

Chapter 12

Phytoremediation of Heavy Metals Contaminated Soils Through Transgenic Plants

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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 non-biodegradable 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 possess 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 fulfills 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

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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 $327.4 \text{ }^\circ\text{C}$, and boiling point $1725 \text{ }^\circ\text{C}$. It is a naturally occurring, bluish gray metal usually found as a mineral combined with other elements, such as sculpture (i.e., Pbs, 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 to 67 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., $\text{Pb}^{2+}\text{SO}_4^{2-}$), whereas Pb(IV) compounds tend to be covalent (e.g., tetraethyl lead, $\text{Pb}(\text{C}_2\text{H}_5)_4$). Some Pb (IV) compounds, such as PbO_2 , are strong oxidants. Lead forms several basic salts, such as $\text{Pb}(\text{OH})_2 \cdot 2\text{PbCO}_3$, 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 $817 \text{ }^\circ\text{C}$, and boiling point $613 \text{ }^\circ\text{C}$, 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 $\text{HAs}(\text{CH}_3)_2$ and trimethylarsine $\text{As}(\text{CH}_3)_3$ 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 $(\text{CH}_3)\text{AsO}_2\text{H}_2$ and dimethylarsinic acid $(\text{CH}_3)_2\text{AsO}_2\text{H}$. 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 $419.5 \text{ }^\circ\text{C}$, and boiling point $906 \text{ }^\circ\text{C}$. Zinc occurs naturally in soil (about 70 mg 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 $320.9 \text{ }^\circ\text{C}$, and boiling point $765 \text{ }^\circ\text{C}$. 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 *itaitai* 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 $-13.6 \text{ }^\circ\text{C}$, and boiling point $357 \text{ }^\circ\text{C}$ 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 ($\text{pH} < 4$) 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 ⁻¹ dw)	Essential for human	Toxicity symptoms in humans
As	No	-	≥20(a)	<i>Pteris vittata</i> L. (f)	>1,000 (f)	No	Cancer (e.g., lung and skin); cardiovascular, gastrointestinal, hepatic, and renal diseases
Cd	No	-	5–10 (b)	<i>Thlaspi caerulescens</i> 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)	<i>Haumaniastrum robertii</i> (Robyns) P.A. Du vign. & Plancke (e)	>1,000 (e)	Yes	Contact dermatitis; mutagenic and carcinogenic effects
Cr	No	-	1–2 (b)	<i>Brassica juncea</i> (L.) Czern.; <i>B.napus</i> (L.); <i>Vallisneria americana</i> (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)	<i>Brassica juncea</i> (L.) Czern.; <i>Vallisneria americana</i> 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)	<i>Agrostis castellana</i> 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.; <i>Thlaspi</i> spp. (e)	>1,000 (e)	Yes	Accumulation in lungs Irreversible neurological damage; renal disease; cardiovascular effects; reproductive toxicity
Pb	No	–	10–20 (b)	<i>Thlaspi rotundifolium</i> (L.) Gaudin ssp. <i>Cepaeifolium</i> (Wulfen) Rouy & Fouc; <i>T. Caerulescens</i> J. & C. Presl.; <i>Alyssum Wulfenianum</i> Bernh.; <i>Arrhenatherumelatius</i> (L) Beauv.; <i>Festuca ovina</i> 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)	<i>Thlaspi</i> spp.; <i>Cardaminopsis</i> 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) Förstner [224]; (c) Li et al. [225]; (d) McCutcheon and Schnoor [226]; (e) Terry and Banuelos [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 break-down 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 than 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]. Contaminants 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,

