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Plant-driven removal of heavy metals from soil: uptake, translocation, tolerance mechanism, challenges, and future perspectives

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Abstract Increasing heavy metal (HM) concentrations in the soil have become a significant problem in the modern industrialized world due to several anthropogenic activities. Heavy metals (HMs) are non-biodegradable and have long biological half lives; thus, once entered in food chain, their concentrations keep on increasing through biomagnification. The increased concentrations of heavy metals ultimately pose threat on human life also. The one captivating solution for this problem is to use green plants for HM removal from soil and render it harmless and reusable. Although this green technology called phytoremediation has many advantages over conventional methods of HM removal from soils, there are also many challenges that need to be addressed before making this technique practically feasible and useful on a large scale. In this review, we discuss the mechanisms of HM uptake, transport, and plant tolerance mechanisms to cope with increased HM concentrations. This review article also comprehensively discusses the advantages, major challenges, and future perspectives of phytoremediation of heavy metals from the soil.

Keywords Phytoremediation · Metal ion uptake · Translocation · Tolerance mechanisms

Introduction

Phytoremediation can be defined as a process in which green plants remove, sequester, or stabilize many organic and inorganic contaminants including heavy metals to render them harmless (Salt et al. 1998). It is very difficult to remove heavy metals (HMs) from soils mainly because, being inorganic contaminants, they are bound to the soil matrix and cannot be easily mineralized. Thus, they can be removed using physical, chemical, and biological methods of remediation (Cunningham and Ow 1996). Different physico-chemical and engineering techniques have been developed and employed for the purpose of removing toxic HM ions from polluted soils and waters. They, however, are associated with several disadvantages including negative effect on soil properties and biodiversity, and being quite expensive (Padmavathiamma and Li 2007). Phytoremediation offers an eco-friendly alternative. It can be used as a very effective technique as metal hyperaccumulator plants accumulate heavy metals in aboveground parts. Then, the plants could be harvested and heavy metals removed and further used for industrial uses (Salt et al. 1998).

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Phytoremediation is classified into five subgroups: (i) phytoextraction, the uptake of HM ions from soil and direct translocation into the plant biomass. Metals are removed by subsequent removal of the plants. (ii) Phytodegradation: Some plants can enhance microbial degradation of organic contaminants in the rhizosphere, implied in both soil and water. (iii) Phytostabilization: Metal ions become less available in soil due to absorption and precipitation within the roots and root zone, implied in both soil and water. (iv) Phytovolatilization: Plants uptake HM ions (Hg, Se, volatile hydrocarbons) from soil and emit them into the air in volatile form through transpiration, implied in both soil and water. (v) Rhizofiltration: Metal ions are uptaken and removed from contaminated water by plant roots, implied in surface water. Among these, phytoextraction, rhizofilteration, and phytostabilization are commercially important.

A hyperaccumulator plant can be distinguished from a non-hyperaccumulator by its capability to absorb and accumulate exceptionally high (50–100 times than non-accumulators) concentrations of HM ions in their aboveground tissues without severe damage to plant growth and vital physiological processes (McGrath and Zhao 2003; Rascio and Navari-Izzo 2011). Hyperaccumulator plants have an exceptional ability to translocate (having translocation factor >1) the high concentrations of toxic metal ions from roots to shoot tissues and render them harmless by sequestering them away from the cytoplasm (Rascio and Navari-Izzo, 2011). Translocation factor is the ratio of heavy metal ions in the shoot to that in the root. The other factor which is very important for phytoremediation and identification of hyperaccumulator species is bioconcentration factor. It is the ratio of metal ion concentration in plant tissue to soil. About 0.2 % of all known plant species are classified as HM accumulators (Rascio and Navari-Izzo, 2011; Sarma 2011). Since the emergence of the idea of phytoremediation (Utsunamyia 1980; Chaney 1983), many studies have been done on this novel technique called phytoremediation, but there are still many knowledge gaps to be addressed before the actual commercial field level utilization of this green technology.

In present review, we discuss the various aspects of phytoremediation including metal ion uptake from soil, translocation, mechanisms of HM hypertolerance in plants, advantages/challenges, and future perspectives for the success of green remediation technology.

