Chapter 1
Detoxification of Heavy Metals: State of Art

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1.1 Introduction

Land and water are precious natural resources on which rely the sustainability of agriculture and the civilization of mankind. Unfortunately, they have been subjected to maximum exploitation and are severely degraded or polluted due to anthropogenic activities. The pollution includes point sources such as emission, effluents, and solid discharge from industries, vehicle exhaustion, and metals from smelting and mining, and nonpoint sources such as soluble salts (natural and artificial), use of insecticides/pesticides, disposal of industrial and municipal wastes in agriculture, and excessive use of fertilizers (McGrath et al. 2001; Nriagu and Pacyna 1988; Schalscha and Ahumada 1998). Each source of contamination has its own damaging effects to plants, animals, and ultimately to human health, but those that add heavy metals to soil and water are of serious concern due to their persistence in the environment and carcinogenicity to human beings. They cannot be destroyed biologically but are only transformed from one oxidation state or organic complex to another (Garbisu and Alkorta 2001; Gisbert et al. 2003). Therefore, heavy metal pollution poses a great potential threat to the environment and human health.

In order to maintain good quality of soil and water and keep them free from contamination, continuous efforts have been made to develop technologies that are easy to use, sustainable, and economically feasible. Physicochemical approaches

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have been widely used for remedying polluted soil and water, especially at a small scale. However, they experience more difficulties for a large scale of remediation because of high costs and side effects. Some microorganism-based remediation techniques, such as bioremediation, show potential for their ability to degrade and detoxify certain contaminants. Although these biological systems are less amenable to environmental extremes than other traditional methods, they have the perceived advantage of being more cost effective (Cunningham et al. 1997). The use of plant species for cleaning polluted soil and water named as phytoremediation has gained increasing attention since last decade, as an emerging cheaper technology. Many studies have been conducted in this field in the last two decades. Numerous plant species have been identified and tested for their traits in the uptake and accumulation of different heavy metals. Mechanisms of metal uptake at whole plant and cellular levels have been investigated. Progresses have been made in the mechanistic and practical application aspects of bioremediation and phytoremediation. They are reviewed in this chapter.

1.2 The Origin of Heavy Metals in Soil

1.2.1 Geochemical Origins of Heavy Metals

Ten 'major elements', O, Si, Al, Fe, Ca, Na, K, Mg, Ti, and P, constitute over 99% of the total element content of the earth's crust. The remaining elements of the periodic table are called 'trace elements' and their individual concentrations do not normally exceed 1,000 mg/kg (0.1%); in fact, most have average concentrations of less than 100 mg/kg (Mitchell 1964).

Trace elements occur as trace constituents of primary minerals in igneous rocks (which crystallize from molten magma). They become incorporated into these minerals by isomorphously substituting in the crystal lattice for ions of one of the major elements at the time of crystallization.

Sedimentary rocks comprise approximately 75% of the rocks outcropping at the earth's surface and are therefore more important than igneous as soil parent materials. They are formed by the lithification of sediments comprising rock fragments or resistant primary minerals, secondary minerals such as clays, or chemical precipitates such as $CaCO_3$. In general, clays and shales tend to have relatively high concentrations of many elements due to their ability to adsorb metal ions. Black (or bituminous) shales contain high concentrations of several metals and metalloids, including Ag, As, Cd, Cu, Pb, Mo, U, V, and Zn. The sediments from which they are formed act both as an adsorbent for heavy metals and as a substrate for microorganisms. The latter catalyzes the development of reducing conditions, which lead to further heavy metal accumulation through the precipitation of metal sulfides.

1.2.2 Sources of Heavy Metals Contaminants in Soils

Although heavy metals are ubiquitous in soil parent materials, the major anthropogenic sources of metals to soils and the environment are given in the following sections.

1.2.2.1 Metaliferous Mining and Materials

Metals utilized in manufacturing are obtained from either the mining of ore bodies in the earth's crust, or the recycling of scrap metal. Ores are naturally occurring concentration of minerals with a sufficiently high concentration of metals to render them economically worthwhile exploiting. With increase in demand for metals and improvements in mineral extraction technology, a higher proportion of ore bodies are being mined and consequently much greater quantities of waste are produced, especially tailings (finely milled fragments of rock and residual particles of ore not removed by the separation process). These tailing particles, which can be transported by either wind or water, constitute a significant source of metal contamination in soils surrounding the mines and in alluvial soils downstream.

Accidental flooding resulting from the failure of dams in tailing lagoons has been responsible for many severe pollution events in several countries. Once in the soil, the ore mineral fragments undergo oxidation and other weathering reactions and as a consequence the metal ions become distributed within the soil system and hence potentially bioavailable. Most ore minerals contain several other metals as minor inclusions as shown in Table 1.1.

1.2.2.2 Agricultural and Horticultural Materials

Agricultural practices constitute very important nonpoint sources of metals which make significant contributions to their total concentrations in soils in many parts of the world, especially in regions of intensive farming. According to the data obtained by the Lowestoft Laboratory of the Ministry of Agriculture, Fisheries and Food (MAFF 1986), the main sources are as follows:

Impurities in fertilizers: Cd, Cr, Mo, Pb, U, V, and Zn
Sewage sludge: especially Cd, Ni, Cu, Pb, and Zn (and many other elements)
Manures from intensive animal production, especially pigs and poultry: Cu, As, and Zn
Pesticides: Cu, As, Hg, Pb, Mn, and Zn
Refuse derived composts (not widely used in agriculture): Cd, Cu, Ni, Pb, and Zn
Desiccants: As
Wood preservatives: As, Cu, and Cr
Corrosion of metal objects (galvanized metal roofs and wire fences): Zn and Cd

Table 1.1 Common ore minerals

Metal	Ore minerals	Associated heavy metals
Ag	Ag_2S, PbS	Au, Cu, Sb, Zn, Pb, Se, Te
As	$FeAsS$, AsS, Cu Ores	Au, Ag, Sb, Hg, U, Bi, Mo, Sn, Cu
Au	Au^a, $AuTe_2$, $(Au, Ag)Te_2$	Te, Ag, As, Sb, Hg, Se
Ba	$BaSO_4$	Pb, Zn
Cd	ZnS	Pb, Zn, Cu
Cr	$FeCr_2O_4$	Ni, Co
Cu	$CuFeS_2$, Cu_5FeS_4, Cu_2S, Cu_3AsS_4, CuS, Cu^a	Zn, Cd, Pb, As, Se, Sb, Ni, Pt, Mo, Au, Te
Hg	HgS, Hg^a, Zn Ores	Sb, Se, Te, Ag, Zn, Pb
Mn	MnO_2	Fe, Co, Ni, Zn, Pb
Mo	MoS_2	Cu, Re, W, Sn
Ni	$(Ni, Fe)_9S_8$, $NiAs$, $(Co,Ni)_3S_4$	Co, Cr, As, Pt, Se, Te
Pb	PbS	Ag, Zn, Cu, Cd, Sb, Ti, Se, Te
Sb	Sb_2S_3, Ag_3SbS_3	Ag, Au, Hg, As
Se	Cu Ores	As, Sb, Cu, Ag, Au
Sn	SnO_2, $Cu_2(Fe, Zn)SnS_4$	Nb, Ta, W, Rb
U	U_2O_3	V, As, Mo, Se, Pb, Cu, Co, Ag
V	C_2O_5, VS_4	U
W	WO_3, $CaWO_4$	Mo, Sn, Nb
Zn	ZnS	Cd, Cu, Pb, As, Se, Sb, Ag, Au, In

[a]Native metal deposits (adapted from Peters 1978)

It should be noted that not all of these sources relate to current practices and materials.

Most agricultural and horticultural soils in technologically advanced countries are regularly amended with fertilizers and many also receive organic manures (usually based on livestock feces and urine) and lime. Typical ranges of heavy metal concentrations found in these materials are given in Table 1.2 (Kabata-Pendias and Pendias 1992; Webber et al. 1984).

1.2.2.3 Sewage Sludge

Sewage sludge is the residue produced from the treatment of domestic and industrial wastewaters and large amounts are produced worldwide.