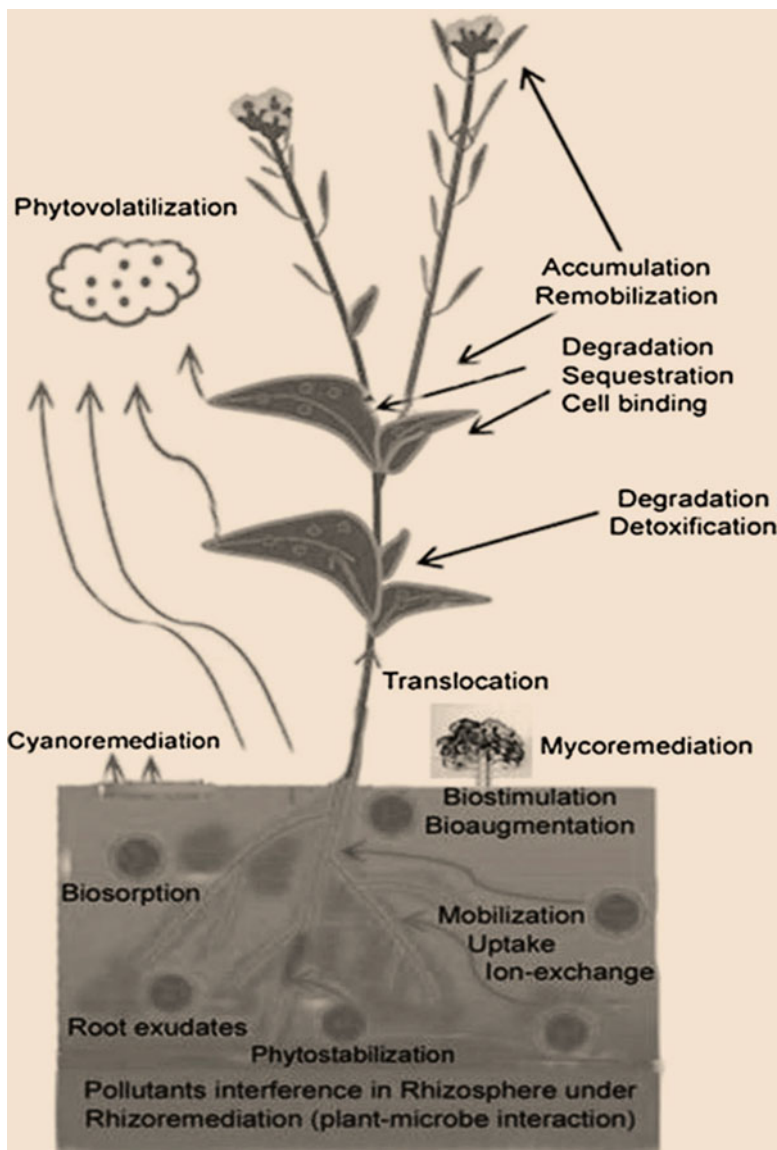


Fig. 12.1 An overview of biotechnological approaches for phytoremediation. Reproduced from Mani and Kumar Int. J. Environ. Sci. Technol. 11:843–872 [70], © 2014 Springer (modified from Dhankher et al. [267])

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 $HgCl_2$ 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 morphophysiological 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	<i>Berkheya coddii</i>	Asteraceae	Robinson et al. [230]; Moradi et al. [231]
		<i>Alyssum serpyllifolium</i> , <i>A. bertolonii</i>	Brassicaceae	Becerra-Castro et al. [232]; Barzanti et al. [233]
		<i>Sebertia acuminata</i>	Sapotaceae	Jaffre et al. [234]; Perrier [235]
		<i>Phidiasia lindavii</i>	Acanthaceae	Reeves et al. [236]
		<i>Bornmuellera kiyakii</i>	Brassicaceae	Reeves et al. [237]
Cu	34	<i>Ipomea alpina</i>	Convolvulaceae	Cunningham and Ow [59]
		<i>Crassula helmsii</i>	Crassulaceae	Küpper et al. [238]
		<i>Commelina communis</i>	Commelinaceae	Wang and Zhong [239]
Co	34	<i>Haumaniastrum robertii</i>	Lamiaceae	Brooks [85]
		<i>Crotalaria cobalticola</i>	Fabaceae	Oven et al. [240]
Se	20	<i>Astragalus bisulcatus</i>	Fabaceae	Galeas et al. [241]
		<i>Stanleya pinnata</i>	Brassicaceae	Freeman et al. [242]; Hladun et al. [243]
Zn	18	<i>Thlaspi caerulescens</i>	Brassicaceae	Kupper and Kochian [244]
		<i>Arabis gemmifera</i> , <i>A. paniculata</i>)	Brassicaceae	Kubota and Takenaka [245]; Tang et al. [246]
		<i>Sedum alfredii</i>	Crassulaceae	Sun et al. [247]
		<i>Arabidopsis halleri</i>	Brassicaceae	Zhao et al. [248]
		<i>Picris divaricata</i>	Asteraceae	Du et al. [249]
Pb	14	<i>Sesbania drummondii</i>	Fabaceae	Sahi et al. [250]; Sharma et al. [251]
		<i>Hemidesmus indicus</i>	Apocynaceae	Chandra Sekhar et al. [252]
		<i>Arabis paniculata</i>	Brassicaceae	Tang et al. [246]
		<i>Plantago orbignyana</i>	Plantaginaceae	Bech et al. [253]
Mn	9	<i>Austromyrtus bidwillii</i>	Myrtaceae	Bidwell et al. [254]
		<i>Phytolacca americana</i>	Phytolaccaceae	Pollard et al. [255]
		<i>Viotia neurophylla</i>	Proteaceae	Fernando et al. [256]
		<i>Gossia bidwillii</i>	Myrtaceae	Fernando et al. [257]
		<i>Maytenus founieri</i>	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	<i>Thlaspi caerulescens</i>	Brassicaceae	Basic et al. [117]
		<i>Arabidopsis halleri</i>	Brassicaceae	Dahmani-Muller et al. [259]; Bert et al. [260]
		<i>Bidens pilosa</i>	Asteraceae	Sun et al. [261]
Cr	na	<i>Salsola kali</i>	Amaranthaceae	Gardea-Torresday et al. [262]
		<i>Leersia hexandra</i>	Poaceae	Zhang et al. [263]
		<i>Gynura pseudochina</i>	Asteraceae	Mongkhonsin et al. [264]
Ti	na	<i>Iberis intermedia</i>	Brassicaceae	Leblanc et al. [265]
		<i>Brassica oleracea</i>	Brassicaceae	Al-Najar et al. [266]