Uptake of metal ions from soil

The total concentrations of heavy metal present in soil or water are not readily available for bioaccumulation. Among the heavy metal ions, some are more mobile and available for plant uptake, e.g., Cd and Zn, than others like Pb which are relatively immobile (Lasat 2000). Thus, to make metal ions available to be taken up by roots, they must be mobilized into the soil solution first. The mobility of metal ions in soil mainly depends on the pH of soil and presence of chelating agents like EDTA. Many other factors such as root size, external metal concentrations, temperature, metal interaction, addition of nutrients, and salinity also seem to play a minor role to influence the metal ions mobility in soil (Rieuwerts et al. 1998). Plants employ various strategies to increase the bioavailability of metal ions. For example, secretion of phytosiderophores, carboxylates, and acidification of the rhizosphere to facilitate the chelation and solubilization of soil bound metals (Kinnersley 1993). Besides, the solubility of metal ions in water is very low and they have strong affinity toward soil particles and many other organic contaminants present in soil. In this case, the use of soil microorganisms to make metal ions available for translocation is a beneficial technique. The soil microorganisms present in rhizosphere and enzymes secreted by them also have a crucial role in controlling the availability of metal ions for absorption by roots (Burns and Dick, R. P. Eds.. 2002). The mobile fraction of metal available in soil which is ready for absorption by plants is known as the bioavailable portion. For the successful reclamation of metal-contaminated soil, it is very important to identify the bioavailable fraction from the total metal concentration present in soil (John and Leventhal 1995; Olaniran et al. 2013).

After absorption by roots, the metal ions first come in contact with the cell wall which is an ion exchanger of low selectivity and affinity (Ghosh and Singh 2005). The uptake of heavy metals into roots occurs either by passive diffusion through the cell membrane or by the more common process of active transport against concentration and/or electrochemical potential gradients mediated by carriers. These carriers can be complexing agents, such as organic acids or proteins that bind to the metal species (Fergusson 1990). For example, under Al stress, the roots of buckwheat have been reported to secret oxalic acid and form a non-toxic Al-oxalate complex which is then translocated into the leaves (Ma et al., 1998; Hall 2002).

Translocation of metal ions in plants

There are two pathways available for metal ions to enter into the roots: apoplastic and symplastic. The apoplastic movement of the metal ions is possible only as non-cationic metal chelates, because cell walls have comparatively high exchange capacity for cations (Raskin et al. 1997). Thus, as the most of the metal ions are insoluble and unable to move on their own in vascular system, they are immobilized in apoplastic and symplastic compartments after forming carbonate, sulphate, or phosphate precipitates (Raskin et al. 1997; Garbisu and Alkorta 2001). Metal hyperaccumulator plants translocate a very high concentration of metal ions into the shoot via symplastic movement through the xylem. Heavy metal ions enter into the xylem stream via root symplasm (Tester and Leigh 2001). For symplastic movement of metal ions, they must have to cross the plasma membrane. The high negative resting potential of plasma membrane facilitates the inward movement of metal ions due to electrochemical gradient (Raskin et al. 1994). Also, the metal ions sequestered inside the cell vacuoles may enter into the xylem stream via the stele. Thus, the entry of metals from the root into the xylem is mainly determined by three processes: metal ion sequestration into root cells, symplasmic transport into the stele, and release into the xylem (Ghosh and Singh 2005; Saxena and Misra 2010). In xylem, the transport of metal is mediated by membrane transport proteins. The transport of metal ions through casparian strip occurs by energy requiring active transport system (Cunningham and Berti 1993). Once inside a cell, the metal ions can move along a concentration gradient or through different types of cation channels in the cell membrane meant for other essential metal ions (Prasad 2004). The relationship between elevated transcript levels of metal transporter genes and metal uptake has been well documented in literature (Pence et al. 2000; Lombi et al. 2002). With techniques like transporter identification by sequence comparison, yeast mutant complementation and regulated gene activities in transgenic plants several gene families have been identified which are likely to aid the transport of HM ions. These gene families include the Zn-regulated transporter (*ZRT*), the heavy metal (or CPx-type) ATPases, the cation diffusion facilitators (CDFs), the natural resistance-associated macrophage proteins (Nramps), cation antiporters, and the ZIP family (Guerinot 2000; Williams et al. 2000; Gaxiola et al. 2002).