Sewage sludge are a significant source of plant nutrients and organic matter and some specially treated sludge, such as those containing lime or cement kiln dust, have useful liming properties as well. However, the beneficial properties of sludge are limited by their contents of potentially harmful substances such as heavy metals and organic micropollutants (PAHs, PCBs, and pesticides). Although all the sludge contain a wide range of metals and other contaminants in varying concentrations, those from industrial catchments generally have higher metal contents than those from mainly suburban domestic areas. The heavy metals most likely to cause problems for crop production on sludge-amended soils are Cd, Cu, Ni, and Zn (Davis and Calton-Smith 1980; McGrath et al. 1994).

Table 1.2 Typical ranges of heavy metal concentration in fertilizers, farmyard manure, lime, and compost (mg/kg)

Metal	Phosphate fertilizers	Nitrate fertilizers	Farmyard manure	Lime	Composted refuse
Ag	–	–	–	–	–
As	2–1,200	2.2–120	3–25	0.1–25	2–52
B	5–115	–	0.3–0.6	10	–
Cd	0.1–170	0.05–8.5	0.1–0.8	0.04– 0.1	0.01–100
Co	1–12	5.4–12	0.3–24	0.4–3	–
Cr	66–245	3.2–19	1.1–55	10–15	1.8–410
Cu	1–300	–	2–172	2–125	13–3,580
Hg	0.01–1.2	0.3–2.9	0.01–0.36	0.05	0.09–21
Mn	40–2,000	–	30–969	40–1,200	–
Mo	0.1–60	1–7	0.05–3	0.1–15	–
Ni	7–38	7–34	2.1–30	10–20	0.9–279
Pb	7–225	2–27	1.1–27	20–1,250	1.3–2,240
Sb	<100	–	–	–	–
Se	0.5	–	2.4	0.08–0.1	–
U	30–300	–	–	–	–
V	2–1,600	–	–	20	–
Zn	50–1,450	1–42	15–566	10–450	82–5,894

Adapted from Kabata-Pendias and Pendias (1992) and Webber et al. (1984)

Due to the relatively high concentrations of heavy metals, the sludges can be major sources of heavy metals in the soil to which they are applied. Several authors have shown that the transfer of heavy metals from sewage sludge-amended soils to crops is significantly lower than those from inorganic sources, such as metal salts or mining wastes (Alloway and Jackson 1991).

1.2.2.4 Fossil Fuel Combustion

In general, fossil fuel combustion results in the dispersion of a wide range of heavy metals, which can include: Pb, Cd, Cr, Zn, As, Sb, Se, Ba, Cu, Mn, U, and V, over a very large area, although not all these elements are present in significant concentrations in all types of coal and petroleum. The metals accumulate in the coal and petroleum deposits as they formed and are either emitted into the environment as airborne particles during combustion or accumulated in the ash which may itself be transported and contaminate soils or waters or may be leached in situ. The combustion of petrol (gasoline) containing Pb additives has been the largest source of this metal in this environment and has affected soils over a high proportion of the earth's terrestrial surface. Pb is emitted in the exhaust of vehicles running on Pb containing petrol as aerosol particles 0.01–0.1 μm in diameter, but these primary particles can cluster to form large particles (0.3–1 μm). These particles comprise mainly PbBrCl but can react with other air pollutants to form more complex compounds such as a-2PbBrCl·NH$_4$Cl.

1.2.2.5 Metallurgical Industries

Metallurgical industries can contribute to soil pollution in several ways: (a) by the emission of aerosols and dusts which are transported in air and eventually deposited on soils or vegetation; (b) by liquid effluents which may pollute soils at times of flooding; (c) by the creation of waste dumps in which metals become corroded and leached into the underlying soils. Many heavy metals are used in specialist alloys and steels – V, Mn, Pb, W, Mo, Cr, Ni, Cu, Zn, Sn, Si, Ti, Te, Ir, Ge, Tl, Sb, In, Cd, Be, Bi, Li, As, Ag, Sb, Pr, Os, Nb, Nd, and Gd (MAFF 1986). Hence, the manufacture of these materials, their disposal, or recycling in scrap metal can lead to environmental pollution by a wide range of metals.

1.2.2.6 Electronics

A large number of heavy metals are used in the manufacture of semiconductors, cables, contacts, and other electrical components. These include: Cu, Zn, Au, Ag, Pb, Sn, Y, W, Cr, Se, Sm, Ir, In, Ga, Ge, Re, Sn, Tb, Co, Mo, Hg, Sb, As, and Gd (MAFF 1986). Environmental pollution can occur from the manufacture of the components, their accidental contact with soils, and their disposal in waste. In addition to metals, old electronic equipment may also often include capacitors and transformers containing polychlorinated biphenyls (PCBs), which are persistent organic pollutants in soils.

1.2.2.7 Chemical and Other Industrial Sources

Other significant sources of heavy metal pollution of soils and the environment can be the manufacture and/or use and disposal of the following (MAFF 1986):

Chlorine manufacture, Hg
Batteries, Pb, Sb, Zn, Cd, Ni, and Hg
Pigments and paints, Pb, Cr, As, Sb, Se, Mo, Cd, Ba, Zn, and Co
Catalysts, Pt, Sm, Sb, Ru, Co, Rh, Re, Pd, Os, Ni, and Mo
Polymer stabilizers, Cd, Zn, Sn, and Pb
Printing and graphics, Se, Pb, Cd, Zn, Cr, and Ba
Medical Uses, Ag, Sn, Ba, Cu, Hg, Sb, Se, Pt, and Zn
Additives in fuels and lubricants, Se, Te, Pb, Mo, and Li

1.2.2.8 Waste Disposable

The disposal of household, municipal, and industrial wastes can lead to soil pollution with heavy metals in various ways. The landfilling of municipal solid

waste can lead to several metals including Cd, Cu, Pb, Sn, and Zn being dispersed into soil, groundwater, and surface water in leachates if the landfill is not managed properly. Temporary waste stockpiles can cause significant soil contamination which may not be discovered until analysis at a later time when the land is no longer used for this purpose. This has been the cause of metal contamination in several urban allotment gardens in the UK.

1.3 Soil–Plant Relationships of Heavy Metals

1.3.1 Soil–Plant System

The major interrelationships affecting the dynamics of heavy metals between the soil and the plant are shown in Fig. 1.1. The soil–plant system is an open system subject to inputs, such as contaminants, fertilizers, and pesticides, and to losses, such as the removal of metals in harvested plant material, leaching, erosion, and vitalization.
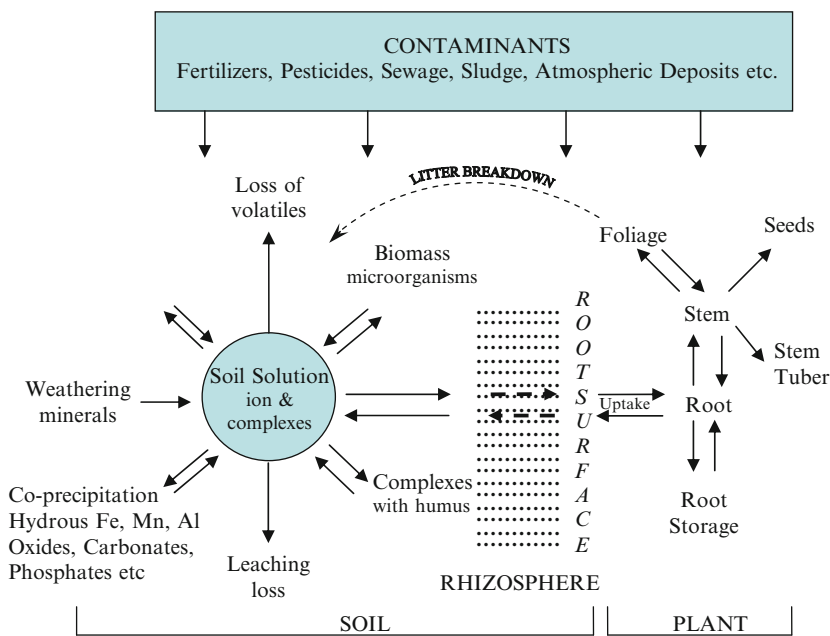
Fig. 1.1 The soil–plant system showing the key components concerned with the dynamics of heavy metals (modified from Peterson and Alloway 1979)

1.3.2   Plant Uptake of Metals

The factors affecting the amounts of metal absorbed by a plant are those controlling: (a) the concentration and speciation of the metal in the soil solution, (b) the movement of the metal from the bulk soil to the root surface, (c) the transport of the metal from the root surface in the root, and (d) its translocation from the root to the shoot (Peterson and Alloway 1979). Plant uptake of mobile ions present in the soil solution is largely determined by the total quantity of this ion in the soil, but, in the case of strongly absorbed ions, absorption is more dependent on the amount of root produced (Krauskopf 1967). Mycorrhizae are symbiotic fungi which effectively increase the absorptive area of the root and can assist in the uptake of nutrient ions, such as orthophosphates and micronutrients.

