for Science (IFS Grant No. W/5698-1) for providing financial support, and the University of Agriculture Faisalabad, Pakistan.

References

1. Niazi NK, Bishop TFA, Singh B (2011) Evaluation of spatial variability of soil arsenic adjacent to a disused cattle-dip site, using model-based geostatistics. Environ Sci Technol 45:10463–10470. doi:10.1021/es201726c
2. Niazi NK, Singh B, Minasny B (2015) Mid-infrared spectroscopy and partial least-squares regression to estimate soil arsenic at a highly variable arsenic-contaminated site. Int J Environ Sci Technol 12:1965–1974. doi:10.1007/s13762-014-0580-5
3. Lessl JT, Luo J, Ma LQ (2014) Pteris vittata continuously removed arsenic from non-labile fraction in three contaminated-soils during 3.5 years of phytoextraction. J Hazard Mater 279:485–492. http://dx.doi.org/10.1016/j.jhazmat.2014.06.056
4. Niazi NK, Singh B, Shah P (2011) Arsenic speciation and phytoavailability in contaminated soils using a sequential extraction procedure and XANES spectroscopy. Environ Sci Technol 45:7135–7142. doi:10.1021/es201677z

5. Niazi NK, Singh B, Zwieten LV, Kachenko AG (2012) Phytoremediation of an arsenic-contaminated site using *Pteris vittata* L. and *Pityrogramma calomelanos* var. *austroamericana*: a long-term study. Environ Sci Pollut Res 19:3506–3515. doi:10.1007/s11356-012-0910-4
6. Shakoor MB, Niazi NK, Bibi I, Rahman MM, Naidu R, Dong Z, Shahid M, Arshad M (2015) Unraveling health risk and speciation of arsenic from groundwater in rural areas of Punjab, Pakistan. Int J Environ Res Public Health 12:12371–12390. doi:10.3390/ijerph121012371
7. Naidu R, Smith E, Owens G, Bhattacharya P, Nadebaum P (2006) Managing arsenic in the environment: from soil to human health. CSIRO, Collingwood
8. Ng JC, Wang J, Shraim A (2003) A global health problem caused by arsenic from natural sources. Chemosphere 52:1353–1359
9. Feldmann J (2001) Appetite for arsenic. Chem Br 37:31–32
10. USEPA (2001) In: U.S.E.P.A. Workshop on managing arsenic risks to the environment: Characterization of waste, chemistry, and treatment and disposal, May 2001, Denver, CO, pp 107. http://www.epa.gov/ord/NRMRL/pubs/625r03010/625r03010total.pdf
11. Singh AP, Goel RK, Kaur T (2011) Mechanisms pertaining to arsenic toxicity. Toxicol Int 18:87
12. Chou W-C, Jie C, Kenedy AA, Jones RJ, Trush MA, Dang CV (2004) Role of NADPH oxidase in arsenic-induced reactive oxygen species formation and cytotoxicity in myeloid leukemia cells. Proc Natl Acad Sci U S A 101:4578–4583
13. Gonzaga MIS, Santos JAG, Ma LQ (2006) Arsenic phytoextraction and hyperaccumulation by fern species. Sci Agric 63:90–101
14. Frankenberger W (2002) Arsenic (V)/(III) cycling in soils and natural waters: chemical and microbiological processes. Marcel Dekker, New York
15. Rahman MA, Reichman SM, De Filippis L, Sany SBT, Hasegawa H (2016) Phytoremediation of toxic metals in soils and wetlands: concepts and applications. Environmental remediation technologies for metal-contaminated soils. Springer, Berlin, pp 161–195
16. Ma LQ, Komar KM, Tu C, Zhang WH, Cai Y, Kennelley ED (2001) A fern that hyperaccumulates arsenic—a hardy, versatile, fast-growing plant helps to remove arsenic from contaminated soils. Nature 409:579
17. Niazi NK (2011) Variability speciation and phytoremediation of soil arsenic at cattle dip sites in NSW, Australia. PhD thesis, The University of Sydney, Australia
18. Kertulis-Tartar GM, Ma LQ, Tu C, Chirenje T (2006) Phytoremediation of an arsenic-contaminated site using *Pteris vittata* L.: a two-year study. Int J Phytoremediation 8:311–322. doi:10.1080/15226510600992873
19. Yang L, Donahoe RJ (2007) The form, distribution and mobility of arsenic in soils contaminated by arsenic trioxide, at sites in southeast USA. Appl Geochem 22:320–341. doi:10.1016/j.apgeochem.2006.11.005
20. Devesa-Rey R, Paradelo R, Diaz-Fierros F, Barral MT (2008) Fractionation and bioavailability of arsenic in the bed sediments of the Anllons River (NW Spain). Water Air Soil Pollut 195:189–199. doi:10.1007/s11270-008-9739-3
21. Ko BG, Anderson CWN, Bolan NS, Huh KY, Vogeler I (2008) Potential for the phytoremediation of arsenic-contaminated mine tailings in Fiji. Aust J Soil Res 46:493–501. doi:10.1071/sr07200
22. Novoa-Munoz JC, Queijeiro JMG, Blanco-Ward D, Alvarez-Olleros C, Garcia-Rodeja E, Martinez-Cortizas A (2007) Arsenic fractionation in agricultural acid soils from NW Spain using a sequential extraction procedure. Sci Total Environ 378:18–22. doi:10.1016/j.scitotenv.2007.01.026
23. Smith E, Naidu R, Alston AM (1998) Arsenic in the soil environment: a review. Adv Agron 64:149–195
24. Xu W, Kachenko AG, Singh B (2010) Effect of soil properties on arsenic hyperaccumulation in *Pteris vittata* L. and *Pityrogramma calomelanos* var. *austroamericana*. Int J Phytoremediation 12:174–187
25. Al-Abed SR, Jegadeesan G, Purandare J, Allen D (2007) Arsenic release from iron rich mineral processing waste: influence of pH and redox potential. Chemosphere 66:775–782. doi:10.1016/j.chemosphere.2006.07.045

26. Carbonell-Barrachina AA, Jugsujinda A, Burlo F, Delaune RD, Patrick WH (2000) Arsenic chemistry in municipal sewage sludge as affected by redox potential and pH. Water Res 34:216–224
27. Mandal BK, Suzuki KT (2002) Arsenic round the world: a review. Talanta 58:201–235
28. Masscheleyn PH, Delaune RD, Patrick WH (1991) Effect of redox potential and pH on arsenic speciation and solubility in a contaminated soil. Environ Sci Technol 25:1414–1419
29. Grafe M, Sparks DL (2006) Solid phase speciation of arsenic. In: Smith E, Owens G, Bhattacharya P, Nadebaum P, Naidu R (eds) Managing arsenic in the environment. From soil to human health. CSIRO, Collingwood, pp 75–91
30. Smith E, Naidu R, Alston AM (1999) Chemistry of arsenic in soils: I. Sorption of arsenate and arsenite by four Australian soils. J Environ Qual 28:1719–1726
31. Xiao R, Bai J, Lu Q, Zhao Q, Gao Z, Wen X, Liu X (2015) Fractionation, transfer, and ecological risks of heavy metals in riparian and ditch wetlands across a 100-year chronosequence of reclamation in an estuary of China. Sci Total Environ 517:66–75. doi:10.1016/j.scitotenv.2015.02.052
32. O'Reilly SE, Strawn DG, Sparks DL (2001) Residence time effects on arsenate adsorption/desorption mechanisms on goethite. Soil Sci Soc Am J 65:67–77
33. Sun XH, Doner HE (1996) An investigation of arsenate and arsenite bonding structures on goethite by FTIR. Soil Sci 161:865–872
34. Sun XH, Doner HE (1998) Adsorption and oxidation of arsenite on goethite. Soil Sci 163:278–287
35. Violante A, Del Gaudio S, Pigna M, Ricciardella M, Banerjee D (2007) Coprecipitation of arsenate with metal oxides. 2. Nature, mineralogy, and reactivity of iron(III) precipitates. Environ Sci Technol 41:8275–8280. doi:10.1021/es070382
36. Goh K-H, Lim T-T (2005) Arsenic fractionation in a fine soil fraction and influence of various anions on its mobility in the subsurface environment. Appl Geochem 20:229–239
37. Frau F, Biddau R, Fanfani L (2008) Effect of major anions on arsenate desorption from ferrihydrite-bearing natural samples. Appl Geochem 23:1451–1466
38. Meng XG, Korfiatis GP, Bang SB, Bang KW (2002) Combined effects of anions on arsenic removal by iron hydroxides. Toxicol Lett 133:103–111
39. Stachowicz M, Hiemstra T, van Riemsdijk WH (2008) Multi-competitive interaction of As(III) and As(V) oxyanions with Ca^{2+}, Mg^{2+}, PO_4^{3-}, and CO_3^{2-} ions on goethite. J Colloid Interface Sci 320:400–414. doi:10.1016/j.jcis.2008.01.007
40. Violante A, Pigna M (2002) Competitive sorption pf arsenate and phosphate on different clay minerals and soils. Soil Sci Soc Am J 66:1788–1796
41. Grafe M, Eick MJ, Grossl PR (2001) Adsorption of arsenate (V) and arsenite (III) on goethite in the presence and absence of dissolved organic carbon. Soil Sci Soc Am J 65:1680–1687
42. Grafe M, Eick MJ, Grossl PR, Saunders AM (2002) Adsorption of arsenate and arsenite on ferrihydrite in the presence and absence of dissolved organic carbon. J Environ Qual 31:1115–1123
43. Gadepalle VP, Ouki SK, Van Herwijnen R, Hutchings T (2008) Effects of amended compost on mobility and uptake of arsenic by rye grass in contaminated soil. Chemosphere 72:1056–1061. doi:10.1016/j.chemosphere.2008.03.048
44. Gustafsson JP (2006) Arsenate adsorption to soils: modelling the competition from humic substances. Geoderma 136:320–330. doi:10.1016/j.geoderma.2006.03.046
45. Lin HT, Wang MC, Seshalah K (2008) Mobility of adsorbed arsenic in two calcareous soils as influenced by water extract of compost. Chemosphere 71:742–749. doi:10.1016/j.chemosphere.2007.10.022
46. Sisr L, Mihaljevic M, Ettler V, Strnad L, Sebek O (2007) Effect of application of phosphate and organic manure-based fertilizers on arsenic transformation in soil columns. Environ Monit Assess 135:465–473. doi:10.1007/s10661-007-9666-6
47. Wang SL, Mulligan CN (2006) Effect of natural organic matter on arsenic release from soils and sediments into groundwater. Environ Geochem Health 28:197–214. doi:10.1007/s10653-005-9032-y