The superfamily of P-type ATPases performs the function of transport of a wide range of cations across the cell membranes. For example, there are 8 heavy metal ATPases, 6 Nramps, and 15 ZIPs present in *Arabidopsis* (Mills et al. 2003; Williams et al. 2000; Mäser et al. 2001; Hall and Williams 2003). The heavy metal ATPases (HMAs) possess eight transmembrane domains with large cytoplasmic loop (Williams et al. 2000; Mills et al. 2003) which contains the phosphorylation site along with many highly conserved motifs (Palmgren and Harper 1999; Hall and Williams 2003). The existence of two main groups of transporters has been suggested by the phylogenetic analysis of a subfamily (P1B) of HMAs: one having specificity for monovalent cations (e.g., Cu^+ and Ag^+) and the other for divalent cations (e.g., Zn^{2+}, Pb^{2+}, and Cd^{2+}) (Axelsen and Palmgren, 2001; Mills et al., 2003; Hall and Williams 2003).

The ATP-binding cassette (ABC) superfamily is another important and diverse family of transmembrane proteins involved in a wide range of transport functions by utilizing energy from ATP hydrolysis (Rea 1999; Martinoia et al. 2002). In plants, 13 subfamilies of this superfamily are identified, the multidrug resistance proteins (MDRs) being the largest (Rea 2007). Other important subfamilies of ATP-binding cassette (ABC) superfamily are multidrug resistance-associated protein homologs (MRPs), peroxisomal membrane protein homologs (PMPs), pleiotropic drug resistance homologs (PDRs), etc. (Rea 2007). They have a role in Mg-ATP-hydrolysis-driven vacuolar sequestration of glutathione S-conjugates (GS-conjugates) (Martinoia et al. 1993).

The natural resistance-associated macrophage proteins (Nramps) is also an important integral membrane protein family involved in transport of metal ions in a wide range of organisms, including both prokaryotes and eukaryotes. Three different Nramps have been identified in yeasts. They mediate the uptake of many heavy metal ions (Cu^{2+}, Mn^{2+}, Co^{2+}, Fe^{2+}, and Cd^{2+}) (Supek et al. 1997; Chen et al. 1999). The occurrence of Nramp in bacteria, yeast, and plants has been well documented in the literature (Eide 1998; van der Zaal et al. 1999). For example, the analysis of expression of AtNramp genes in *Arabdopsis* has established their role as constitutive metal transporters involved in transport of Fe, Mn, and Cd (Thomine et al. 2000).

The ZIP (ZRT, IRT-like proteins) family of genes is involved in the transport of many cations (e.g., Fe, Mn, and Zn) (Guerinot 2000). Different subfamilies of ZIP gene superfamily exhibit variations in terms of substrate and specificity (Guerinot 2000; Mäser et al. 2001). The ZIP family members have been identified from both prokaryotes and eukaryotes. The 2 ZIP family members are identified in *Oryza sativa* and 15 in *Arabidopsis* (Grotz et al. 1998; >Mäser et al. 2001). On the basis of alignment of the predicted amino acid sequences, ZIPs could be grouped into four subfamilies (Gaither and Eide, 2001). However, *ZIP* higher plant genes could be grouped together (Mäser et al. 2001).

The cloning of various *ZIP/NRAMP* transporter genes from plant species and other organisms has shown a wide range of metal specificity and sequences (Guerinot 2000; Williams et al. 2000). These investigations of ZIP and NRAMP gene families suggested the existence of consensus regions on the amino acid sequences which could be responsible for the determination of the metal transport. For example, the histidine-rich region found between transmembrane domains III and IV of *AtIRT1* is a cytoplasmic metal ion-binding site (Zhao and Eide 1996).

The available literature clearly shows that there is an array of transporters/pathways for the cellular uptake of diverse HMs including both essential and non-essential ones. They do exhibit considerable non-specificity.

Mechanisms of heavy metal tolerance in plants

Heavy metal tolerance is the key prerequisite for the successful implementation of phytoremediation. Plants are equipped with multiple means of HM detoxification and tolerance. They are mutually exclusive and contribute to the varying extents to detoxification of individual HMs. Through these, plants try to keep the cellular concentrations of HMs below the toxicity threshold levels (Hall 2002; Sharma and Dietz 2006). Broadly, there are two defense strategies adopted by plants to prevent the accumulation of excess metal concentrations in the cytoplasm: avoidance and tolerance. According to Verkleij and Schat (1990), avoidance refers to the ability of plants to hinder excessive metal uptake. On the other hand, tolerance refers to the ability to cope with the accumulated metal ions by using different mechanisms. These mechanisms depend upon the metal involved and its concentration, plant species, organs, and developmental stage (Navari-Izzo and Quartacci 2001).