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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. caentescens* 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 phytoextractor 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 over-express 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. x 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 (35S²). 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 distribution. 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 tobacco; the CaMV 35S promoter was not affected by Cd exposure in tobacco, whereas the ribulose biphosphate 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 (γ ECS) 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, γ ECS 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 ionic mercury 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 oxy-anion 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 [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-carboxylic acid (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^0 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
<i>me r A</i>	Hg(II) reductase	Gram-negative bacteria	<i>Liriodendron tulipifera</i>	50 $\mu\text{mol l}^{-1}$ HgCl ₂ ; 500 mg HgCl ₂ kg ⁻¹
			<i>Nicotiana tabacum</i>	V: Hg volatilization rate increase tenfold
<i>mer A</i>	Hg(II) reductase	Gram-negative bacteria	<i>Arabidopsis thaliana</i>	T: 10 $\mu\text{mol l}^{-1}$ CH ₃ HgCl (>40-fold)
<i>mer B</i>	Organomercurial lyase	Gram-negative bacteria	<i>A. thaliana</i>	V: Up to 59 pg Hg(0) mg ⁻¹ fresh biomass min ⁻¹
<i>APSI</i>	ATP sulfurylase	<i>A. thaliana</i>	<i>B. juncea</i>	A: Twofold increase in Se concentration
<i>MT-I</i>	MT	Mouse	<i>N. tabacum</i>	T: 200 $\mu\text{mol l}^{-1}$ CdCl ₂ (20-fold)
<i>CUP1</i>	MT	<i>Saccharomyces cerevisiae</i>	<i>B. oleracea</i>	T: 400 $\mu\text{mol l}^{-1}$ CdCl ₂ (approximately 16-fold)
<i>gsh2</i>	GSH synthase	<i>E. coli</i>	<i>B. juncea</i>	A: Cd concentrations 125 %
<i>gsh1</i>	γ -Glu-Cys synthase	<i>E. coli</i>	<i>B. juncea</i>	A: Cd concentrations 190 %
<i>NtCBP4</i>	Cation channel	<i>N. tabacum</i>	<i>N. tabacum</i>	T: 250 mmol l^{-1} NiCl ₂ (2.5-fold), Pb sensitive
				A: Pb concentrations 200 %
<i>ZATI</i>	Zn transporter	<i>A. thaliana</i>	<i>A. thaliana</i>	T: Slight increase
<i>TaPCS1</i>	PCs	Wheat	<i>Nicotiana glauca</i> R. Graham	A: Pb concentrations 200 %

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^aRelative values refer to control plants not expressing the transgene. A accumulation in the shoot; GSH glutathione, MT metallothionein; T tolerance; V volatilization; PCs Phytochelatin

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 volatilizing 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, Parlayıcı S (2009) Adsorption of Cu²⁺ and Pb²⁺ 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, *Dissertationes De Agricultura, Doctoraatsproefschrift 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](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 availability 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://tradedetalfutures.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, Mikhailovsky 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. Baelos GS, Meek DW (1990) Accumulation of selenium in plants grown on selenium-treated soil. *J Environ Qual* 19:772
73. Baelos GS, Cardon G, Mackey B, Ben-Asher J, Wu L, Beuselinck P, Akohoue S, Zambruski 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, Reimdsijk 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 Environ 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, Brooks 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. Padmavathamma 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. *BioTechnol* 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, Svatoš 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 Plant Biology* 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 1*). *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 Ni₂-tolerance and Pb₂-hypersensitivity in transgenic plants. *Plant J* 20:171–182
153. Van der Zaal BJ, Neuteboom LW, Pinas JE, Chardonens AN, Schat H, Verkleij JAC, Hooykaas PJJ (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* 119:1047–1055
154. Curie C, Alonso JM, Le Jean V, Ecker JR, Briat JF (2000) Involvement of Nramp1 from *Arabidopsis thaliana* in iron transport. *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. Guo J 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, Martínez 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, Ibrahim K, Alib N, Ghanem I, Al-Daoud 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. Herbig 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, Ganai 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. *EMBO J* 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. Visoottiviset 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, Ametoli M, Gallo A, Pignattelli S, Gabbriellini 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 Kızıldaây 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, Batianoff 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