48. McLaren RG, Megharaj M, Naidu R (2006) Fate of arsenic in the soil environment. In: Naidu R, Smith E, Owens G, Bhattacharya P, Nadebaum P (eds) Managing arsenic in the environment: from soil to human health. CSIRO, Collingwood, pp 157–182
49. Frankenberger WT, Arshad M (2002) Volatilization of arsenic. In: Frankenberger WT Jr (ed) Environmental chemistry of arsenic. Marcel Dekker, New York, pp 363–380
50. Agely A, Sylvia D, Ma LQ (2005) Mycorrhizae increase arsenic uptake by the hyperaccumulator Chinese brake fern (*Pterisvittata* L.). J Environ Qual 34:2181–2186
51. Fitz WJ, Wenzel WW (2006) Sequestration of arsenic by plants. In: Naidu R, Smith E, Owens G, Bhattacharya P, Nadebaum P (eds) Managing arsenic in the environment: from soil to human health. CSIRO, Collingwood, pp 209–222
52. Jiang QQ, Singh BR (1994) Effect of different forms and sources of arsenic in crop yield and arsenic concentration. Water Air Soil Pollut 74:321–343
53. Adriano DC (2001) Trace elements in terrestrial environments. Biogeochemistry, bioavailability and risks of metals. Springer, New York
54. Anawar HM, Garcia-Sanchez A, Regina IS (2008) Evaluation of various chemical extraction methods to estimate plant-available arsenic in mine soils. Chemosphere 70:1459–1467. doi:10.1016/j.chemosphere.2007.08.058
55. Smith E, Juhasz AL, Weber J (2009) Arsenic uptake and speciation in vegetables grown under greenhouse conditions. Environ Geochem Health 31:125–132. doi:10.1007/s10653-008-9242-1
56. Asher CJ, Reay PF (1979) Arsenic uptake by barley seedlings. Aust J Plant Physiol 6:459–466
57. Meharg AA, Jardine L (2003) Arsenite transport into paddy rice (*Oryza sativa*) roots. New Phytol 157:39–44. doi:10.1046/j.1469-8137.2003.00655.x
58. Hartley-Whitaker J, Ainsworth G, Meharg AA (2001) Copper- and arsenate-induced oxidative stress in Holcus lanatus L. clones with differential sensitivity. Plant Cell Environ 24:713–722. doi:10.1046/j.0016-8025.2001.00721.x
59. Kertulis-Tartar GM (2005) Arsenic hyperaccumulation by *Pteris vittata* L. and its potential for phytoremediation of arsenic contaminated soils. PhD thesis, University of Florida
60. Srivastava M, Ma LQ, Singh N, Singh S (2005) Antioxidant responses of hyper-accumulator and sensitive fern species to arsenic. J Exp Bot 56:1335–1342. doi:10.1093/jxb/eri134
61. Salt DE, Smith R, Raskin I (1998) Phytoremediation. Annu Rev Plant Biol 49:643–668
62. Glass DJ (1999) Economic potential of phytoremediation. In: Raskin I, Ensley BD (eds) Phytoremediation of toxic metals. Wiley, New York, pp 15–31
63. Ma JF, Goto S, Tamai K, Ichii M (2001) Role of root hairs and lateral roots in silicon uptake by rice. Plant Physiol 127:1773–1780
64. Kramer U (2005) Phytoremediation: novel approaches to cleaning up polluted soils. Curr Opin Biotechnol 16:133–141. doi:10.1016/j.copbio.2005.02.006
65. Brooks RR, Lee J, Reeves RD, Jaffre T (1977) Detection of nickeliferous rocks by analysis of herbarium specimens of indicator plants. J Geochem Explor 7:49–57. doi:10.1016/0375-6742(77)90074-7
66. Wild H (1974) Arsenic tolerant plant species established on arsenical mine dumps in Rhodesia. Kirkia 9:265–278
67. Brooks RR, Malaisse F (1985) Heavy metals in pteridophytes. In: The heavy metal-tolerant flora of South-Central Africa: a multidisciplinary approach. A.A. Balkema, Rotterdam
68. Tu C, Ma LQ (2002) Effects of arsenic concentrations and forms on arsenic uptake by the hyperaccumulator ladder brake. J Environ Qual 31:641–647
69. Francesconi K, Visoottiviseth P, Sridokchan W, Goessler W (2002) Arsenic species in an arsenic hyperaccumulating fern, *Pityrogramma calomelanos*: a potential phytoremediator of arsenic-contaminated soils. Sci Total Environ 284:27–35
70. Zhao FJ, Dunham SJ, McGrath SP (2002) Arsenic hyperaccumulation by different fern species. New Phytol 156:27–31

71. Meharg AA (2003) Variation in arsenic accumulation-hyperaccumulation in ferns and their allies. New Phytol 157:25–31
72. Wang HB, Ye ZH, Shu WS, Li WC, Wong MH, Lan CY (2006) Arsenic uptake and accumulation in fern species growing at arsenic-contaminated sites of southern China: field surveys. Int J Phytoremediation 8:1–11. doi:10.1080/16226510500214517
73. Kachenko AG, Bhatia NP, Singh B, Siegele R (2007) Arsenic hyperaccumulation and localization in the pinnule and stipe tissues of the gold-dust fern (*Pityrogramma calomelanos* (L.) Linkvar. *austroamericana* (Domin) Farw. using quantitative micro-PIXE spectroscopy. Plant and Soil 300:207–219. doi:10.1007/s11104-007-9406-2
74. Luu Thai D, Truong P, Mammucari R, Foster N (2014) A Critical Review of the Arsenic Uptake Mechanisms and Phytoremediation Potential of Pteris Vittata. Int J Phytoremediation 16:429–453. doi:10.1080/15226514.2013.798613
75. Salt DE, Smith RD, Raskin I (1998) Phytoremediation. Annu Rev Plant Physiol Plant Mol Biol 49:643–668. doi:10.1146/annurev.arplant.49.1.643
76. Reina SV, Esteban E, Goldsbrough P (2005) Arsenate-induced phytochelatins in white lupin: influence of phosphate status. Physiol Plant 124:41–49. doi:10.1111/j.1399-3054.2005.00484.x
77. Zhang W, Cai Y, Downum KR, Ma LQ (2004) Thiol synthesis and arsenic hyperaccumulation in *Pteris vittata* (Chinese brake fern). Environ Pollut 131:337–345
78. Pickering IJ, Prince RC, George MJ, Smith RD, George GN, Salt DE (2000) Reduction and coordination of arsenic in Indian mustard. Plant Physiol 122:1171–1177. doi:10.1104/pp.122.4.1171
79. Raab A, Feldmann J, Meharg AA (2004) The nature of arsenic-phytochelatin complexes in Holcus lanatus and Pteris cretica. Plant Physiol 134:1113–1122. doi:10.1104/pp.103.033506
80. Kachenko AG, Grafe M, Singh B, Heald SM (2010) Arsenic speciation in tissues of the hyperaccumulator *P. calomelanos* var. *austroamericana* using X-ray absorption spectroscopy. Environ Sci Technol 44:4735–4740. doi:10.1021/es1005237
81. Shetty K, Hetrick B, Figge D, Schwab A (1994) Effects of mycorrhizae and other soil microbes on revegetation of heavy metal contaminated mine spoil. Environ Pollut 86:181–188
82. Weissenhorn I, Leyval C, Belgy G, Berthelin J (1995) Arbuscular mycorrhizal contribution to heavy metal uptake by maize (Zea mays L.) in pot culture with contaminated soil. Mycorrhiza 5:245–251
83. Chaudhry T, Hayes W, Khan A, Khoo C (1998) Phytoremediation-focusing on accumulator plants that remediate metal-contaminated soils. Aust J Ecotoxicol 4:37–51
84. Ka K, Firestone M (1983) Vesicular arbuscular mycorrhizal mediation of grass response to acidic and heavy metal depositions. Plant Soil 72:39–48
85. Schüepp H, Miller DD, Bodmer M (1987) A new technique for monitoring hyphal growth of vesicular-arbuscular mycorrhizal fungi through soil. Trans Br Mycol Soc 89:429–435
86. El-Kherbawy M, Angle J, Heggo A, Chaney R (1989) Soil pH, rhizobia, and vesicular-arbuscular mycorrhizae inoculation effects on growth and heavy metal uptake of alfalfa (Medicago sativa L.). Biol Fertil Soils 8:61–65
87. Heggo A, Angle J, Chaney R (1990) Effects of vesicular-arbuscular mycorrhizal fungi on heavy metal uptake by soybeans. Soil Biol Biochem 22:865–869
88. Meharg A (1994) Integrated tolerance mechanisms: constitutive and adaptive plant responses to elevated metal concentrations in the environment. Plant Cell Environ 17:989–993
89. Wright W, Fitter A, Meharg A (2000) Reproductive biomass in Holcus lanatus clones that differ in their phosphate uptake kinetics and mycorrhizal colonization. New Phytol 146:493–501
90. Fitz WJ, Wenzel WW (2002) Arsenic transformations in the soil–rhizosphere–plant system: fundamentals and potential application to phytoremediation. J Biotechnol 99:259–278
91. Al Agely A, Sylvia DM, Ma LQ (2005) Mycorrhizae Increase Arsenic Uptake by the Hyperaccumulator Chinese Brake Fern (L.). J Environ Qual 34:2181–2186