Avoidance mechanisms adapted by plants under HM stress

Avoidance mechanisms used by plants include metal exclusion, complexation to various ligands, and translocation (Navari-Izzo and Quartacci 2001). Plants avoid toxic metal ion accumulation in the cytoplasm by preventing metal ion transport across the plasma membrane (Tong et al. 2004; Yang et al. 2005) and altering membrane permeability, changing metal–cell wall binding capability, increasing the exudation of metal-chelating substances, and stimulating the efflux pumping (Hall 2002; Yang et al. 2005). Embedding the toxic metals in the plant cell walls is another distinct mechanism of metal tolerance and accumulation by plants (Memon and Schröder 2009). The amount of free metal ions entering the cells can be reduced by binding them to negatively charged polygalacturonic acids (Ernst et al. 1992).

Plasma membrane is the primary site of heavy metal toxicity. Plants tend to control the entry of heavy metal ions at plasma membrane level as its failure may lead to the disruption of normal cellular functions and ultimately the whole plant functions (Dietz et al. 1999). Toxic effects of heavy metal ions on plasma membrane include K^+ leakage, oxidation, and cross-linking of protein thiols and inhibited activity of membrane proteins (Ernst et al. 1992; Hall 2002) and alteration of membrane lipid composition and fluidity. Active efflux mechanisms of plasma membrane prevent the accumulation of metal ions into the cytosol. Avoiding the entry of heavy metal cations in cytoplasm and preventing the development of oxidative stress are the major strategies employed by metal-tolerant plants (Dietz et al. 1999).

Heavy metal ion chelation and compartmentalization

Once the metal ions get entry into cytosol, they can be removed by chelation (Clemens 2001). The chelating and compartmentalization of metal ions are the most likely defense mechanisms implied by plants to resist the detrimental effects of metals (Cunningham et al. 1995). There are many organic and inorganic ligands present in the cytoplasm that are capable of heavy metal chelation. The main organic compounds involved in metal ion chelation are phytochelatins (PCs), metallothioneins (MTs), organic acids, amino acids, and cell wall proteins/pectins/polyphenols (Hall 2002; Sharma and Dietz 2006) whereas the main inorganic compounds are phosphates and silicates (Bourg and Loch 1995). These metal-chelating compounds reduce heavy-metal-induced phytotoxicity by reducing the free metal ion concentrations through chelation (Salt et al. 1998). For example, Al is converted to non-toxic Al-oxalate in wheat roots and leaves (Hall 2002), Zn to Zn-phytate, and Pb to Pb-carbonate, -sulphate, and -phosphate (Salt et al. 1998). The resulting metal–chelator complex is transported actively from the cytosol across the tonoplast into the vacuoles. For example, Vögeli-Lange and Wagner (1990) studied the mesophyll protoplasts isolated from tobacco plants (*Nicotiana rustica* var. *pavonii*) exposed to Cd and reported that Cd was chelated by PCs and the Cd-PC complex was transported actively into the vacuole across the tonoplast. The role of other organic metal chelators in HM ions detoxification from cytoplasm is also well documented in the literature. For example, Van Hoof et al. (2001) suggested that MTs were involved in the Cu tolerance of Cu-tolerant *Silene vulgaris* populations. And, Boominathan and Doran (2003) studied the heavy metal uptake and organic acid response in the hairy roots of Cd and Ni hyperaccumulator *Thlaspi caerulescens* and *Alyssum bertolonii*, respectively. They observed that the levels of citric, malic, and malonic acids were constitutively high in the hairy roots of both species and reported 13 % of the total Cd and 28 % of the total Ni in *T. caerulescens* and *Alyssum bertolonii* hairy roots, respectively, to be associated with organic acids. Nicotinamine (NA) is another important metal chelator found in plants that forms strong complexes with most of the transition metal ions. The role for NA was proposed in Ni hyperaccumulation after the identification of Ni-NA complexes in Ni-exposed roots of *T. caerulescens* (Vacchina et al. 2003; Mari et al. 2006). The vacuoles are the last destination for these metal–chelator complexes as these complexes are removed from the cytoplasm by efflux of ions into vacuoles (Hall 2002). This is achieved by the increased ability to transport metals into the vacuoles. For example, overexpression of a Zn transporter (ZAT) has been shown to increase the Zn tolerance by its sequestration into the vacuoles (Hall 2002). A mutant yeast (*hmt1*) incapable of accumulating Cd-PC complex was found to be Cd-sensitive (Salt et al. 1998). Also, barley roots accumulated elevated transcript levels of two vacuolar ATPase subunits, namely, VHA-E and VHA-c, under Cd and Fe stress (Sharma et al. 2004).