Absorption of metals by plant roots can be by both passive and active processes. Passive uptake involves diffusion of ions in the soil solution into the root endodermis. On the other hand, active uptake takes place against a concentration gradient but requires metabolic energy and can therefore be inhibited by toxins. The mechanisms appear to differ between metals: for instance, Pb uptake is generally considered to be passive, while that of Cu, Mo, and Zn is thought to be either active metabolic uptake, or a combination of both active and passive uptake (Kabata-Pendias and Pendias 1992).

Absorption mechanism can vary for different metal ions, but ions that are absorbed in the roots by the same mechanism are likely to compete with each other. For example, Zn absorption is inhibited by Cu and H^+, but not by Fe and Mn; Cu absorption is inhibited by Zn, NH_4^+, Ca, and K (Barber 1984).

The rhizosphere is the zone about 1–2 mm wide between plant roots and the surrounding soil. It receives appreciable amounts of organic material from the roots, including exudates, mucilage, sloughed-off cells, and their lysates (Marschner 1986). These organic compounds give rise to intense microbiological and biochemical activity in the rhizosphere, which enables roots to mobilize some of the metals that are strongly absorbed in the soil, by acidification, redox changes, or the formation of organic complexes.

The uptake of metals from soil is greater in plants grown in pots in greenhouse than from the same soil in the field (Graham 1981; Marschner 1986). This is probably due to differences in microclimate and soil moisture, and to the roots of container-grown plants in contaminated soil, whereas those of field-grown plants may reach down to less contaminated soil.

1.3.3   The Biological Essentiality of Trace Elements

There are three criteria to say whether a trace element is essential for the normal growth of plants or not:
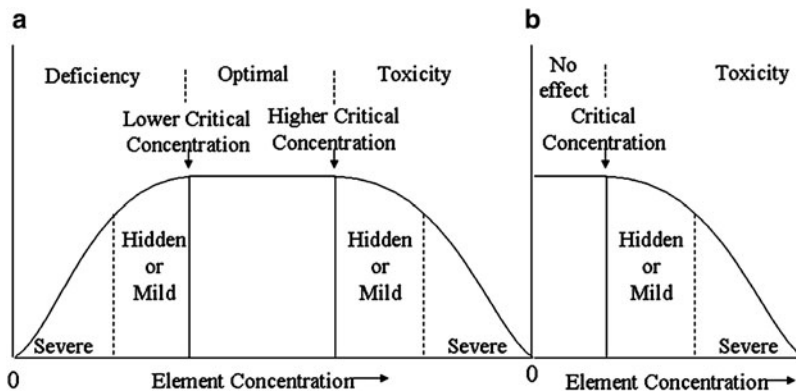
Fig. 1.2 Typical dose–response curves for (**a**) essential trace elements (micronutrients) and (**b**) nonessential trace elements (modified from Alloway 1995)

(a) The plant can neither grow nor complete its life cycle without an adequate supply of the element.
(b) The element cannot be wholly replaced by any other element.
(c) The element has a direct influence on the plant and is involved in its metabolism (Bowen 1979).

Apart from C, H, O, N, P, K, and S, the elements which have been shown to be essential for plants are Al, B, Br, Ca, Cl, Co, Cu, F, Fe, I, K, Mg, Mn, Mo, Na, Ni, Rb, Si, Ti, V, and Zn (Kabata-Pendias and Pendias 1992).

Essential trace elements are frequently referred to as micronutrients. If a plant supply of a micronutrient is inadequate, its growth is adversely affected. At the other extreme, an excessive supply of a micronutrient will cause toxicity. Typical dose–response curves for micronutrients and for nonessential trace elements (Fig. 1.2) show that when the supply of a micronutrient to a plant is inadequate, growth and yield are severely reduced and symptoms of deficiency are manifested. With an increasing supply of micronutrient, the yield reduction becomes progressively lower and the symptoms are less marked. As the supply of the micronutrient increases beyond the lower critical concentration, there is a zone of luxury consumption with no effect on yield. The upper critical concentration heralds the commencement of yield reductions due to toxicity, which becomes more severe until the lethal dose is reached.

The curve of nonessential elements in Fig. 1.2 shows that there is no deficiency effect with low concentration of the element; yield is not affected until the upper concentration limit is reached, after which toxicity occurs in the same way as with an excess of a micronutrient.

1.3.4 Heavy Metal Toxicity in Plants

Excessive concentrations of both essential and nonessential metals result in phytotoxicity. Kabata-Pendias and Pendias (1992) list the following possible causal mechanisms:

1. Changes in the permeability of the cell membrane: Ag, Au, Br, Cd, Cu, F, Hg, I, Pb, and UO_2
2. Reaction of sulphydryl (–SH) groups with cations: Ag, Hg, and Pb
3. Competition for sites with essential metabolites: As, Sb, Se, Te, W, and F
4. Affinity for reacting with phosphate groups and active groups of ADP or ATP: Al, Be, Y, Zr, lanthanides, and, possibly, all heavy metals
5. Replacement of essential ions (mainly major cations): Cs, Li, Rb, Se, and Sr
6. Occupation of sites for essential groups such as phosphate and nitrate: arsenate, fluorate, borate, bromate, selenate, tellurate, and tungstate.

In excessive amounts, the relative toxicity of different metals to plants can vary with plant genotype and experimental conditions. The most toxic metals to higher plants and microorganisms are Hg, Cu, Ni, Pb, Co, Cd, and possibly also Ag, Be, and Sn (Mench and Martin 1991). Food plants which tolerate relatively high concentrations of these potentially hazardous metals are likely to create a greater health risk than those which are more sensitive and show definite symptoms of toxicity.

1.3.5 Effects of Heavy Metals on the Soil Microbial Mass

Several authors have shown that high concentrations of various heavy metals in soils had inhibitory effects on microbial activity. Tyler et al. (1989) reported that the normal decomposition of conifer litter and recycling of plant nutrients were inhibited in the forest surrounding a brass foundry which had emitted large amounts of Cu, Zn, and other metals as aerosols over many years. The reason for the inhibition of microbial activity was that the growth of trees in the area was retarded due to deficiencies in plant macronutrients. However, other authors, such as Olson and Thornton (1982), have reported that soils from severely metal contaminated sites, such as Shipham in Somerset, contained bacteria that showed tolerance to Cd relative to bacteria in uncontaminated soils. Doelman and Haanstra (1979) showed that Pb inhibited both microbial respiration and dehydrogenase activity in polluted soils. Although tolerant populations of microorganisms were found in polluted soils, there was a change in the balance of the different types of the microorganisms present which could have an impact on soil fertility.

It was found that the metals from sewage sludge had a marked inhibitory effect on symbiotic nitrogen fixation in the roots of white clover due to toxicity affecting *Rhizobium leguminosum bv trifolii*. Experiments conducted in vitro showed a

decreasing order of toxicity as being: Cu > Cd > Ni > Zn (Chaudri et al. 1992). Other workers studying rhizobia in more recent field experiments elsewhere in UK concluded that the low number of these bacteria present was probably due to inhibitory effect of Cd (Obbard and Jones 1993). An independent study in Japan on the effects of Cd, Cr, Cu, Ni, and Pb on organic decomposition in gley and adosol soils showed that all the metals inhibited the evolution of CO_2. In this study, Cd and Cu showed the highest inhibitory effect, while Pb showed the smallest one (Hattori 1992).

1.4 Heavy Metal Detoxification of Soil

Lone et al. (2008) classified different approaches used to reclaim metal polluted soils into physicochemical and biological ones.

1.4.1 Physiochemical Methods of Remediating Metal Polluted Soil

The physiochemical approaches involved in soil remediation include as follows.