92. Leyval C, Turnau K, Haselwandter K (1997) Effect of heavy metal pollution on mycorrhizal colonization and function: physiological, ecological and applied aspects. Mycorrhiza 7:139–153
93. Singh N, Ma LQ (2007) Assessing plants for phytoremediation of arsenic-contaminated soils. Phytoremed Methods Rev 319–347
94. Wang J, Zhao F-J, Meharg AA, Raab A, Feldmann J, McGrath SP (2002) Mechanisms of arsenic hyperaccumulation in Pteris vittata. Uptake kinetics, interactions with phosphate, and arsenic speciation. Plant Physiol 130:1552–1561
95. Tu S, Ma L, MacDonald G, Bondada B (2004) Effects of arsenic species and phosphorus on arsenic absorption, arsenate reduction and thiol formation in excised parts of Pteris vittata L. Environ Exp Bot 51:121–131
96. Santos JA, Gonzaga MI, Ma LQ, Srivastava M (2008) Timing of phosphate application affects arsenic phytoextraction by Pteris vittata L. of different ages. Environ Pollut 154:306–311
97. Lessl JT, Ma LQ (2013) Sparingly-soluble phosphate rock induced significant plant growth and arsenic uptake by Pteris vittata from three contaminated soils. Environ Sci Technol 47:5311–5318. doi:10.1021/es400892a
98. Luikham E, Lhungdim J, Singh AI (2005) Influence of sources and levels of phosphorus on growth and yield of green gram (Vigna radiata L. Wilczek). Legume Res Int J 28:59–61
99. Srivastava S, D'Souza SE, Sen U, States JC (2007) In utero arsenic exposure induces early onset of atherosclerosis in ApoE−/− mice. Reprod Toxicol 23:449–456
100. Chavan P, Shinde V, Kote G, Solunke P, Bhondve A (2008) Response of sources and levels of phosphorus with and without PSB inoculation on growth, yield and quality of soybean. Res Crops 9:286–289
101. Raskar B (1999) Effect of source and level of phosphorus on the yield of sorghum (Sorghum bicolor) under dryland conditions. Indian J Agron 44:760–764
102. Kidd P, Mench M, Alvarez-Lopez V, Bert V, Dimitriou I, Friesl-Hanl W, Herzig R, Janssen JO, Kolbas A, Mueller I, Neu S, Renella G, Ruttens A, Vangronsveld J, Puschenreiter M (2015) Agronomic practices for improving gentle remediation of trace element-contaminated soils. Int J Phytoremediation 17:1005–1037. doi:10.1080/15226514.2014.1003788
103. Wan C, Wang Y, Wang N, Norimatsu W, Kusunoki M, Koumoto K (2016) Development of novel thermoelectric materials by reduction of lattice thermal conductivity. Sci Technol Adv Mater 11(4):044306

Chapter 20
Phytoremediation of Boron-Contaminated Sites

Consuelo d.P. Rámila, Gonzalo E. Pizarro, and Carlos Alberto Bonilla

20.1 Introduction

Boron is essential for plant growth [1] but it is required only in trace concentrations as a result of its high toxicity [2]. Boron toxicity produces different symptoms, including leaf burn-chlorotic and/or necrotic patches often at the margins and tips of older leaves [2], and reduced root and shoot elongation [3, 4]. Many countries present toxic levels of boron in their soils and water, especially in arid and semiarid regions [5]. Boron toxicity has been reported in South Australia, the USA, Turkey, Iraq, Syria, Italy, and Chile, among others [5]. This limits agriculture activities by reducing crop yields [6] and the variety of species that can be cultivated. Unfortunately, current technologies for treating boron-polluted soils are not cost-effective and therefore, new technologies are needed. Boron in soils cannot be easily removed owing to its sorption onto soil surfaces [7]. The most common practice is leaching [8], but it presents several limitations that prevent its use in arid and semiarid regions. Other treatments include replacing the surface soil with low-boron soil, and adding lime to increase boron sorption onto soil surfaces. However, these treatments are short-term solutions and also present several disadvantages [9].

As current practices to remediate high-boron soils are often ineffective or impracticable, researchers are exploring alternative technologies, such as phytoremediation. Phytoremediation is the use of plants and associated soil microbes to reduce the concentration or toxic effects of contaminants in the environment. It has the advantage of being potentially cost-effective, efficient, and environmentally friendly [10]. During the last two decades, different phytoremediation strategies for boron-polluted sites have been investigated. These can be divided into three main groups: phytoextraction, phytorestoration, and phytomanagement. The aim of this

---

C.d.P. Rámila • G.E. Pizarro • C.A. Bonilla (✉)
Department of Hydraulic and Environmental Engineering, Pontificia Universidad Católica de Chile, Santiago, Chile
e-mail: cbonilla@ing.puc.cl

A.A. Ansari et al. (eds.), *Phytoremediation*, DOI 10.1007/978-3-319-40148-5_20

chapter is to review the current knowledge on phytoremediation of boron-polluted sites. First, the background of the boron toxicity problem is exposed. Then, we present the studies done to date in boron phytoextraction and discuss the main factors affecting boron uptake by plants and different strategies to enhance it (i.e., adding chelates, microorganisms' inoculation, or genetically modifying plants). We address the phytomanagement of boron-contaminated sites using poplars, which can prevent leaching while extracting boron. Finally, we present a study of the potential of a Gramineae species to restore soil properties in a boron-contaminated mine.

20.2 Boron in the Environment

Boron (B) is a metalloid widely distributed in the lithosphere and hydrosphere, in spite of its low abundance [11]. In nature, boron is always found bound to oxygen and occurs mainly as boric acid (H_3BO_3) and its salts (borates) or as borosilicate minerals [12]. There are at least 200 minerals that contain boron [13]. The average soil boron concentration ranges from 10 to 20 mg/kg, and freshwater boron concentration is normally between less than 0.01 and 1.5 mg/L. Seawater has an average boron concentration much higher than fresh water (4.6 mg/L [14]).

20.2.1 Biogeochemical Cycle of Boron

Boron enters the environment in a variety of ways (Fig. 20.1). Natural processes represent the main pathway of boron release and include: (1) weathering of boron-containing minerals [13], (2) volcanic activity, which can release boron to the atmosphere that can be afterward introduced to the environment through precipitation events [13], and (3) codistillation of boron from seawater to the atmosphere [15].

Anthropogenic activities may also greatly contribute to boron environmental cycles. The main human activities that mobilize boron are mining, biomass burning, and fossil fuel combustion [16]. Also, leaching of treated wood/paper, sewage, and sludge disposal [17], as well as wastes originating from industrial applications such as manufacturing of glass and porcelain, fertilizers, herbicides, and detergents, contribute to the entrance of boron to the environment [2].

20.2.2 Boron Mobility in Water and Soil

Boric acid (H_3BO_3), a relatively unreactive molecule, is the main boron species in water and soil solution at neutral pH. It is very soluble (55 g/L at 25 °C [13]) and a weak Lewis acid (pKa 9.24 at 25 °C [14]). Only at very high concentrations (>216 mg/L) and at high pH it forms polynuclear ions or ring structures [13]. Boric

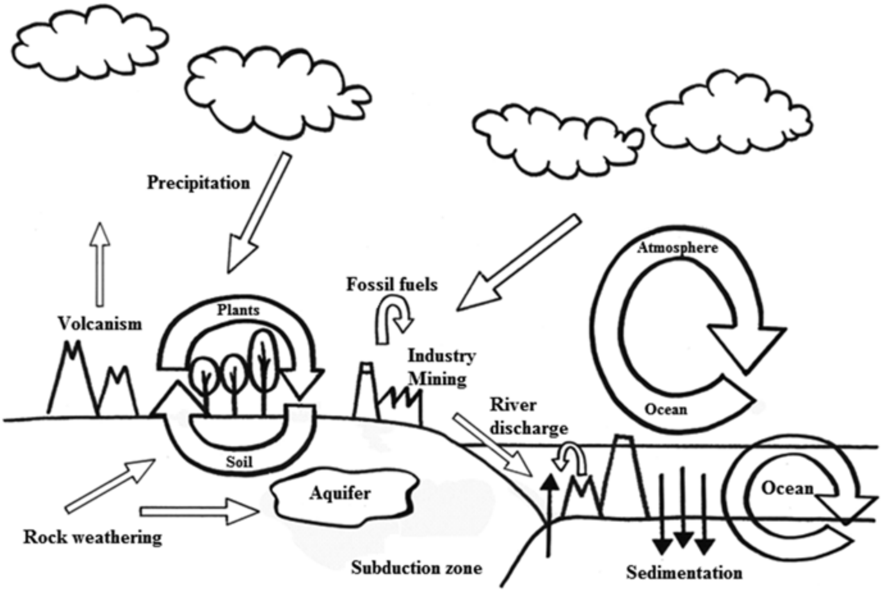


Fig. 20.1 Biogeochemical cycle of boron, redrawn from Kot (2009)

acid volatilization is not significant at ambient temperatures; however, it co-evaporates with moisture to form borate aerosols in the atmosphere [15]. Boric acid neither undergoes oxidation-reduction reactions nor precipitates with the majority of cations, nevertheless it coprecipitates with calcium carbonate at high concentrations [13].

Boron mobility in soil is controlled fundamentally by sorption–desorption processes (Fig. 20.2), because boric acid is not a very reactive molecule, and boron minerals are in general too soluble or insoluble [18]. Boron sorption onto soil depends on the soil's composition. Aluminum and iron oxides, organic matter, clay minerals, and, to a lesser extent, calcium carbonate are the main constituents involved in boron adsorption by soils [7]. The principal mechanism of boron adsorption is ligand exchange with hydroxyl groups on the surface. Soil parameters that control boron adsorption are temperature, ionic strength, humidity content, and pH [19]. Soil pH is the most important [7]; boron adsorption increases in soil with pH in the range of 3–9 and decreases at pH over 10–11.5 [18].