A few other compartmentalization strategies are also employed by plants other than metal ion sequestration into vacuoles. These include the heavy metal ion sequestration in the apoplast or specialized cell types, e.g., epidermal cells, mesophyll cells, and trichomes (Eapen and D'souza 2005). For example, Cd, Mn, and Pb were found to be sequestered in trichomes (Salt et al. 1998). Another effective way to get rid of excess ions is the translocation of metals into old leaves, which are then removed as a result of natural leaf shedding. For example, Zn was reported to accumulate in leaves during the last week prior to leaf shedding; thereby, plants use the leaf fall as a means of reducing the level of toxic metals inside the plant body (Ernst et al. 1992).

Role of amino acids in heavy metal tolerance mechanism

Nitrogen donor ligands, particularly free amino acids, are thought to play a role in heavy metal tolerance. Histidine is regarded as one of the most important amino acids involved in HM ion hyperaccumulation, particularly for Ni hyperaccumulation (Callahan et al. 2006). For example, Krämer et al. (1996) using HPLC reported a substantial increase (36-fold) in histidine concentration in xylem sap of *Alyssum lesbiacum*, a Ni hyperaccumulator plant, when exposed to 0.3 mM Ni compared to control plants. Earlier, Farago et al. (1980) also reported the dominance of amino acids serine, aspartic acid, and isoleucine in Ni hyperaccumulator species *Hybanthus floribundus*.

Accumulation of another amino acid, free proline, in response to heavy metal exposure is also reported in many studies (Schat et al. 1997; Sharma and Dietz 2009). Approximately 20-fold increase in proline content was observed under metal (Cd, Cu, Zn) stress in *S. vulgaris* (Schat et al. 1997). The functions of proline seem to be manifold. It plays a major role in adjustment to osmotic stresses, maintaining the water balance as it stabilizes the subcellular structures, and has a role in free radical scavenging (Kavi Kishor et al. 1995; Sharma and Dietz 2006). Accumulation of proline has also been suggested to be related to the changes of water status of the plants under heavy metal stress. The exposure to heavy metals such as Cd and Ni is known to deteriorate the plant water balance (Barceló and Poschenrieder 1990; Costa and Morel 1994). The alleviation of metal-induced water stress in plants by elevated proline concentration could have a possible role in the heavy metal tolerance. Besides, proline has also been reported to have a role as free radical scavenger, having been demonstrated in a number of in vitro assay systems (Alia et al. 2001; Kaul et al. 2008). $OH^{\cdot}$ radical scavenging properties of proline were reported by Smirnoff and Cumbes (1989).

At high metal concentrations, avoidance mechanisms do not work. The inadequate avoidance mechanisms result in the increased metal ion concentrations in the cytoplasm causing the formation of free radicals that leads to the oxidative stress. Thereafter, plants need to undergo some biochemical changes to defend against oxidative stress (Navari-Izzo and Quartacci 2001). The degree of heavy-metal-induced cell damage depends on the rate of free radical and reactive oxygen species formation, and the efficiency of detoxification and repair mechanisms (Dietz et al. 1999).

Tolerance mechanisms adapted by plants under HMs stress

Plants need to implement a rapid and effective strategy of detoxification to minimize the extent of reactive oxygen species (ROS)-induced cellular damage. They are able to induce an array of enzymatic and non-enzymatic antioxidative defense mechanisms upon initiation of ROS burst. Superoxide dismutase (SOD) is included in the first line of enzymatic antioxidative defense system within a cell. It catalyzes the dismutation of superoxide anion ($O_2^{\bullet-}$) into hydrogen peroxide (H_2O_2) and oxygen (O_2). The metal-cofactor-dependent isoforms of SOD are located in different cell compartments (Dalton 1995). Catalase (CAT), another important antioxidative enzyme, converts hydrogen peroxide (H_2O_2) to water (H_2O) and oxygen (O_2). It is found predominantly in peroxisomes (Dalton 1995). The SOD-CAT equilibrium is essential for maintaining steady-state level of $O_2^{\bullet-}$ and H_2O_2. Peroxidase (POD) is a heme-containing protein and utilizes H_2O_2 to oxidize organic and inorganic substrates. Glutathione reductase (GR) is a flavoprotein that is distributed in cytosol, mitochondria, and endoplasmic reticulum. It is an integral part of ascorbate–glutathione cycle and catalyzes the glutathione disulphide (GSSG) reduction in to glutathione (GSH) via NADPH-dependent reduction pathway. Glutathione peroxidase (GPx) converts H_2O_2 to H_2O using GSH as reducing agent in GPx cycle. It also catalyzes the oxidation of lipid hydroperoxides to other hydroperoxides (Ahmad 1995).