1.4.1.1 Excavation Method

This involves the excavation and reburial of polluted soils in special landfills (Conder et al. 2001; Jing et al. 2007; Lombi et al. 2002; Neilson et al. 2003; Bennett et al. 2003). Although the excavation method is the most used approach to reclaim contaminated soils (Lombi et al. 2001), it does not remediate the soil (Neilson et al. 2003).

1.4.1.2 Capping of the Polluted Soil

This involves top soiling of the polluted soils with uncontaminated soils from offsite to a depth that would minimize uptake of heavy metals by vegetation (Neilson et al. 2003; Okoronkwo et al. 2005). Still, this does not give a permanent solution to the problem since the metal can still be leached into the underground water.

1.4.1.3 Fixation and Inactivation (Stabilization) of the Polluted Soil

This involves the conversion of the heavy metals into those forms that are less mobile and available for plants and microflora (Lone et al. 2008; Conder

et al. 2001). Usually, the essence of stabilization is to reduce the amount of phytoavailable metal and thus reduce their toxicities to plants, animals, and soil organisms. Some commonly used chemical immobilization agents include zeolite, gravel sludge, beringite, alkaline materials, organic materials (sewage sludge and compost), phosphate (Conder et al. 2001), and lime stabilized municipal biosolids (Stuczynski et al. 2007; Conder et al. 2001).

1.4.1.4   Soil Washing

This technique involves the use of acids (HCI and HNO_3), chelators (EDTA, Nitriloacetic acid, DTPA, etc.), and other anionic surfactant (biosurfactant) (Neilson et al. 2003) to solubilize the polluting metals. It may take the form of in situ treatment, which involves soil flushing with pumps (Neilson et al. 2003), or ex situ treatment, which involves washing an excavated portion of the contaminated site with these agents followed by the return of clean soil residue to the site (Lone et al. 2008). This method is generally expensive and it is fraught with lots of side effects (Lone et al. 2008).

Other physicochemical methods include: thermal treatment (Jing et al. 2007), precipitation, or flocculation followed by sedimentation, ion exchanges, reverse osmosis, and microfiltration (Lone et al. 2008). These physicochemical approaches are not suitable for practical purposes because of their high cost, low efficiency, destruction of soil structure, and fertility (Lone et al. 2008; Jing et al. 2007).

1.4.2   Biological Approaches of Remediating Metal Polluted Soil

The biological approaches involved in soil remediation include:

- Use of microorganisms to detoxify metal by valence transformation, extracellular chemical precipitation, or volatilization (some microorganisms can enzymatically reduce a variety of metals in metabolic processes that are not related to metal assimilation) (Lone et al. 2008).
- Use of special type of plants to decontaminate soil or water by inactivating metals in the rhizosphere or translocating them in their aerial parts. This approach is called phytoremediation.

These new techniques are cheaper, efficient, and more environmental friendly (Lone et al. 2008; Jing et al. 2007).

1.4.2.1 Microorganism-Based Remediation

Selective pressures from a metal-containing environment have led to the development of resistance systems in microorganisms to virtually all toxic metals (Rouch et al. 1995). These systems are mostly plasmid-mediated and very specific and have been found in virtually all eubacterial groups studied (Silver and Misra 1984; Ji and Silver 1995). These reports included mostly aerobic microorganisms, with prominent examples being resistance in *Staphylococcus* sp., *Escherichia coli*, *Pseudomonas aeruginosa*, and *Bacillus* sp. (Nakahara et al. 1977; Marques et al. 1979; Harnett and Gyles 1984; Schwarz and Hobel 1989; Belliveau et al. 1991; Wang and Shen 1995). Resistance has been reported for mercury [Hg(II)] and organomercurials in obligate anaerobes like Bacteroides and Clostridium species.

There are differences between chromosomal and plasmid-based metal resistance systems. Essential metal resistance systems are usually chromosome-based and more complex than plasmid systems. Plasmid-encoded systems, on the other hand, are usually toxic-ion efflux mechanism.

A cell may develop metal resistance systems in an attempt to protect sensitive cellular components. Limiting metal access or altering cellular components decreases their sensitivity to metals. Several factors determine the extent of resistance in a microorganism: the type and number of mechanisms for metal uptake, the role each metal plays in normal metabolism, and the presence of genes located on plasmids, chromosomes, or transposons that control metal resistance. Six mechanisms are postulated to be involved in resistance to metals (Silver 1992; Rouch et al. 1995).

Metal Exclusion by Permeability Barrier

Alterations in the cell wall, membrane, or envelope of a microorganism are examples of metal exclusion by permeability barrier. This mechanism is an attempt by the organism to protect metal-sensitive, essential cellular components. A prominent example is the exclusion of Cu(II) resulting from altered production of the membrane channel protein porin by *E. coli* B (Rouch et al. 1995). This is usually a single gene mutation, which decreases the permeability of the membrane to metal ions (Ji and Silver 1995). Another example is nonspecific binding of metals by the outer membrane or envelope. This offers limited metal protection due to saturation of binding sites (Beveridge and Murray 1976; Hoyle and Beveridge 1983).

Bacteria that naturally form an extracellular polysaccharide coating demonstrate the ability to bioabsorb metal ions and prevent them from interacting with vital cellular components (Scott et al. 1988; Scott and Palmer 1990). The exopolysaccharide coating of these bacteria may provide sites for the attachment of metal cations (Scott and Palmer 1988). Exopolysaccharide by itself is not as efficient in binding Cd(II) as an organism with intact extracellular coating

(Scott and Palmer 1988). This protective layer appears to prevent uptake, keeping metal ions away from sensitive cellular components.

Periplasmic binding of Cu(II) is found in *Pseudomonas* sp. where resistance is coded for by an operon of four genes: *copA*, *copB*, *copC*, and *copD*; *copA* and *copB* confer partial resistance with the addition of *copC* and *copD*, providing for full Cu(II) resistance (Silver and Walderhaug 1992; Silver and Ji 1994). In some species of *Staphylococcus aureus*, penicillinase plasmids can mediate resistance by changing cell membrane permeability to Cd(II) as well as to other metals. In this case in the membrane appear conformational changes that prevent metal ions from entering.

Active Transport of the Metal Away from the Microorganism

Active transport or efflux systems represent the largest category of metal resistance systems. Microorganisms use active transport mechanisms to export toxic metals from their cytoplasm. These mechanisms can be chromosomal or plasmid-encoded. These efflux systems can be non-ATPase or ATPase-linked and highly specific for the cation or anion they export (Silver et al. 1989; Nies and Silver 1995). Evidence for ATP-dependence comes from the use of energy uncouplers and ionophore antibiotics (Silver and Misra 1984; Rensing et al. 1998). Uncouplers prevent the formation of ATP, which results in a decline in the efflux of cations or anions. Prominent examples are inducible plasmid-encoded resistance for As(V), arsenite [As(III)], and antimonite mediated by the *ars* operon in *E. coli* and *S. aureus*; Cd(II) resistance encoded by the *cad* operon in *S. aureus*, *Bacillus* sp., and *Listeria* sp. or the *czc* operon found in *Alcaligenes eutrophus*; and Pb(II) resistance mediated by *ZntA* in *E. coli* and *CadA* in *S. aureus* (Silver and Walderhaug 1992; Ji and Silver 1995; Rensing et al. 1998).

Intracellular Sequestration of Metals by Protein Binding

Intracellular sequestration is the accumulation of metals within the cytoplasm to prevent exposure to essential cellular components. Metals commonly sequestered are Cd(II), Cu(II), and Zn(II). Two examples exist for this form of metal resistance: metallothionein production in *Synechococcus* sp. and cysteine-rich proteins in *Pseudomonas* sp. (Rouch et al. 1995; Silver and Phung 1996). The metal resistance system in *Synechococcus* sp. consists of two genes: *smtA* and *smtB*. *smtA* encodes a metallothionein that binds to Cd(II) and Zn(II) (Silver et al. 1989; Silver 1992). Cysteine residues in SmtA metallothionein may act as a sink for excess toxic cations.