20.3 Boron and Plants

Boron is an essential micronutrient for plant growth and is required at different concentrations depending on the species and stage of development [20]. The main role of boron in plants is the cross-linking of the cell wall rhamnogalacturonan II

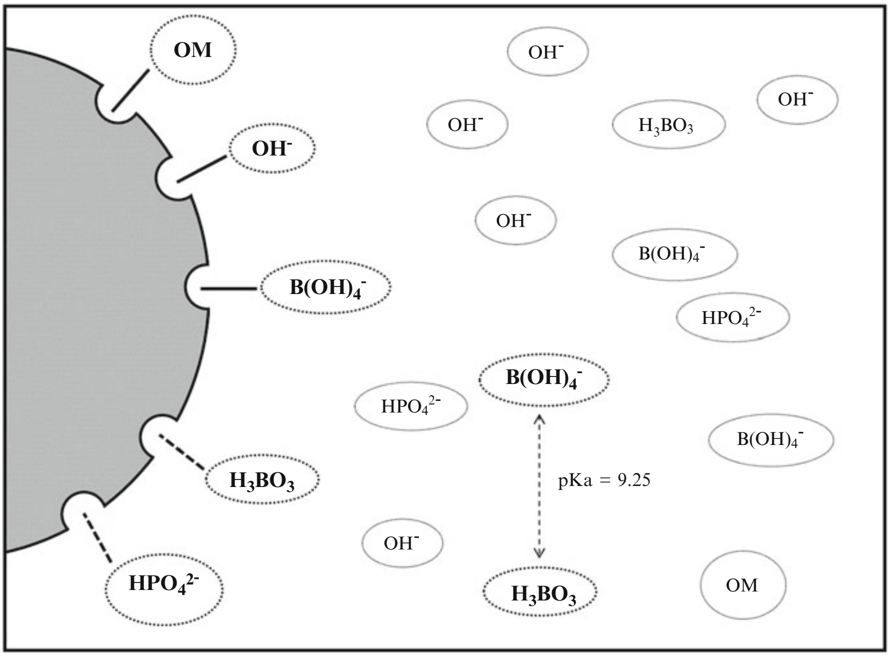


Fig. 20.2 Schematic representation of boron adsorption onto soil surfaces. The main species of boron in soil solution are boric acid and borate, whose relative abundance depends on the soil solution pH (pKa 9.25). The main mechanism of boron adsorption is ligand exchange with hydroxyl groups on the surface. Both H_3BO_3 and $B(OH)_4^-$ are adsorbed onto soil surfaces, but borate presents a stronger affinity than boric acid. Organic matter (OM) can adsorb boron, but it may also occlude or compete for adsorption sites. Phosphate may also compete with boron in some soils

and pectin assembly, which is essential for the cell wall structure and function [21]. Boron deficiency can cause sterility and necrosis of meristematic tissue, among other symptoms [1]. However, boron is required only in trace concentrations as a result of its high toxicity. Sensitive species show severe toxicity symptoms when the available boron concentration in soil exceeds 3 mg/kg [22], and when boron concentrations in irrigation water are over 0.5 mg/L [23]. In most plants, leaves with boron concentrations higher than 250 mg/kg DW exhibit symptoms of toxicity [24] while sufficient boron levels are 20 mg/kg [25]. Toxic levels of boron inhibit photosynthesis [26–29] and may cause general oxidative stress to membranes or other cell compartments [17]. Visible boron toxicity symptoms include leaf burn-chlorotic and/or necrotic patches often at the margins and tips of older leaves [2] and reduced root and shoot elongation [3, 4]. High boron concentrations in soils can significantly reduce crop yield [6].

Although high boron concentration is toxic for most plants, some species are able to resist it. Plants can do this through two main strategies: lowering boron uptake and/or harboring internal tolerance mechanisms. Mechanisms that decrease boron uptake in plants include: (a) lowering the expression of membrane channels involved in boron uptake [30] and (b) active efflux pumping of boron back to the medium [31, 32]. Described internal tolerance mechanisms are: (a) compartmentation of boron in vacuoles, e.g., by tonoplast intrinsic proteins [33], (b) active efflux of boron from the intracellular phase into the apoplast [34], and (c) effective antioxidant response [35–37].

20.4 Boron Toxicity

Many countries throughout the world present toxic levels of boron in their soils and water, especially in arid and semiarid regions [5]. Boron toxicity limits agriculture activities in these areas because it is toxic to plants, and therefore it reduces crop yields [6] and the variety of species that can be cultivated. High levels of boron in soil occur naturally in places such as those originated from marine sediments and volcanic activities. Also, mining, industrial activities (e.g., the production of glass, ceramics, and detergents), overfertilization, and especially irrigation with water containing high levels of boron have led to boron toxicity problems in some sites [2]. Boron toxicity has been reported in Australia [38], West Asia and North Africa [39], Turkey [40, 41], Italy [42], the USA [43], Brazil [44], Argentina [45], and Chile [46].

Boron toxicity in soils cannot be easily treated owing to its interaction with soil surfaces (Fig. 20.2). The most common practice is leaching [8]. This old practice is still applied in farming for soil recovery [9], but it presents several limitations: (1) leaching requires large volumes of water as a result of boron adsorption onto soil surfaces [47], which is impractical in arid and semiarid regions where little water is available; (2) because the washing fraction should not be so large as to result in the loss of soil nutrients that are essential for plant growth [9], it may not be possible to leach boron in highly contaminated sites without affecting the soil nutritional status; (3) the adsorbed boron fraction of the soil is often not removed, therefore it can recharge the soil solution later [48]; and (4) soils must have good drainage capacity [2, 43]. In addition, this method can lead to the contamination of adjacent sites and groundwater if boron-enriched leachates are not properly collected [49]. Boron toxicity in soils can also be treated by adding lime to increase soil pH and therefore boron adsorption [50], but this is a short-term solution and highly saline soils cannot be treated this way. Finally, surface soil can be replaced by soils with low boron levels, but this treatment is expensive and boron from subsurface layers can migrate with water evaporation and recharge non-contaminated soil [9]. As current practices to remediate high-boron soils are often ineffective or impracticable, researchers are exploring two alternative strategies: (1) enhance crops' tolerance to boron, and (2) use plants to phytoremediate boron-contaminated sites [51].

20.5 Boron Phytoremediation

A promising technology for treating contaminated matrices is phytoremediation. Phytoremediation is the use of plants and associated soil microbes to reduce the concentration or toxic effects of contaminants in the environment [52]. Phytoremediation presents several advantages over physical and chemical remediation. It is an in situ, passive, solar-driven technique, and therefore is less expensive. It is also aesthetically pleasing, environmentally friendly, and offers benefits to contaminated sites [53]. In these sites, it increases microbial activity, reduces soil erosion, decreases dust formation, and, as plants may transpire large amounts of water, they may prevent downward migration of contaminants by percolation [54]. Phytoremediation also sequestrates carbon, and its by-products can be utilized in many ways [55, 56]. During the last two decades, scientists have studied tolerant plants to solve boron toxicity problems through three main phytoremediation strategies: phytoextraction, phytomanagement, and phytorestoration (Table 20.1). The use of constructed wetlands and aquatic plants to remove boron from water has also been studied (e.g., 57–59), but this topic is beyond the scope of this chapter.

Table 20.1 Plant species studied for phytoremediation of boron-contaminated sites

Plant species	Strategy of phytoremediation	Type of experiment	Reference
Brassica juncea, *Festuca arundinacea*, and *Brassica napus*	Phytoextraction	Pot experiments	Bañuelos et al. [49]
Brassica juncea, *Festuca arundinacea*, *Lotus corniculatus*, and *Hibiscus cannabinus*	Phytoextraction	Field trials	Bañuelos et al. [63]
Festuca arundinacea	Phytoextraction	Field trials	Bañuelos [62]
Salsola soda	Phytoextraction	Pot experiments	Centofanti and Bañuelos [65]
Helianthus annuus, *Zea mays*, and *Brassica juncea*	Phytoextraction	Pot experiments	Tassi et al. [42]
Brassica juncea	Phytoextraction	Lysimeter	Giansoldati et al. [66]
Brassica juncea, *Raphanus sativus*, *Amaranthus cruentus*, and *Hybiscus cannabinus*	Phytoextraction	Lysimeter	dos Santos et al. [68]
Sorghum halepense, *Cyperus rotundus*, *Cynodon dactylon*, *Amaranthus retroflexus*, *Echinochloa crus-galli*, and *Chenopodium album*	Phytoextraction	Pot experiments	Aydın and Çakır [72]
Chrysopogon zizanioides	Phytoextraction	Pot experiments	Smolcz and Cortés [78]
Brassica napus	Enhanced phytoextraction	Field trials	Esringü et al. [80]

(continued)

Table 20.1 (continued)

Plant species	Strategy of phytoremediation	Type of experiment	Reference
Meadow plant species	Enhanced phytoextraction	Field trials	Gullap et al. [81]
Zea mays and *Helianthus annuus*	Enhanced phytoextraction	Pot experiments	Turan and Angin [82]
Vetiveria zizanioides	Enhanced phytoextraction	Pot experiments	Angin et al. [83]
Nicotiana glauca (transgenic line)	Enhanced phytoextraction	Pot experiments	Martínez et al. [85]
Brassica juncea (three transgenic lines)	Enhanced phytoextraction	Pot experiments	Bañuelos et al. [86]
Populus deltoides	Phytomanagement	Lysimeter	Robinson et al. [84]
Populus deltoides	Phytomanagement	Field trials	Robinson et al. [84]
Populus euramericana x Populus yunnanensis	Phytomanagement	Lysimeter	Robinson et al. [93]
Populus deltoides x yunnanensis, *Populus euramericana x yunnanensis*, and *Populus alba x glandulosa*	Phytomanagement	Field trials	Robinson et al. [93]
Populus canadensis	Phytomanagement	Field trials	Rees et al. [95]
Puccinellia distans	Phytorestoration	Pot experiments	Stiles et al. [43]
Gypsophila sphaerocephala, *Gypsophila perfoliata*, *Puccinellia distans*, and *Elymus elongatus*	Hyperaccumulation	Field observations	Babaoglu et al. [70]
Puccinellia distans and *Gypsophila arrostil*	Hyperaccumulation	Hydroponic tests	Stiles et al. [43, 51]
Puccinellia distans, *Gypsophila perfoliata*, *Isatis glauca*, *Elymus elongatus*, *Glaucium corniculatum*, *Alyssum sibiricum*, *Polygonum equisetiforme*, *Chenopodium album*, and *Tamarix tetrandra*	Hyperaccumulation	Field observations	Bocuk and Ture [71]
Puccinellia frigida	Hyperaccumulation	Field observations	Rámila et al. [75]
Puccinellia frigida	Hyperaccumulation	Hydroponic tests	Rámila et al. [77]

20.5.1 *Phytoextraction of Boron*

Phytoextraction consists of planting a tolerant plant species that can take up a contaminant and translocate it to its upper parts. The contaminant is then removed by harvesting the plant shoots. The plant species used are often hyperaccumulators

[60]. Phytoextraction is the most widely studied phytoremediation strategy for boron toxicity, and has been investigated in different countries with promising results (e.g., USA, Italy, Brazil, Turkey, and Chile).