Responses of antioxidative enzyme activities play a crucial role in determination of the tolerance and sensitivity levels of different species/genotypes under HM stress. For example, Wang et al. (2008) concluded that metal accumulator plants (*T. caerulescens* and *Brassica juncea*) are equipped with superior antioxidative defense system as compared to non-accumulators (*Nicotiana tabacum*) under Cd stress. Similarly, roots of hyperaccumulator *Alyssum bertolonii* were found to be better protected by higher endogenous activities of antioxidative enzymes like CAT and SOD as compared to non-accumulator *N. tabacum* under Ni stress (Boominathan and Doran, 2002). Therefore, the way plant's antioxidative defense system responds under any particular HM stress plays a very important role in HM tolerance.

Low molecular weight antioxidants such as ascorbate, glutathione, and tocopherols are also known to have an important role in plant responses to the HM stress. They fulfill multiple roles in plant defense reactions under HM stress and may accumulate in many tissues at millimolar concentrations (Foyer et al. 1983; Caregnato et al. 2008). Glutathione and ascorbate also have a role in ROS elimination as they act as electron donor for a group of peroxidases involved in H_2O_2 scavenging (Verma and Dubey 2003). Besides, role of glutathione is also evidenced as chelator and signaling component under metal-stressed conditions (Jozefczak et al. 2012).

Advantages and challenges of phytoremediation

There are many advantages offered by the phytoremediation technology for the reclamation of HM-contaminated soils over conventional methods. These are as follows: (i) low input costs and aesthetically pleasing, (ii) soil stabilization and reduced leaching of water and inorganics in the soil, (iii) valuable metals can be regenerated from plant biomass after harvesting, (iv) this green remediation technology have a wide range of applicability in terms of toxic heavy metals, (v) environment friendly, (vi) removal of secondary toxic materials from soil, air, and water, and (vii) enhanced regulatory and public acceptance (Glass 1999; Ghosh and Singh, 2005).

Despite the apparent advantages, all aspects of phytoremediation are still not fully understood. Before this technology could become efficient and cost-

Table 1 List of transgenic plants having higher biomass and better metal accumulation potential as compared to their control counterparts

Name of plant	Gene introduced	Result	References
Nicotiana glauca	TaPCS1	High biomass, very high increase in zinc, lead, cadmium, nickel, and boron accumulation potential	Martínez et al. (2006)
Arabidopsis	SMTA	8 times more selenium concentration in biomass increased 8 times compared to non-transgenic controls	Cherian and Oliveira (2005)
Arabidopsis, tobacco, poplar trees, Indian mustard, and eastern cotton wood	merA and merB	8 times more selenium concentration in biomass increased 8 times compared to non-transgenic controls	Gratão et al. (2005) Cherian and Oliveira (2005) Bizily et al. (2000)
Arabidopsis	*arsC* and *y-E*	High tolerance to arsenic and cadmium, high biomass	Cherian and Oliveira (2005)

effective on a commercial scale, there are several limitations that need to be overcome. For phytoremediation to be effective and to occur within a reasonable time frame, plant yield (biomass production) and contaminant accumulation have to be dramatically enhanced. This may be achieved by cultivation of rapidly growing hyperaccumulating plants or genetically engineered plants with hyperaccumulating genes. Examples of few genetically engineered plants having better metal accumulating capability are given in Table 1. The major disadvantages that must be taken into account before implementation of the phytoremediation commercially on large scale are as follows: (i) The plant must have the capability to grow in the contaminated soil or material, (ii) the plant can remediate only the soil area which is in reach of its root system and the contaminants must be soluble in soil water, (iii) process can take years for contaminant removal and to make the soil reusable, (iv) climatic conditions and soil health are also limiting factors, (vi) introduction of non-indigenous species for phytoremediation purpose may affect biodiversity, (v) possible contamination of the food chain as a result of grazing on heavy-metal-contaminated vegetation (Glass 1999; Ghosh and Singh 2005). However, in spite of many limitations, interest in phytoremediation is increasing in the mining sector due to the recovery of rare and expensive trace metals from harvested biomass (phytomining) and the low cost of using plants to remediate mining areas (Mendez and Maier 2008). Selection of proper plant species is also one of the very important factors in determination of success rate of phytoremediation. Ideally, a plant should possess some properties to become suitable for phytoremediation (depicted in Fig. 1).