Extracellular Sequestration

Metal resistance based on extracellular sequestration has been hypothesized only in bacteria, but it is found in several species of yeast and fungi (Joho et al. 1995). One of the forms of Ni(II) resistance in yeast may be based on this mechanism. *Saccharomyces cerevisiae* may reduce absorption of Ni(II) by excreting large amounts of glutathione (Murata et al. 1985). Glutathione binds with great affinity to heavy metals. Yeast carrying the methylglyoxal resistance gene demonstrates the ability to form extracellular metal glutathione complexes in metal-rich media (Murata et al. 1985). Resistance results when the toxic metal is bound in a complex and cannot enter the cell membrane. A similar mechanism exists in Cu(II)-resistant fungi (Murphy and Levy 1983). These fungi secrete oxalate to form a metal–oxalate complex.

Enzymatic Detoxification of a Metal to a Less Toxic Form

Mercury resistance is a model example of an enzymatic detoxification system in microorganisms. Both Gram-positive (*S. aureus*, *Bacillus* sp.) and Gram-negative bacteria (*E. coli*, *P. aeruginosa*, *Serratia marcescens*, and *Thiobacillus ferrooxidans*) demonstrate resistance to Hg(II) (Misra 1992). Mercury is toxic because it binds to and inactivates essential thiols that are part of enzymes and proteins. Some bacteria contain a set of genes that form a Hg(II) (*mer*) resistance operon. This operon not only detoxifies Hg(II) but also transports and self-regulates resistance (Misra 1992; O'Halloran 1993; Ji and Silver 1995). The same set of genes also encodes the production of a periplasmic binding protein and membrane-associated transport proteins. The periplasmic binding protein collects Hg(II) in the surrounding environment and transport proteins take it to the cytoplasm for detoxification.

The Hg(II) resistance or *mer* operon codes for the production of two enzymes. The first organomercurial lyase catalyzes the following reaction (Weiss et al. 1977; Misra 1992):

$$RHgX + H^+ + X^- \Leftrightarrow RH + HgX_2$$
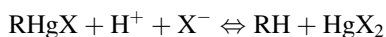
Mercuric ion reductase catalyzes the following reaction (Weiss et al. 1977; Misra 1992):

$$Hg(SR)_2 + NADPH + H^+ \Leftrightarrow Hg(0) + NADP^+ + 2RSH$$
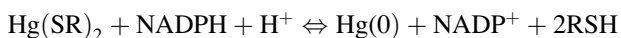
Another enzymatic detoxification system includes plasmid-mediated As(V) resistance in *B. subtilis*, *S. aureus*, and *E. coli* species (Rouch et al. 1995; Tsutomu

and Kobayashi 1998). The *arsC* gene of the *ars* operon codes for arsenate reductase, which reduces intracellular As(V) to the more toxic As(III) form (Nies and Silver 1995). As(III) is then removed from the organism by an efflux pump encoded by other genes in the *ars* operon (Wang and Shen 1995). This enzyme does not function on its own; instead it requires a coupling protein to reduce arsenate. These coupling proteins can vary among microorganisms; *S. aureus*, for example, uses thioredoxin, whereas *E. coli* uses glutaredoxin (Silver and Walderhaug 1992).

Reduction in Metal Sensitivity of Cellular Targets

Some microorganisms adapt to the presence of toxic metals by altering the sensitivity of essential cellular components; this provides a degree of natural protection (Rouch et al. 1995). Protection is achieved by mutations that decrease sensitivity but do not alter basic function or by increasing production of a particular cellular component to keep ahead of metal inactivation. DNA repair mechanisms also provide limited protection to plasmid and genomic DNA. The microorganism may also protect itself by producing metal-resistant components or alternate pathways in an effort to bypass sensitive components. Adaptation has been found in *E. coli*. Upon exposure to Cd(II), unadapted *E. coli* demonstrate considerable DNA damage; however, after subculture the same organisms show resistance (McEntee et al. 1986; Mergeay 1991). Natural resistance can result from normal cellular functions that give the organism a basic level of tolerance (Rouch et al. 1995). An example is glutathione, which may offer protection to metal ions such as Ag(I), Cu(I,II), Cd(II), and Hg(II) (Ni'bhriain et al. 1983) by suppressing free radical formation (Rouch et al. 1995).

1.4.2.2 Phytoremediation of Heavy Metal Polluted Soil

Phytoremediation is an integrated multidisciplinary approach to the cleanup of contaminated soils, which combines the disciplines of plant physiology, soil chemistry, and soil microbiology (Cunningham and Ow 1996). These contaminants include heavy metals, radionuclides, chlorinated solvents, petroleum hydrocarbons, PCBs, PAHs, organophosphate insecticides, explosives, and surfactants (Khan et al. 2004).

This technique involves the use of green plants to decontaminate soil, water, and air. Its application spans through the remediation of both organic and inorganic pollutants (Lone et al. 2008).

Certain species of higher plants can accumulate very high concentration of metals in their tissues without showing toxicity (Klassen et al. 2000; Bennett et al. 2003). Such plants can be used successfully to clean up heavy metal polluted soils. For having a feasible cleanup method, the plants must (1) extract large concentrations of heavy metals into their roots, (2) translocate the heavy metal

into the surface biomass, and (3) produce a large quantity of plant biomass. In addition, remediative plants must have mechanism(s) to detoxify and/or tolerate high metal concentrations accumulated in their shoots. In the natural setting, certain plants that have the potential of uptaking heavy metals have been identified. Indian mustard (*B. juncea*) is a high biomass, rapidly growing plant that has an ability to accumulate Ni and Cd in its shoots. It is a promising plant for phytoremediation (Terry et al. 1992).

In the following section, we mention different categories of phytoremediation such as phytoextraction, phytostabilization, rhizofiltration, and phytovolatilization (Lone et al. 2008).

Phytoextraction

This technology involves the extraction of metals by plant roots and the translocation thereof to shoots. The roots and shoots are subsequently harvested to remove the contaminants from the soil. With successive cropping and harvesting, the levels of contaminants in the soil can be reduced (Vandenhove et al. 2001). Usually, the shoot biomass is harvested for proper disposal in special site or is burnt to recover the metal (Bennett et al. 2003; Islam et al. 2007; Peciulytė et al. 2006). Researchers at the University of Florida have discovered the ability of the Chinese brake fern, *P. vittata*, to hyperaccumulate arsenic (Ma et al. 2001a). Sunflower, *Halianthus annus*, has proved effective in the remediation of radionuclides and certain other heavy metals (Schnoor 1997).

Phytostabilization

Phytostabilization, also referred to as in-place inactivation, is primarily used for the remediation of soil, sediment, and sludges (United States Protection Agency 2000). It is the use of plant roots to limit contaminant mobility and bioavailability in the soil. The plants' primary purposes are 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 a barrier to prevent direct contact with the contaminated soil, and (3) prevent soil erosion and the distribution of the toxic metal to other areas (Raskin and Ensley 2000). Phytostabilization can occur through the sorption, precipitation, complexation, or metal valence reduction. It is useful for the treatment of lead (Pb) as well as arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), and zinc (Zn). Some of the advantages associated with this technology are that the disposal of hazardous material/biomass is not required (United States Protection Agency 2000) and it is very effective when rapid immobilization is needed to preserve groundwater and surface water.

Rhizofiltration

Rhizofiltration is primarily used to remediate extracted groundwater, surface water, and wastewater with low contaminant concentrations (Ensley 2000). It is defined as the use of plants, both terrestrial and aquatic, to absorb, concentrate, and precipitate contaminants from polluted aqueous sources in their roots. Rhizofiltration can be used for Pb, Cd, Cu, Ni, Zn, and Cr, which are primarily retained within the roots (United States Protection Agency 2000; Jing et al. 2007). The advantages associated with rhizofiltration are the ability to use both terrestrial and aquatic plants for either in situ or ex situ applications. Another advantage is that contaminants do not have to be translocated to the shoots. Thus, species other than hyperaccumulators may be used. Terrestrial plants are preferred because they have a fibrous and much longer root system, increasing the amount of root area (Raskin and Ensley 2000). Dushenkov et al. (1995) observed that roots of many hydroponically grown terrestrial plants such as Indian mustard (*B. juncea* L.) and sunflower (*H. annuus* L.) effectively removed the potentially toxic metals, Cu, Cd, Cr, Ni, Pb, and Zn, from aqueous solutions. An experiment on rhizofiltration by Karkhanis et al. (2005) was conducted in a greenhouse, using *Pistia*, duckweed, and water hyacinth (*Eichornia crassipes*), to remediate aquatic environment contaminated by coal ash containing heavy metals.