20.5.1.1 Phytoextraction Tests

In the west side of central California, USA, more than 200,000 ha of the land present high levels of boron and salinity in its soils [61]. This negatively impacts agronomic production, and therefore its management has been studied during the past decades. A series of boron phytoextraction assays has been performed in this region [49, 62–64]. In 1993, the ability to phytoextract boron from contaminated soil in Fresno was studied in pot experiments (90–160 days) using Indian mustard (*Brassica juncea*), tall fescue (*Festuca arundinacea*), and canola (*Brassica napus*). Extractable soil boron concentrations were lowered at least 32 % by all the species (initial concentration = 3 mg/kg). It was concluded that planting any of them could reduce water-extractable B in the soil [49].

In field experiments conducted in Los Baños (west side of central California), four species were evaluated for boron phytoextraction purposes: Indian mustard, tall fescue, birdsfoot trefoil (*Lotus corniculatus*), and kenaf (*Hibiscus cannabinus*). In less than 1 year, the initial extractable soil boron concentration in the 0–60 cm stratum (that ranged from 1 to 10 mg/kg) was reduced by 52 % [63]. The mean boron concentration in shoots of the four plant species ranged from 96 to 684 mg/kg DW, reaching its highest value in kenaf foliage. The researchers concluded that planting any of these four species may reduce boron concentrations in the studied soil.

Later in Los Baños, a 1.5-year field experiment was performed using tall fescue for boron phytoextraction [64]. Tall fescue successfully tolerated the high native soil boron concentration (5 mg/L of water-extractable boron) and reduced it by 35 %. Control bare plots treated under the same irrigation regime did not exceed 13 % of reduction. It was also observed that the mass of boron extracted by plants exceeded the theoretical one that should have been removed from soil. This could be explained because additional amounts of boron would have been made available for plant uptake. Also, the amount of boron extracted decreased during the 2nd year, which may be because it becomes more difficult for plants to extract available boron after the more easily extractable fraction has already been removed. The researchers concluded that planting tall fescue alone may not be the long-term solution for removing boron. Nevertheless, it could be employed as one component of an overall strategy to lower extractable soil native boron levels in agricultural soils, and reduce leaching of boron into shallow groundwater [64].

Recently, Centofanti and Bañuelos [65] studied the potential of agretti (*Salsola soda*) to phytomanage boron in Fresno (CA, USA). In pot experiments, they found that this alternative crop resisted high boron concentrations in the soil (10 mg/L of water-extractable boron), and also accumulated high boron levels in its shoots without symptoms of toxicity (100 mg/kg DW in 4 weeks). They concluded that agretti has the potential to be grown in boron-laden saline soils and manage excessive soluble boron in soil.

In the Cecina River basin, Italy, the residues of boron-related mine activities were spilled into its two tributaries for more than 150 years. This led to boron contamination that is still present in some areas of the basin [42]. To assess the potential for treating this area through boron phytoextraction, boron uptake was studied using different plant species (*Helianthus annuus*, *Zea mays*, and *B. juncea*).

In pot experiments, *B. juncea* removed 45 % of the initial available boron concentration (40 mg/kg) from the contaminated soil after two consecutive growing cycles, which was considered a promising result [42]. Boron phytoextraction using *B. juncea* was later studied on a larger scale (lysimeter), and the effect of nitrogen fertilization was also assessed [66]. Fertilizing the contaminated soil (that had originally low amounts of nitrogen) significantly enhanced boron phytoextraction (the total boron phytoextracted increased threefold). Nitrogen fertilization increased the biomass production while maintaining boron uptake levels unaltered in the shoots of *B. juncea*. It was concluded that nitrogen fertilization improved boron phytoextraction, and that phytoextraction could reduce boron levels in the sediments of the Cecina River basin and limit its diffusion through the environment [66, 67].

In Piracicaba, Sao Paulo, Brazil, three hectares of an agricultural soil were contaminated with heavy metals and boron as a result of an accident with fertilizer's raw material. In order to test the potential of mustard, kenaf, turnip (*Raphanus sativus*), and amaranth (*Amaranthus cruentus*) to phytoextract boron from this area, pot experiments were conducted [68]. Considering four cycles per year and a constant rate of boron uptake, all the species tested would remove 50 % of boron in a soil with initial available concentration of 3.8 mg/kg in less than 1 year. *Ricinus communis* was also tested for its ability to remove boron from this area [44]. In pot experiments, this species accumulated 626 mg/kg DW in its shoots in 74 days. Factoring one growing cycle per year, the time required to phytoextract 50 % of the initial boron concentration was calculated to be between 12 and 16 years. To our knowledge, field experiments have not been conducted.

Boron toxicity is also a major problem in Turkey. Turkey possesses most of the boron reserves in the world, and it is one of the greatest borate suppliers [40]. Within a region of 3.5 million ha of cultivated land, 18 % of soils presented boron levels above the concentration considered toxic for most crops [69]. To tackle this problem, some researchers have looked for phytoremediator candidate species in different contaminated places. Babaglou et al. [70] searched for tolerant species in an actively boron-mined area of Kõrka. *Gypsophila sphaerocephala*, *Gypsophila perfoliata*, *Puccinellia distans*, and *Elymus elongatus* were found growing in an area that presented extremely high total and available soil boron concentrations (8900 mg/kg and 227 mg/kg, respectively). *G. sphaerocephala* presented the highest boron concentration in its leaves (3345 mg/kg DW). It was concluded that the two *Gypsophila* species found could be considered to be hyperaccumulators, and that their phytoremediation potential should be further studied. The tolerance and accumulation of *P. distans* and *Gypsophila arrostil* were later investigated under controlled conditions in hydroponic experiments [51]. *G. arrostil* was found to be moderately tolerant to boron whereas *P. distans* suffered no significant reduction in plant biomass until boron concentration was higher than 250 mg/L, which made it one of the most boron-tolerant plants reported.

Recently, Bocuk and Ture [71] searched for potential boron-phytoremediator plant species in seven open boron-mine sites located in four different provinces of Turkey. They found nine wild terrestrial plant species with potential for boron phytoremediation. Of these, *P distans* and *G. perfoliata* showed the greatest tolerance (45 mg/kg of available boron in soil) and the highest boron accumulation in shoots (270 mg/kg DW and 267 mg/kg DW, respectively), which was consistent with the study by Babaglou et al. [70]. An attempt was made to study boron phytoextraction in Turkey by Aydin and Cakir [72]. In pot experiments, the effect of increasing boron application on the growth and uptake of common weed species (*Sorghum halepense*, *Cyperus rotundus*, *Cynodon dactylon*, *Amaranthus retroflexus*, *Echinochloa cruss-galli*, and *Chenopodium album*) was studied. Unfortunately, they found that none of these species had potential for boron phytoextraction.

Northern Chile naturally presents high levels of boron in soils and water [73–75]. Solubilization of natural deposits of borate salts via weathering and geochemical transformations in the soil, lead to boron migration into rivers and groundwater in this area. The use of these boron-rich waters to irrigate agricultural soils contributes most significantly to the deposition of boron [76]. Recently, phytoremediation has been seen as a possible alternative for managing boron-contaminated soils in Northern Chile. Rámila et al. [75] found a boron hyperaccumulator plant able to resist extremely high concentrations of boron available in soil (>4000 mg/kg), water (>800 mg/L), and shoots (>5000 mg/kg DW). This Chilean native plant species (*Puccinellia frigida*) is one of the most boron-tolerant species reported and could have potential for phytoextraction purposes (Figs. 20.3 and 20.4). *P. frigida*'s tolerance to different levels of boron in hydroponic culture was later explored. We found that

Fig. 20.3 Hydrothermal springs in the Colpitas River basin, Northern Chile. Soils and water present extremely high boron concentrations (>4000 mg/kg and 800 mg/L, respectively). Soils in this area are strongly alkaline (pH 8.4–8.9) and present high salinity levels (EC: 4.9–8.8 mS/cm), conditions often present in boron-contaminated sites

Fig. 20.4 *Puccinellia frigida* specimens growing under extremely toxic conditions in Northern Chile. This species is one of the most boron-tolerant species reported

P. frigida resisted extremely high boron concentrations in the medium (>500 mg/L) and accumulate it in its shoots, and therefore it had potential for phytoremediation purposes [77]. Nevertheless, the rate of biomass production should be further investigated to confirm its phytoextraction potential.

In Arica (Northern Chile), a phytoextraction test was recently performed in pot experiments using vetiver grass (*Chrysopogonzizanioides*) [78]. Plants irrigated for three months with water with different boron levels efficiently removed boron. Vetiver grass showed up to 96 % of efficiency, and it accumulated more than 300 mg/kg DW in shoots without symptoms of toxicity. Smolcz and Cortés concluded that this technology could be a good alternative for lowering boron in soils across more than 3000 ha in the Lluta Valley.

20.5.1.2 Enhanced Phytoextraction

A major disadvantage of phytoextraction is the large amount of time required to remove a contaminant [79]. In order to reduce this time, different strategies have been investigated: (1) inoculating microorganisms to increase soil boron availability and plant biomass, (2) adding chelates to increase soil boron availability, and (3) increasing the tolerance and accumulation of boron in plants by genetically modifying them.

Adding soil microorganisms and plant root-associated bacteria may enhance the bioavailability of metals and also increase biomass production [80]. Recently, the effect of adding *Bacillus megaterium* (a plant growth-promoting rhizobacteria) on the boron phytoremediation potential of *B. napus* was studied in field experiments [80]. Adding *B. megaterium* increased the boron availability in soil and the boron accumulation in *B. napus*, while also ameliorating the negative effects of toxicity in the dry mass yield. It was concluded that the addition of *B. megaterium* may increase phytoremediation efficiency. In another study, the addition of *B. megaterium* also increased the boron availability in soil and the boron accumulation by meadow plants, but it had no effect on the plants' biomass yield [81].