Future perspectives

For the success of phytoremediation, it is critically important to understand the response of plants at molecular and physiological level to heavy metal toxicity development, tolerance, and hyperaccumulation. Most of the studies to date focus on the study of HM effect on adult plant growth stage, but it is of immense significance to get information on the plant responses to HM stress right from the seed germination/seedling growth stage, because these are the most sensitive stages of plant growth cycle when facing external stress, as the plants that are used for phytoremediation have to face stress right from early stages of growth (Pulford and Watson 2003; Thakur and Sharma 2015). Seed germination and early seedling growth are also the most suitable stages for biomonitoring. Another important factor to keep in mind is that the plants grown on contaminated soils

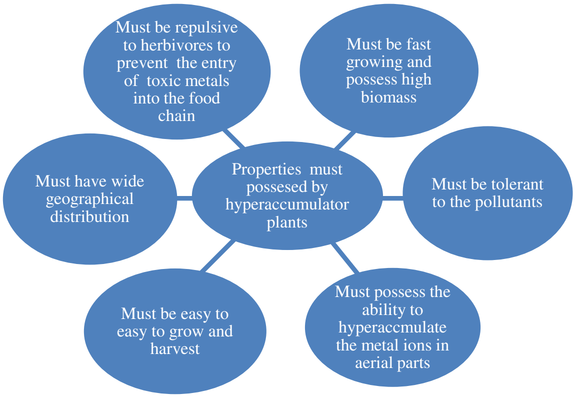

Fig. 1 Properties that must be possessed by a plant before qualifying as hyperaccumulator (Pilon-Smits 2005; Yang et al. 2005; Kotrba et al. 2009)

have to face multiple metal stress rather than that of a single metal. It is apparent that metals interact differently when they are present in a mixture. For example, ions of different metals will compete for binding sites of soil particles or plant metal transporters (Woolson 1973; Clemens et al. 1998). Thus, to understand the response mechanism of plant of interest under multiple metal stress is also a very important aspect for the successful realization of phytoremediation in field conditions.

The use of plant-growth-promoting rhizobacteria in contaminated soils to enhance the phytoremediation capacity of plants is a relatively new technique. For example, Babu et al. 2013 have shown the enhancement of heavy metal phytoremediation by *Alnus firma* when inoculated with endophytic *Bacillus thuringiensis* GDB-1. The use of metal-resistant rhizobacteria can hasten the process of phytoremediation via various direct and indirect mechanisms, e.g., alteration in the bioavailability of metal ions for absorption, increased root growth, solubilization of phosphate, production of siderophores, nitrogen fixation, production of 1-aminocyclopropane-1-carboxylate deaminase (ACC), etc. (Glick 2010; Ma et al. 2011). Rhizobacteria can alleviate the root growth inhibition due to HM ion exposure in turn increasing the phytoremediation capacity of plants by extensive root system growth (Arshad et al. 2007). Being relatively a new technique, the use of plant-growth-promoting bacteria (PGPR) for phytoremediation needs to be further explored in terms of understanding of plant and microorganism interactions in field conditions, using genetically engineered bacteria, etc. (Arshad et al. 2007).

Conclusions

The use of green plants to remove HMs from soil is a process which involves many factors for its successful commercial application. These factors include the understanding of plant growth and tolerance mechanisms under stress, nature and quantity of HM ions in soil, and soil health parameters (pH, binding properties, temperature, other organic contaminants, etc.). Future research needs to be focused on the factors that limit or enhance the uptake, translocation, and sequestration of HM ions in plants and genetically modified plant species with high biomass, fast growth rate, and metal hyperaccumulator genes. The future of this green remediation technology relies on the synchronized research in the fields of plant physiology/biochemistry, plant genetics, soil microbiology, and environmental engineering.

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