Phytovolatilization

Phytovolatilization is the uptake and release into the atmosphere of volatile material such as mercury or arsenic containing compound (Jing et al. 2007; Lone et al. 2008). It involves the use of plants to take up contaminants from the soil, transforming them into volatile forms and transpiring them into the atmosphere (United States Protection Agency 2000). This process primarily has been used to remediate Hg^{2+} contaminated soil. The advantage of this method is that the contaminant, mercuric ion, may be transformed into a less toxic substance (i.e., elemental Hg). The disadvantage to this is that the mercury released into the atmosphere is likely to be recycled by precipitation and then redeposited back into lakes and oceans, repeating the production of methyl-mercury by anaerobic bacteria. Indian mustard and canola (*Brassica napus*) may be effective for phytovolatilization of selenium, and, in addition, accumulate the selenium (Bañuelos et al. 1997).

Lombi et al. (2001) suggested two approaches for the phytoextraction of heavy metals. The first is the continuous or natural phytoextraction. This involves the use of natural hyperaccumulator plants with exceptional metal accumulating capacity to remediate the soil.

The second approach suggested (Lombi et al. 2001) is the chemically enhanced phytoextraction. This involves the use of high biomass crops that are induced to take up large amount of metals when their mobility in soil is enhanced by chemical

treatment. The chemicals employed are mostly chelating agents such as EDTA, NTA, and citric acid (Lombi et al. 2001).

1.5   Heavy Metal Tolerance Mechanism(s) in Plants

Heavy metals such as Cu and Zn are essential for normal plant growth, although elevated concentrations of both essential and nonessential metals can result in growth inhibition and toxicity symptoms. Some plant species, however, have evolved tolerant races that can survive and thrive on such metalliferous soils, presumably by adapting mechanisms that may also be involved in the general homeostasis of, and constitutive tolerance to, essential metal ions as found in all plants. Plants have a range of potential mechanisms at the cellular level that might be involved in the detoxification and thus tolerance to heavy metal stress. The strategies for avoiding heavy metal buildup are diverse. Extracellularly they include roles for mycorrhizas and for cell wall and extracellular exudates. Tolerance could also involve the plasma membrane, either by reducing the uptake of heavy metals or by stimulating the efflux pumping of metals that have entered the cytosol. Within the protoplast a variety of potential mechanisms exist, for example, for the repair of stress-damaged proteins involving heat shock proteins or metallothioneins, and for the chelation of metals by organic acids, amino acids, or peptides, or their compartmentation away from metabolic processes by transport into the vacuole. This range of mechanisms is summarized in Fig. 1.3.

1.5.1   Extracellular Avoidance of Metal Buildup

1.5.1.1   Mycorrhizas

Although mycorrhizas are not always considered as plant metal tolerance mechanisms, they, and particularly ectomycorrhizas that are characteristic of trees and shrubs, can be effective in ameliorating the effects of metal toxicity on the host plant (Marschner 1995; Hüttermann et al. 1999; Jentschke and Godbold 2000). However, the mechanisms involved in conferring this increase in tolerance are difficult to understand as they may be quite diverse. Since large differences in response to metals have been observed, both between fungal species and to different metals within a species, these mechanisms show considerable species and metal specificity (Hartley et al. 1997; Hüttermann et al. 1999). For example, Colpaert and van Assche (1992) showed that the ectomycorrhizal fungus *Paxillus involutus* retained Zn and that this reduced the Zn content of *Pinus sylvestris*, whereas another species *Thelephora terrestris* retained little Zn and even increased the Zn content of the host (Colpaert and van Assche 1992). The mechanisms employed by the fungi at the cellular level to tolerate heavy metals are probably similar to some of the
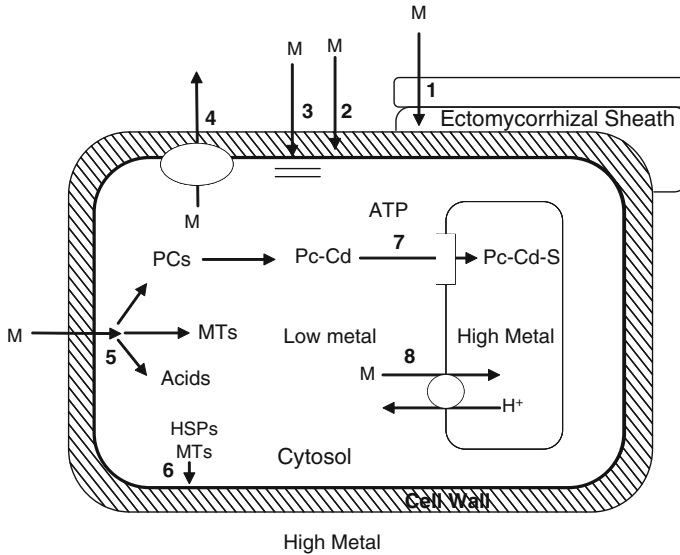
Fig. 1.3 Summary of potential cellular mechanisms available for metal detoxification and tolerance in higher plants. 1. Restriction of metal movement to roots by mycorrhizas. 2. Binding to cell wall and root exudates. 3. Reduced influx across plasma membrane. 4. Active efflux into apoplast. 5. Chelation in cytosol by various ligands. 6. Repair and protection of plasma membrane under stress condition. 7. Transport of PC–Cd complex into the vacuole. 8. Transport and accumulation of metals in vacuole (modified from Marschner 1995)

strategies employed by higher plants, namely binding to extracellular materials or sequestration in the vacuolar compartment. Thus in the fungus *Pisolithus tinctorius*, tolerance to Cu and Zn was achieved by binding to extrahyphal slime (Tam 1995), whereas detoxification of Cd in *P. involutus* involved binding of Cd to the cell walls and accumulation of Cd in the vacuole (Blaudez et al. 2000).

In relation to the role of ectomycorrhizas in metal tolerance by the host plant, most mechanisms that have been proposed involve various exclusion processes that restrict metal movement to the host roots. These have been extensively reviewed and assessed (Jentschke and Godbold 2000) and include absorption of metals by the hyphal sheath, reduced access to the apoplast due to the hydrophobicity of the fungal sheath, chelation by fungal exudates, and adsorption onto the external mycelium. Clearly, from the variation between species described above, these different exclusion mechanisms are likely to vary in significance between different plant and fungal interactions.

There are fewer reports on the role played by arbuscular mycorrhizas in metal tolerance. Weissenhorn et al. (1995) showed that the effects of maize root colonization by arbuscular mycorrhiza could either reduce the heavy metal content of the plants or increase metal absorption from polluted soils, depending on growth conditions, the fungus, and the metal. However, a *Glomus* isolate (Br1) obtained from zinc violets (*Viola calaminaria*) growing on a heavy metal soil was shown to

support the growth of maize and alfalfa on heavy metal soils more effectively than a commonly used *Glomus* isolate (Hildebrandt et al. 1999).

1.5.1.2 The Cell Wall and Root Exudates

The binding properties of the cell wall and the role on metal tolerance have been controversial. Although the root cell wall is directly in contact with metals in the soil solution, adsorption onto the cell wall must be of limited capacity and thus have a limited effect on metal activity at the surface of the plasma membrane. It is also difficult to explain metal-specific tolerance by such a mechanism (Ernst et al. 1992). However, Bringezu et al. (1999) reported that the heavy metal-tolerant *Silene vulgaris* ssp. *humilis* accumulated a range of metals in the epidermal cell walls, either bound to a protein or as silicates.

One related process concerns the role of root exudates in metal tolerance. Root exudates have a variety of roles (Marschner 1995) including that of metal chelators that may enhance the uptake of certain metals. In an investigation into the role of Ni-chelating exudates in Ni-hyperaccumulating plants, it was observed that the Ni-chelating histidine and citrate accumulated in the root exudates of nonhyperaccumulating plants and thus could help to reduce Ni uptake and so play a role in a Ni-detoxification strategy (Salt et al. 2000). Since the range of compounds exuded is wide, other exudates could play a role in tolerance to other metals. The clearest example of a role for root secretions in tolerance is in relation to organic acids and the detoxification of the light metal Al (Ma et al. 2001b). Buckwheat, for example, secretes oxalic acid from the roots in response to Al stress and accumulates nontoxic Al-oxalate in the leaves (Ma et al. 1997); thus, detoxification occurs both externally and internally.