Bioavailability of metals in soil can also be increased by adding chelates. The effect of organic chelates in boron availability was studied by Turan and Angin [82], and Angin et al. [83]. In pot experiments, humic acid addition increased the availability of boron, and also the boron content in shoots and roots of the studied species (*Z. mays* and *H. annuus*). Therefore, humic acid addition could facilitate boron phytoextraction [82]. Later, the effect of adding humic acid to a contaminated soil on boron phytoextraction was investigated using vetiver grass [83]. In pot experiments, with initial extractable boron concentrations that ranged from 0.85 to 44.75 mg/kg, humic acid addition increased boron removal. The effect depended on the initial level of boron contamination and the amount of humic acid applied.

The use of genetically modified plants to improve biomass production and increase the tolerance and accumulation of high-biomass plants has potential for phytoremediation purposes [84]. Transgenic plants have been rarely studied for boron phytoremediation. To the best of our knowledge, only two studies address this issue. In 2005, *Nicotiana glauca* transformed with a gene encoding a phytochelatin synthase (*TaPCS1*) was tested for its application in phytoremediation in Spain [85]. In pot experiments using different mine soils (contaminated with various heavy metals and boron), the lines overexpressing *TaPCS1* presented a higher biomass production. In some soils, they also exhibited more than three times the boron concentration found in wild-type plants. It was concluded that these plants could become a good alternative for remediating boron and metals in highly contaminated soils. In a field study, three transgenic Indian mustard lines were tested for their ability to remove selenium from a selenium- and boron-contaminated saline sediment [86]. These lines overexpressed genes encoding the enzymes adenosine triphosphate sulfurylase, ç-glutamyl-cysteine synthetase, and glutathione synthetase. Although every transgenic line significantly increased selenium uptake compared to the wild type, boron uptake was lower in all of them. Therefore, the overexpression of any of these enzymes did not enhance the boron-phytoextraction potential of *B. juncea*.

20.5.1.3 Environmental Conditions That Affect Boron Uptake

Different soil and site conditions influence boron uptake by plants. Plants can absorb only the bioavailable fraction of boron in the soil. As discussed in Sect. 20.2.2, boron availability in the soil will be determined by the soil's composition (i.e., types and amount of minerals and organic matter), temperature, ionic strength, humidity content, and pH [19]. Plants absorb boron only in its uncharged form (H_3BO_3, pKa 9.25 (25 °C) [14]), whose amount will be determined by the soil's pH. Salinity also affects boron uptake by plants [87–89]; temperature, light intensity, wind velocity, soil properties, and humidity determine transpiration, which strongly influences boron uptake [90], and this also depends on the exposure time [91].

20.5.2 Phytomanagement of Boron-Contaminated Sites

Phytomanagement is the use of plants and soil amendments to reduce the environmental risk posed by contaminated sites [62]. Ideal candidates to be used in this technology are poplars (*Populus* sp.) because they are perennial, fast-growing, produce large amounts of foliage, have an extensive and deep root system [92], and are tolerant of different metals and metalloids. Poplars can pump large amounts of water by transpiration, which prevents the percolation of rainwater, and control the pollutant migration from shallow aquifers. Poplars are highly tolerant of boron [84], and therefore could be used to phytomanage boron-contaminated places. During the last decade, some researchers have studied the phytomanagement of boron-contaminated sites using poplar trees [84, 93–95]. All of them agree in that these trees might be useful in reducing leaching by evapotranspiration while also extracting boron from the contaminated sites.

In New Zealand, phytoremediation studies using poplars have been performed in a wood-waste site in North Island called the Kopu site. In this 3.6-ha site, sawdust and yard scrapings from timber milling had been dumped for 30 years, which led to boron contamination. Leachate resulting from the annual rainfall frequently entered the local stream, elevating boron concentrations over the drinking water standard (1.4 mg/L). In the year 2000, the pile was planted with two *Populus deltoids* hybrid clones (7000 trees/ha) and lysimeter assays were performed [84]. Using a computer model, with experimental data as input, Robinson et al. estimated that poplars would substantially reduce leaching during the summer months when trees are fully leafed and transpire at their highest capacity. In addition, poplars accumulated high amounts of boron in shoots (700 mg/kg DW). Therefore, poplars would both control leaching and reduce boron loading via phytoextraction. As expected, boron concentration in the drainage water from lysimeter assays decreased during the course of the experiment (from 2.2 to ~0.5 mg/L, after 2 years). The estimated cost of capping the site was six times higher than using phytoremediation, and would need ongoing maintenance to ensure its integrity. This study showed that phytoremediation could be a cost-effective solution to reducing boron leaching from the Kopu site. Nevertheless, to obtain robust predictions of the efficacy of this system, further investigation was required.

A 3-year study in the Kopu site was performed by Robinson et al. [93]. In this study, in situ and lysimeter assays were performed to assess the effect of planting three varieties of poplars on the boron concentration and amount of the leachate from the site. Leaching was substantially reduced and *Populus euramericana* x *yunnanensis* leaves reached a boron concentration of 1000 mg/kg DW. Because the poplars' leaves accumulated high amounts of boron, their eventual decomposition would release it, hence, it was concluded that coppicing must be considered. According to simulations, annually removing 50 % of the poplars' above biomass would only slightly increase the leaching from the site, but it would remove high amounts of leachable boron (1 kg/ha). The researchers concluded that phytomanagement using poplars would be most effective when trees are partially coppiced and where effluent is re-irrigated onto trees.

Boron accumulation and tolerance of poplars in a boron-laden mixed paper mill waste landfill in the USA was recently reported by Rees et al. [95]. This landfill (Pine Lake Landfill, Wisconsin, USA) was planted with four poplar clones of *Populus canadensis* for 39 months. Total boron concentration in the landfill ranged from 60 to 300 mg/kg, and available boron concentration was between 6.1 and 23 mg/kg. All clones accumulated high boron concentrations in their leaves (up to 1020 mg/kg DW). Also, the survival and growth of trees were in the range of normal performance, despite the fact that some leaves showed signs of toxicity. The researchers concluded that poplars are useful in establishing vegetation in this area for the purpose of stabilizing soil and controlling trace element leaching.

20.5.3 Phytorestoration of a Boron-Contaminated Mine

Phytorestoration consists of restoring a polluted place by revegetating it with an adequate tolerant plant species. Plants increase the soil's organic matter content and thereby renovate soil properties. Also, plant roots support the growth of microbes, including arbuscular mycorrhizal fungi, which can help the establishment of other subsequent species. With this approach, a diverse and completely sustainable ecosystem could be developed in an initially contaminated place [43].

Phytorestoration of a boron-contaminated place using *P. distans* was investigated in a mine in the USA [43]. In pot experiments using the mine soil (with some amendments), the species was able to germinate and survive, and it was proposed as a possible initial vegetation cover for the mine in the study. It was hypothesized that planting this species would restore soil properties, and thus allow the subsequent colonization of the mine with native plant species. This would eventually allow natural succession to occur. The capability of the species to grow successfully under the environmental conditions of the actual mining site has not been reported.

20.6 Conclusions and Perspectives

Phytoremediation of boron-contaminated sites is a promising alternative to conventional treatments, which are often too expensive or impractical to be applied in arid and semiarid regions. Various pot and field experiments demonstrate that plants can significantly reduce available boron concentration in conditions of low-to-moderate soil contamination, in relatively short periods of time. Also, poplars can be used to adequately phytomanage contaminated sites, preventing the leaching of contaminated water. Despite the promising results, there are several issues that still need to be addressed before boron phytoremediation can be considered a real alternative. As is common in the phytoremediation of metals and metalloids, there is a lack of long-lasting field studies and, as a consequence, uncertainty in the long-term effectiveness of boron phytoremediation. For example, a short-term phytoextraction field experiment indicates that boron uptake by plants is unlikely to remain constant over time, and also, that the initial available boron concentration in a soil would not be the sole target, but that some adsorbed boron that may be released as phytoextraction progresses may be targeted as well [64]. The simple extrapolation of a boron removal rate estimated in a short-term field experiment will probably not reflect what will actually occur in the polluted site in the long term.

Another critical point in evaluating the feasibility of phytoremediation is estimating the costs involved. One should consider the costs of planting, field management, agricultural practices, and also the cost of taking the land out of productive use. More studies in which all of these factors are considered are needed in order to evaluate the economic feasibility of phytoremediation. Boron phytoremediation shares disadvantages with general phytoremediation of metals and metalloids. All phytoremediation strategies are site-specific, climate-dependent, and require that the site be amenable to plant growth. Phytoextraction is generally limited to sites polluted to a depth of less than 30–40 cm (root zone), with low-to-moderate levels of contamination [96], plants can generally only treat one contaminant, but many polluted sites contain a suite of contaminants. Another important drawback is that plants may take a long time to clean up a site. The cost of taking the land out of productive use during this time may be very high. This cost can be lowered if biomass with commercial value is produced while phytoremediating the site [79]. The produced biomass may be used as timber, bioenergy, or animal fodder. This last application will depend on the biomass boron concentration, because it has to be lower than the maximum level tolerable by animals [97].

The boron-rich biomass produced in phytoextraction could be used as organic fertilizers in boron-deficient agricultural soils, which have been reported in over 80 countries [98]. Also, using alternative crop species to phytomanage contaminated soils is an alternative that is conducive to profit and therefore may decrease the phytoremediation costs [65]. It is important to ensure that other potentially toxic elements are not present in the biomass if alternative crops, animal fodder, or fertilizers are considered as by-products options of phytoremediation. There are several research areas that can potentially help in bringing boron phytoremediation to widespread application, such as (a) understanding the plant–microorganism interactions, (b) creating models to predict the performance of boron phytoextraction, and (c) using genetically modified plants for phytoremediation. This last application has high potential for phytoremediation. Studies of transgenic plants carrying specific genes for boron tolerance have not been performed. Identifying and transferring the genes responsible for the high boron tolerance in some plants (e.g., *P. distans* and *P. frigida*) to high-biomass plant species is a promising approach toward increasing boron phytoextraction. Finally, it would be very interesting to screen boron tolerance and accumulation in other species from the genus *Puccinellia*, as both *P. frigid* [75] and *P. distans* [51] are extremely boron-tolerant species. This may open the possibility of finding new tolerant/accumulating species that could be used for phytoremediation strategies in sites with different characteristics.

Acknowledgements We are grateful to Dr. Samuel Contreras, Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile, for his valuable suggestions on this manuscript. C.d.P. Rámila acknowledges the support from a postdoctoral grant of the School of Engineering of the Pontificia Universidad Católica de Chile.

References

1. Dell B, Huang L (1997) Physiological response of plants to low boron. Plant Soil 193(1):103–120
2. Nable RO, Bañuelos GS, Paull JG (1997) Boron toxicity. Plant Soil 193(1):181–198
3. Lovatt CJ, Bates LM (1984) Early effects of excess boron on photosynthesis and growth of *Cucurbita pepo*. J Exp Bot 35(3):297
4. Nable RO, Cartwright B, Lance R (1990) Genotypic differences in boron accumulation in barley: relative susceptibilities to boron deficiency and toxicity, Genetic aspects of plant mineral nutrition. Kluwer Academic, Dordrecht, pp 243–251
5. Yau SK, Ryan J (2008) Boron toxicity tolerance in crops: a viable alternative to soil amelioration. Crop Sci 48(3):854–865
6. Cartwright B, Zarcinas B, Mayfield A (1984) Toxic concentrations of boron in a red-brown earth at Gladstone, South Australia. Aust J Soil Res 22(3):261–272
7. Goldberg S (1997) Reactions of boron with soils. Plant Soil 193(1):35–48
8. Leyshon AJ, Jame YW (1993) Boron toxicity and irrigation management. In: Gupta UC (ed) Boron and its role in crop production. CRC Press, Boca Raton, pp 207–226
9. Moraga NB, Amoroso MJ, Rajal VB (2014) Strategies to ameliorate soils contaminated with boron compounds. In: Bioremediation in Latin America. Springer, Cham, pp 41–51
10. Ali H, Khan E, Sajad MA (2013) Phytoremediation of heavy metals—concepts and applications. Chemosphere 91(7):869–881
11. Power PP, Woods WG (1997) The chemistry of boron and its speciation in plants. Plant Soil 193(1-2):1–13
12. Holleman A, Wiberg E (2001) Inorganic chemistry. Academic, San Diego
13. Parks JL, Edwards M (2005) Boron in the environment. Crit Rev Environ Sci Technol 35(2):81–114
14. Woods WG (1994) An introduction to boron: history, sources, uses and chemistry. Environ Health Perspect 102:5–11
15. Argust P (1998) Distribution of boron in the environment. Biol Trace Elem Res 66(1):131–143
16. Kot F (2009) Boron sources, speciation and its potential impact on health. Rev Environ Sci Biotechnol 8(1):3–28
17. Landi M, Innocenti ED, Pardossi A, Guidi L (2012) Antioxidant and photosynthetic responses in plants under boron toxicity: a review. Am J Agric Biol Sci 7:255–270
18. Goldberg S, Su C (2007) New advances in boron soil chemistry. Advances in plant and animal boron nutrition. Springer, Dordrecht, pp 313–330
19. Majidi A, Rahnemaie R, Hassani A, Malakouti MJ (2010) Adsorption and desorption processes of boron in calcareous soils. Chemosphere 80(7):733–739
20. Bonilla I, Blevins D, Bolaños L (2009) Essay 5.1: Boron functions in plants: looking beyond the cell wall. Plant Physiol Dev, 5th online edn. http://www.plantphys.net
21. Camacho-Cristóbal JJ, Rexach J, González-Fontes A (2008) Boron in plants: deficiency and toxicity. J Integr Plant Biol 50(10):1247–1255
22. Hakki E, Atalay E, Harmankaya M, Babaoglu M, Hamurcu M, Gezgin S, Kampus KT (2007) Determination of suitable maize (*Zea mays L.*) genotypes to be cultivated in boron-rich Central Anatolian soil. Advances in plant and animal boron nutrition. Springer, Dordrecht, pp 231–247

23. Maas E (1990) Crop salt tolerance. In: Tanji KK (ed) Agriculture salinity assessment and management, ASCE manuals and reports on engineering practice, vol 71. American Society of Civil Engineers, New York, pp 262–304
24. Gupta UC (1993) Deficiency, sufficiency, and toxicity levels of boron in crops. In: Gupta UC (ed) Boron and its role in crop production. CRC Press, Boca Raton, pp 137–145
25. Epstein E (1965) Mineral metabolism. In: Bonner J, Vemer JE (eds) Plant biochemistry. Academic, New York, pp 438–466
26. Chen L-S, Han S, Qi Y-P, Yang L-T (2012) Boron stresses and tolerance in citrus. Afr J Biotechnol 11(22):5961–5969
27. Guidi L, Degl'Innocenti E, Carmassi G, Massa D, Pardossi A (2011) Effects of boron on leaf chlorophyll fluorescence of greenhouse tomato grown with saline water. Environ Exp Bot 73:57–63
28. Landi M, Pardossi A, Remorini D, Guidi L (2013) Antioxidant and photosynthetic response of a purple-leaved and a green-leaved cultivar of sweet basil (*Ocimum basilicum*) to boron excess. Environ Exp Bot 85:64–75
29. Landi M, Remorini D, Pardossi A, Guidi L (2013) Boron excess affects photosynthesis and antioxidant apparatus of greenhouse *Cucurbita pepo* and *Cucumis sativus*. J Plant Res 126(6):775–786
30. Schnurbusch T, Hayes J, Hrmova M, Baumann U, Ramesh SA, Tyerman SD, Langridge P, Sutton T (2010) Boron toxicity tolerance in barley through reduced expression of the multifunctional aquaporin HvNIP2; 1. Plant Physiol 153(4):1706
31. Hayes JE, Reid RJ (2004) Boron tolerance in barley is mediated by efflux of boron from the roots. Plant Physiol 136(2):3376
32. Reid R (2007) Identification of boron transporter genes likely to be responsible for tolerance to boron toxicity in wheat and barley. Plant Cell Physiol 48(12):1673
33. Pang Y, Li L, Ren F, Lu P, Wei P, Cai J, Xin L, Zhang J, Chen J, Wang X (2010) Overexpression of the tonoplast aquaporin AtTIP5; 1 conferred tolerance to boron toxicity in *Arabidopsis*. J Genet Genomics 37(6):389–397
34. Reid R, Fitzpatrick K (2009) Influence of leaf tolerance mechanisms and rain on boron toxicity in barley and wheat. Plant Physiol 151(1):413
35. Cervilla LM, Blasco B, Ríos JJ, Romero L, Ruiz JM (2007) Oxidative stress and antioxidants in tomato (*Solanum lycopersicum*) plants subjected to boron toxicity. Ann Bot 100(4):747–756
36. Hassan M, Oldach K, Baumann U, Langridge P, Sutton T (2010) Genes mapping to boron tolerance QTL in barley identified by suppression subtractive hybridization. Plant Cell Environ 33(2):188–198
37. Gunes A, Soylemezoglu G, Inal A, Bagci E, Coban S, Sahin O (2006) Antioxidant and stomatal responses of grapevine (*Vitis vinifera* L.) to boron toxicity. Sci Hortic 110(3):279–284
38. Brennan R, Adcock K (2004) Incidence of boron toxicity in spring barley in southwestern Australia. J Plant Nutr 27(3):411–425
39. Yau S, Hamblin J, Ryan J (1994) Phenotypic variation in boron toxicity tolerance in barley, durum and bread wheat. Rachis 13:20–25
40. Gemici Ü, Tarcan G, Helvacı C, Somay AM (2008) High arsenic and boron concentrations in groundwaters related to mining activity in the Bigadiç borate deposits (Western Turkey). Appl Geochem 23(8):2462–2476
41. Türker OC, Böcük H, Yakar A (2013) The phytoremediation ability of a polyculture constructed wetland to treat boron from mine effluent. J Hazard Mater 252–253:132–141
42. Tassi EL, Pedron F, Barbafieri M (2011) Evaluating the absorption of boron by plants: a potential tool to remediate contaminated sediments from Cecina River Basin in Italy. Water Air Soil Pollut 216:275–287
43. Stiles AR, Liu C, Kayama Y, Wong J, Doner H, Funston R, Terry N (2011) Evaluation of the boron tolerant grass, *Puccinellia distans*, as an initial vegetative cover for the phytorestoration of a boron-contaminated mining site in Southern California. Environ Sci Technol 45(20):8922–8927