1.5.1.3 Plasma Membrane

The plant plasma membrane may be regarded as the first 'living' structure that is a target for heavy metal toxicity. Plasma membrane function may be rapidly affected by heavy metals as seen by an increased leakage from cells in the presence of high concentrations of metals, particularly of Cu. For example, it was shown that Cu, but not Zn, caused increased K^+ efflux from excised roots of *Agrostis capillaris* (Wainwright and Woolhouse 1977). Similarly, others concluded that damage to the cell membrane, monitored by ion leakage, was the primary cause of Cu toxicity in roots of *Silene vulgaris*, *Mimulus guttatus*, and wheat, respectively (De Vos et al. 1991; Strange and Macnair 1991; Quartacci et al. 2001). Certainly direct effects of Cu and Cd treatments on the lipid composition of membranes have been reported (Ros et al. 1990; Fodor et al. 1995; Hernandez and Cooke 1997; Quartacci et al. 2001), which may have a direct effect on membrane permeability. In addition, Cd treatments have been shown to reduce the ATPase activity of the plasma membrane fraction of wheat and sunflower roots (Fodor et al. 1995), while, in *Nitella*, Cu-induced changes in cell

permeability were attributed to nonselective conductance increases and inhibition of the light-stimulated H^+-ATPase pump (Demidchik et al. 1997).

Thus, tolerance may involve the protection of plasma membrane integrity against heavy metal damage that would produce increased leakage of solutes from cells (De Vos et al. 1991; Strange and Macnair 1991; Meharg 1993). However, there is little evidence to show how this might be achieved. For example, metal-tolerant plants do not appear to possess enhanced tolerance to free radicals or reactive oxygen species, but rather rely on improved mechanisms for metal homeostasis (Dietz et al. 1999). Again these effects on membranes are metal-specific since, in contrast to Cu, Zn protects membranes against oxidation and generally does not cause membrane leakage (Ernst et al. 1992; Cakmak 2000). Another factor that may be involved in the maintenance of plasma membrane integrity in the presence of heavy metals could be enhanced membrane repair after damage (Salt et al. 1998). This could involve heat shock proteins or metallothioneins, and evidence for this is discussed in the following sections.

Apart from tolerance involving a more resistant plasma membrane or improved repair mechanisms, the cell membrane may play an important role in metal homeostasis, either by preventing or reducing entry into the cell or through efflux mechanisms. Many of these cations, of course, are essential and so complete exclusion is not possible; selective efflux may be more realistic. In bacteria, most resistance systems are based on the energy-dependent efflux of toxic ions (Silver 1996). It appears that the metabolic penalty for having more specific uptake mechanisms, and thus restricting the entry of toxic ions, is greater than that of having inducible efflux systems (Silver 1996).

The number of examples of exclusion or reduced uptake mechanisms in higher plants is quite limited. The clearest example of reduced uptake as an adapted tolerance mechanism is in relation to arsenic toxicity (Meharg and Macnair 1990, 1992). In *Holcus lanatus* roots, phosphate and arsenate appear to be taken up by the same systems. However, an arsenate-tolerant genotype showed a much lower rate of uptake for both anions than the nontolerant genotype, and also showed an absence of the high-affinity uptake system. The altered phosphate and arsenate uptake system was genetically correlated to arsenate tolerance (Meharg and Macnair 1992). More recently, a plasma membrane transporter in tobacco that confers Ni tolerance and Pb hypersensitivity has been described (Arazi et al. 1999). The transporter, designated Nt CBP4, is a calmodulin-binding protein that is structurally similar to certain K^+ and nonselective cation channels. Transgenic plants that overexpressed this transporter showed improved Ni tolerance and hypersensitivity to Pb, which were associated with reduced Ni accumulation and enhanced Pb accumulation.

An alternative strategy for controlling intracellular metal levels at the plasma membrane involves the active efflux of metal ions; although there is no direct evidence for a role for plasma membrane efflux transporters in heavy metal tolerance in plants, recent research has revealed that plants possess several classes of metal transporters that must be involved in metal uptake and homeostasis in general and thus could play a key role in tolerance. These include the heavy metal CPx-ATPases, the Nramps, and the CDF (cation diffusion facilitator) family

(Williams et al. 2000), and the ZIP family (Guerinot 2000). Recently, a role for the Nramps in Fe and Cd uptake has been reported (Thomine et al. 2000); interestingly, disruption of an *AtNramp 3* gene slightly increased Cd resistance, whereas overexpression resulted in Cd hypersensitivity in Arabidopsis.

1.5.2 Intracellular Detoxification Pathways

1.5.2.1 Heat Shock Proteins

Heat shock proteins (HSPs) characteristically show increased expression in response to the growth of a variety of organisms at temperatures above their optimal growth temperature. They are found in all groups of living organisms, can be classified according to molecular size, and are now known to be expressed in response to a variety of stress conditions including heavy metals (Vierling 1991; Lewis et al. 1999); they act as molecular chaperones in normal protein folding and assembly, but may also function in the protection and repair of proteins under stress conditions.

Several authors have reported an increase of HSP expression in plants in response to heavy metal stress. Tseng et al. (1993) showed that, in rice, both heat stress and heavy metal stress increased the levels of mRNAs for low molecular mass HSPs (16–20 kDa), while Neumann et al. (1995) indicated that HSP17 is expressed in roots of *Armeria maritima* plants grown on Cu-rich soils. Small heat shock proteins (e.g., HSP17) were also shown to increase in cell cultures of *Silene vulgaris* and *Lycopersicon peruvianum* in response to a range of heavy metal treatments (Wollgiehn and Neumann 1999); however, no or very low amounts of HSPs were found in plants growing on metalliferous soils, suggesting that HSPs are not responsible for the heritable metal tolerance of *Silene*.

1.5.2.2 Phytochelatins

Chelation of metals in the cytosol by high-affinity ligands is potentially a very important mechanism of heavy metal detoxification and tolerance. Potential ligands include amino acids and organic acids, and two classes of peptides, the phytochelatins and the metallothioneins (Rauser 1999; Clemens 2001). The phytochelatins have been the most widely studied in plants, particularly in relation to Cd tolerance (Cobbett 2000; Goldsbrough 2000).

The phytochelatins (PCs) are a family of metal complexing peptides that have a general structure $(\gamma \text{ Glu Cys})_n$ Gly, where $n = 2\text{--}11$, and are rapidly induced in plants by heavy metal treatments (Rauser 1995; Zenk 1996; Cobbett 2000; Goldsbrough 2000). PCs are synthesized nontranslationally using glutathione as a substrate by PC synthase (Grill et al. 1989; Rauser 1995), an enzyme that is activated in the presence of metal ions (Cobbett 2000). The genes for PC synthase

have now been identified in Arabidopsis and yeast (Clemens et al. 1999; Ha et al. 1999; Vatamaniuk et al. 1999).

It is shown that in *Brassica juncea* Cd accumulation is accompanied by a rapid induction of PC biosynthesis and that the PC content is theoretically sufficient to chelate all Cd taken up; this protects photosynthesis but did not prevent a decline in transpiration rate (Haag-Kerwer et al. 1999). In Arabidopsi*s*, Xiang and Oliver (1998) showed that treatment with Cd and Cu resulted in increased transcription of the genes for glutathione synthesis. Zhu et al. (1999) overexpressed the γ-glutamyl-cysteine synthetase gene from *E. coli* in *Brassica juncea* resulting in increased biosynthesis of glutathione and PCs and an increased tolerance to Cd.

The final step in Cd detoxification, certainly in the fission yeast and probably in higher plants, involves the accumulation of Cd and PCs in the vacuole (Salt et al. 1998; Schat et al. 2000). This accumulation appears to be mediated by Cd/H^+ antiporter and an ATP-dependent ABC transporter, located at the tonoplast (Salt and Wagner 1993; Salt and Rauser 1995; Rea et al. 1998); the stabilization of the Cd–PC complex in the vacuole involves the incorporation of acid-labile sulfide.