44. de Abreu CA, Coscione AR, Pires AM, Paz-Ferreiro J (2012) Phytoremediation of a soil contaminated by heavy metals and boron using castor oil plants and organic matter amendments. J Geochem Explor 123:3–7
45. Franco SA, Parada GL, Quiroga M, de Viana M (2008) Reducción del boro en sustratos por acción de especies nativas. Avances en Energías Renovables y Medio Ambiente 12:127–132
46. Torres A, Acevedo E (2008) El problema de salinidad en los recursos suelo y agua que afectan el riego y cultivos en los valles de Lluta y Azapa en el norte de Chile. Idesia (Arica) 26:31–44
47. Bingham FT, Marsh AW, Branson R, Mahler R, Ferry G (1972) Reclamation of salt affected high B soils in Western Kern County. Hilgardia 41:195–211
48. Peryea FJ, Bingham FT, Rhoades JD (1985) Mechanisms for boron regeneration. Soil Sci Soc Am J 49:840–843
49. Bañuelos G, Cardon G, Phene C, Wu L, Akohoue S, Zambrzuski S (1993) Soil boron and selenium removal by three plant species. Plant Soil 148(2):253–263
50. Bartlett R, Picarelli C (1973) Availability of boron and phosphorus as affected by liming an acid potato soil. Soil Sci 116(2):77–83
51. Stiles A, Bautista D, Atalay E, Babaoğlu M, Terry N (2010) Mechanisms of boron tolerance and accumulation in plants: a physiological comparison of the extremely boron-tolerant plant species, *Puccinellia distans*, with the moderately boron-tolerant *Gypsophila arrostil*. Environ Sci Technol 44(18):7089–7095
52. Greipsson S (2011) Phytoremediation. Nat Educ Knowl 2(7)
53. Vigil M, Marey-Pérez MF, Huerta GM, Cabal VÁ (2015) Is phytoremediation without biomass valorization sustainable? Comparative LCA of landfilling vs. anaerobic co-digestion. Sci Total Environ 505:844–850
54. Cameselle C, Chirakkara RA, Reddy KR (2013) Electrokinetic-enhanced phytoremediation of soils: status and opportunities. Chemosphere 93(4):626–636
55. Mench M, Lepp N, Bert V, Schwitzguébel J-P, Gawronski S, Schröder P, Vangronsveld J (2010) Successes and limitations of phytotechnologies at field scale: outcomes, assessment and outlook from COST Action 859. J Soil Sediment 10(6):1039–1070
56. Sarma H (2011) Metal hyperaccumulation in plants: a review focusing on phytoremediation technology. J Environ Sci Technol 4(2):118–138
57. Del-Campo Marín CM, Oron G (2007) Boron removal by the duckweed *Lemna gibba*: a potential method for the remediation of boron-polluted waters. Water Res 41(20):4579–4584
58. Tatar ŞY, Öbek E (2014) Potential of *Lemna gibba* L. and *Lemna minor* L. for accumulation of boron from secondary effluents. Ecol Eng 70:332–336
59. Türker OC, Türe C, Böcük H, Yakar A (2014) Constructed wetlands as green tools for management of boron mine wastewater. Int J Phytoremediat 16(6):537–553
60. McGrath SP, Zhao FJ (2003) Phytoextraction of metals and metalloids from contaminated soils. Curr Opin Biotechnol 14(3):277–282
61. Bañuelos GS (2015) Coping with naturally-high levels of soil salinity and boron in the westside of central California. Perspect Sci 3(1):4–6
62. Bañuelos G (1996) Managing high levels of boron and selenium with trace element accumulator crops. J Environ Sci Health A 31(5):1179–1196
63. Bañuelos G, Cardon G, Mackey B, Ben-Asher J, Wu L, Beuselinck P, Akohoue S, Zambrzuski S (1993) Boron and selenium removal in boron-laden soils by four sprinkler irrigated plant species. J Environ Qual 22(4):786–792
64. Bañuelos G, Mackey B, Wu L, Zambrzuski S, Akohoue S (1995) Bioextraction of soil boron by tall fescue. Ecotoxicol Environ Saf 31(2):110–116
65. Centofanti T, Bañuelos G (2015) Evaluation of the halophyte *Salsola soda* as an alternative crop for saline soils high in selenium and boron. J Environ Manag 157:96–102
66. Giansoldati V, Tassi EL, Morelli E, Gabellieri E, Pedron F, Barbafieri M (2012) Nitrogen fertilizer improves boron phytoextraction by *Brassica juncea* grown in contaminated sediments and alleviates plant stress. Chemosphere 87(10):1119–1125

67. Barbafieri M, Giorgetti L, Tassi E, Morelli E, Pedron F (2014) Investigations on Boron contaminated sediment (Tuscany, Italy): phytotoxicity and phytoextraction test. In: XII International Conference on Protection and Restoration of the Environment Skiathos Island, Greece, June 29–July 3, pp 665–670
68. dos Santos GCG, Rodella AA, Abreu CAD, Coscione AR (2010) Vegetable species for phytoextraction of boron, copper, lead, manganese and zinc from contaminated soil. Sci Agric 67(6):713–719
69. Gezgin S, Dursun N, Hamurcu M, Harmankaya M, Önder M, Sade B, Topal A, Soylu S, Akgün N, Yorgancilar M (2002) Boron content of cultivated soils in central-southern Anatolia and its relationship with soil properties and irrigation water quality. In: Boron in plant and animal nutrition. Springer, New York, pp 391–400
70. Babaoglu M, Gezgin S, Topal A, Sade B, Dural H (2004) Gypsophila sphaerocephala Fenz ex Tchihat.: a boron hyperaccumulator plant species that may phytoremediate soils with toxic B levels. Turk J Bot 28:273–278
71. Bocuk H, Ture C (2014) Phytoremediation possibilities of boron-contaminated environments by wild plants growing on a boron reserve area in Turkey. Commun Soil Sci Plant Anal 45(13):1784–1798
72. Aydın M, Çakır F (2009) Research on weed species for phytoremediation of boron polluted soil. Afr J Biotechnol 8(18):4514–4518
73. Cáceres VL, Gruttner DE, Contreras NR (1992) Water recycling in arid regions: Chilean case. Ambio 21:138–144
74. Tapia Y, Diaz O, Pizarro C, Segura R, Vines M, Zúñiga G, Moreno-Jiménez E (2013) *Atriplex atacamensis* and *Atriplex halimus* resist As contamination in Pre-Andean soils (northern Chile). Sci Total Environ 450:188–196
75. Rámila CDP, Leiva ED, Bonilla CA, Pastén PA, Pizarro GE (2015) Boron accumulation in *Puccinellia frigida*, an extremely tolerant and promising species for boron phytoremediation. J Geochem Explor 150:25–34
76. Bañuelos G, Ajwa H, Caceres L, Dyer D (1999) Germination responses and boron accumulation in germplasm from Chile and the United States grown with boron-enriched water. Ecotoxicol Environ Saf 43(1):62–67
77. Rámila CP, Contreras SA, Di Domenico C, Molina-Montenegro MA, Vega A, Handford M, Bonilla CA, Pizarro GE (2016) Boron stress response and accumulation potential of the extremely tolerant species *Puccinellia frigida*. J Hazard Mater 317:476–484
78. Smolcz SU, Cortés VG (2015) Remediation of boron contaminated water and soil with vetiver phytoremediation technology in Northern Chile. In: 6th International Conference on Vetiver (ICV6), Da Nang, Vietnam, May 5–8
79. Robinson B, Anderson C, Dickinson N (2015) Phytoextraction: where's the action? J Geochem Explor 151:34–40
80. Esringü A, Turan M, Güneş A, Karaman MR (2014) Roles of *Bacillus megaterium* in remediation of boron, lead, and cadmium from contaminated soil. Commun Soil Sci Plant Anal 45(13):1741–1759
81. Gullap MK, Dasci M, Erkovan Hİ, Koc A, Turan M (2014) Plant growth-promoting rhizobacteria (PGPR) and phosphorus fertilizer-assisted phytoextraction of toxic heavy metals from contaminated soils. Commun Soil Sci Plant Anal 45(19):2593–2606
82. Turan M, Angin I (2004) Organic chelate assisted phytoextraction of B, Cd, Mo and Pb from contaminated soils using two agricultural crop species. Acta Agric Scand Sect B Soil Plant Sci 54(4):221–231
83. Angin I, Turan M, Ketterings QM, Cakici A (2008) Humic acid addition enhances B and Pb phytoextraction by Vetiver grass (Vetiveria zizanioides (L.) Nash). Water Air Soil Pollut 188(1):335–343
84. Robinson B, Green S, Mills T, Clothier B, van der Velde M, Laplane R, Fung L, Deurer M, Hurst S, Thayalakumaran T (2003) Phytoremediation: using plants as biopumps to improve degraded environments. Soil Res 41(3):599–611

85. Martínez M, Bernal P, Almela C, Vélez D, García-Agustín P, Serrano R, Navarro-Aviñó J (2006) An engineered plant that accumulates higher levels of heavy metals than *Thlaspi caerulescens*, with yields of 100 times more biomass in mine soils. Chemosphere 64(3): 478–485
86. Bañuelos G, Terry N, LeDuc DL, Pilon-Smits EA, Mackey B (2005) Field trial of transgenic Indian mustard plants shows enhanced phytoremediation of selenium-contaminated sediment. Environ Sci Technol 39(6):1771–1777
87. Yermiyahu U, Ben-Gal A, Keren R, Reid RJ (2008) Combined effect of salinity and excess boron on plant growth and yield. Plant Soil 304(1–2):73–87
88. Wimmer MA, Goldbach HE (2012) Boron and salt interactions in wheat are affected by boron supply. J Plant Nutr Soil Sci 175(2):171–179
89. Ismail A (2003) Response of maize and sorghum to excess boron and salinity. Biol Plant 47(2):313–316
90. Hu H, Brown PH (1997) Absorption of boron by plant roots. Plant Soil 193(1–2):49–58
91. Reid R (ed) (2007) Update on boron toxicity and tolerance in plants. Advances in plant and animal boron nutrition. Springer, Dordrecht
92. Bañuelos G, Shannon M, Ajwa H, Draper J, Jordahl J, Licht J (1999) Phytoextraction and accumulation of boron and selenium by poplar (*Populus*) hybrid clones. Int J Phytoremediat 1(1):81–96
93. Robinson B, Green S, Chancerel B, Mills T, Clothier B (2007) Poplar for the phytomanagement of boron contaminated sites. Environ Pollut 150(2):225–233
94. Rees R, Robinson BH, Menon M, Lehmann E, Günthardt-Goerg MS, Schulin R (2011) Boron accumulation and toxicity in hybrid poplar (Populus nigra × euramericana). Environ Sci Technol 45(24):10538–10543
95. Rees R, Robinson BH, Rog CJ, Papritz A, Schulin R (2013) Boron accumulation and tolerance of hybrid poplars grown on a B-laden mixed paper mill waste landfill. Sci Total Environ 447:515–524
96. Prasad M, Freitas H, Fraenzle S, Wuenschmann S, Markert B (2010) Knowledge explosion in phytotechnologies for environmental solutions. Environ Pollut 158(1):18–23
97. Bañuelos GS, Wu L, Akohoue S, Zambrzuski S, Mead R (1993) Trace element composition of different plant species used for remediation of boron-laden soils. In: Barrow NJ (ed) Plant nutrition—from genetic engineering to field practice. Springer, Dordrecht, pp 425–428
98. Shorrocks VM (1997) The occurrence and correction of boron deficiency. Plant Soil 193(1–2): 121–148

Index

A.A. Ansari et al. (eds.), *Phytoremediation*, DOI 10.1007/978-3-319-40148-5