1.5.2.3   Metallothioneins

Higher plants contain two major types of cysteine-rich metal binding peptides: the metallothioneins (MTs) and the phytochelatins. MTs are gene-encoded polypeptides that are usually classified into two groups. Class 1 MTs possess cysteine residues that align with a mammalian (equine) renal MT; Class 2 MTs also possess similar cysteine clusters, but these cannot be easily aligned with Class 1 MTs (de Miranda et al. 1989; Robinson et al. 1993; Prasad 1999). In plants, there is a lack of information concerning the metals likely to be bound by MTs, although Cu, Zn, and Cd have been the most widely studied (Tomsett and Thurman 1988; Robinson et al. 1993; Goldsbrough 2000).

Although MTs can be induced by Cu treatments and there is evidence for a role in heavy metal tolerance in fungi and animals (Hamer 1986), the role of MTs in heavy metal detoxification in plants remains to be established (Zhou and Goldsbrough 1994; Zenk 1996; Giritch et al. 1998; Schat et al. 2000). However, it has been reported that MT2 mRNA was strongly induced in Arabidopsis seedlings by Cu, but only slightly by Cd and Zn (Zhou and Goldsbrough 1994); when genes for MT1 and MT2 from Arabidopsis were expressed in an MT-deficient yeast mutant, both genes complemented the mutation and provided a high level of resistance to Cu. Van Vliet et al. (1995) showed that MT genes can be induced by Cu and that the expression of MT2 RNA is increased in a Cu-sensitive mutant of Arabidopsis that accumulates high concentrations of Cu. In contrast, in a study of the effects of Cd exposure on *Brassica juncea*, it was reported that MT2 expression was delayed relative to PC synthesis (Haag-Kerwer et al. 1999) and they argued against a role for MT2 in Cd detoxification. MTs could clearly play a role in metal metabolism, but their precise function is not clear; they may have distinct functions for different metals (Hamer 1986).

1.5.2.4   Organic Acids and Amino Acids

Carboxylic acids and amino acids such as citric, malic, and histidine are potential ligands for heavy metals and so could play a role in tolerance and detoxification (for reviews, see Rauser 1999; Clemens 2001); however, strong evidence for a function in tolerance, such as a clear correlation between amounts of acid produced and exposure to a metal, is not yet existing. For example, a 36-fold increase was reported in the histidine content of the xylem sap on exposure to Ni in the Ni-hyperaccumulating plant *Alyssum lesbiacum* (Krämer et al. 1996). In addition, supplying histidine to a nonaccumulating species greatly increased both its Ni tolerance and the capacity for Ni transport to the shoot. However, the histidine response may not be a widespread mechanism of Ni tolerance since it was not observed in another Ni hyperaccumulator, *Thlaspi goesingense* (Persans et al. 1999). A possible role of the histidine in the root exudates as a Ni detoxifying agent has been discussed earlier (see Sect. 1.5.1.2).

1.5.2.5   Antioxidative Defense Mechanism

Buildup of toxic concentration of heavy metals within the plant tissues results, at some stage of stress exposure, in an increased formation of reactive oxygen species (ROS) (Shah et al. 2001; Verma and Dubey 2003). In general, ROS (O_2^-, OH, and H_2O_2) are products of normal cellular metabolism, production of which is under tight control due to cellular antioxidative defense system. Presence of ROS causes oxidative damage to biomolecules such as lipids, proteins, nucleic acids, etc. (Shah et al. 2001; Blokhina et al. 2003). Induction in the activities of antioxidative enzymes and increase in the level of nonenzymic antioxidants are strategies that plants have adopted to scavenge and to reduce oxidative damage caused due to ROS under heavy metal stress (Shah et al. 2001; Fecht-Christoffers et al. 2003; Verma and Dubey 2003).

Recent reports suggest that though antioxidative defense system is not directly involved in heavy metal detoxification, yet ROS play important role as intermediate signaling molecules to regulate the expression of genes for plant's defense system (Orozco-Cardenas et al. 2001; Vranova et al. 2002).

1.5.2.6   Heavy Metal Sequestration

In Vacuoles

Various metabolites and ions are stored inside the vacuoles. Vacuolar sequestration of a number of heavy metals such as Cd, Ni, As, and Zn is known, which diverts metal ions from metabolically active compartments (cytosol, chloroplasts, and mitochondria) and minimizes the harmful effects of metal ions to vital cellular processes. Transporters are present in internal membranes to allow regulation of

stored metals in organelles. Active accumulation of most of the metal ions is driven by the electrochemical potential by electrogenic proton influxes via the vacuolar H^+-ATPase (Kakinuma et al. 1993). Cd is transported across the tonoplast by a Cd^2/H^+ antiport mechanism (Carrier et al. 2003). Both vacuolar (Bidwell et al. 2004) and extravacuolar localization of Ni ion occurs via a pH-gradient-dependent manner in yeast (Nishimura et al. 1998), whereas in Ni-hyperaccumulator plant *Thlaspi goesingense* vacuolar metal transport proteins termed as metal-tolerance proteins (TgMTPs) are involved in the compartmentalization of Ni in vacuoles (Lombi et al. 2002). Transport of Zn to the vacuoles is mediated by 'Zn-malate shuttle', malate being liberated in exchange for oxalate or citrate and is shuttled back to cytoplasm (Ernst et al. 1992).

In Trichome and Hydropotes

Apart from vacuolar sequestration, plants possess additional morphological features that are also involved in heavy metal sequestration and detoxification. Several reports have confirmed the involvement of glandular trichomes and epidermal structures (hydropotes) in the chelation, sequestration, and detoxification of the metals.

Trichomes are epidermal hairs present at the surface of plant leaves and have diversified roles in exudation of various molecules, protection against wind and sunlight, storage of metals, etc. Retardation in growth and about twofold increases in the number of trichomes were observed in Cd-exposed tobacco seedlings (Choi et al. 2001). A significant proportion of Ni has been found in trichomes of *Alyssum lesbiacum* plants (Krämer et al. 1996). At the bases of *Arabidopsis halleri* trichomes, elevated concentrations of Zn have been found (Sarret et al. 2002). Specific overexpression of a gene coding for a metallothionein (MT2) has been reported in trichomes (Garcia–Hernandez et al. 1998), which suggests that trichomes constitute important sites for accumulation as well as detoxification of toxic metal ions.

In the semiaquatic and aquatic plants of the families Menyanthaceae and Nymphaceae, hydropotes located on the abaxial epidermis of the leaf laminae accumulate Cd (Lavid et al. 2001a). It is suggested that usual polymerization of polyphenols by peroxidase in hydropotes gets enhanced after uptake of heavy metals and thereby detoxification of metals occurs by their binding with polyphenols in these glands (Lavid et al. 2001b, c).

1.6 Conclusion

The pollution of soil and water with heavy metals is an environmental concern today. Metals and other inorganic contaminants are among the most prevalent forms of contamination found at waste sites, and their remediation in soils and sediments is among the most technically difficult. The high cost of existing cleanup

technologies led to the search for new cleanup strategies that have the potential to be low cost, low impact, visually benign, and environmentally sound.

Phytoremediation is a potential remediation strategy that can be used to decontaminate soils contaminated with inorganic pollutants. But phytoremediation technology is still in its early development stages and full-scale applications are still limited. For widespread future use of this technique, it is important that public awareness about this technology is considered and clear and precise information is made available to the general public to enhance its acceptability as a global sustainable technology to be widely used.

The recent advances in plant biotechnology have created a new hope for the development of hyperaccumulating species. However, research work is needed in this respect such as metal uptake studies at cellular level including efflux and influx of different metal ions by different cell organelles and membranes. Rhizosphere studies under the control and field conditions are also needed to examine the antagonistic and synergistic effects of different metal ions in soil solution and the polluted waters. In-depth soil microbial studies are required to identify the microorganisms highly associated with metal solubility and/or precipitations. To date, the available methods for the recovery of heavy metals from plant biomass of hyperaccumulators are limited. Traditional disposal approaches such as burning and ashing are not applicable to volatile metals; therefore, investigations are needed to develop new methods and strategies for effective recovery of metals from the hyperaccumulator plant biomass.

New approaches of genetic manipulation techniques increase the possibility of creating super-accumulator bacteria or plants that can help decontaminate polluted soils, water, and sewage.

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