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Phytoremediation of Heavy Metals: Physiological and Molecular Mechanisms

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Abstract Heavy metals (HM) are a unique class of toxicants since they cannot be broken down to non-toxic forms. Concentration of these heavy metals has increased drastically, posing problems to health and environment, since the onset of the industrial revolution. Once the heavy metals contaminate the ecosystem, they remain a potential threat for many years. Some technologies have long been in use to remove, destroy and sequester these hazardous elements. Even though effective techniques for cleaning the contaminated soils and waters are usually expensive, labour intensive, and often disturbing. Phytoremediation, a fast-emerging new technology for removal of toxic heavy metals, is cost-effective, non-intrusive and aesthetically pleasing. It exploits the ability of selected plants to remediate pollutants from contaminated sites. Plants have inter-linked physiological and molecular mechanisms of tolerance to heavy metals. High tolerance to HM toxicity is based on a reduced metal uptake or increased internal sequestration, which is manifested by interaction between a genotype and its environment. The growing interest in molecular genetics has increased our understanding of mechanisms of HM tolerance in plants and many transgenic plants have displayed increased HM tolerance. Improvement of plants by genetic engineering, i.e., by modifying characteristics like metal uptake, transport and accumulation and plant's tolerance to metals, opens up new possibilities of phytoremediation. This paper presents an overview of the molecular and physiological mechanisms involved in the phytoremediation process, and discusses strategies for engineering plants genetically for this purpose.

Introduction

Global industrialization has resulted in a widespread contamination of environment with persistent addition of organic and inorganic wastes. The contaminants enter the environment either by natural processes or through human activity. The natural

contamination originates from excessive withering of minerals from rocks or displacement from the groundwater or subsurface layers of the soil. Disposal of industrial effluents, sewage sludges, deposition of air-borne industrial wastes, military operations, mining, land-fill operations, industrial solid-waste disposal and the growing use of agricultural chemicals such as pesticides, herbicides and fertilizers are sources of human-assisted contamination of the environment.

Heavy metals (HMs) are among the major environmental contaminants and pose a severe threat to human and animal health by their long-term persistence in the environment (Gisbert et al., 2003; Halim et al., 2003). For instance, lead (Pb) may have a soil-retention time of 150–5,000 years and was reported to maintain a high concentration for as long as 150 years after application of sludge to the soil (Nandakumar et al., 1995). The biological half-life of cadmium (Cd) is about 18 years (Forstner, 1995). Given this, a long-term plan of pollution remediation measures that may lower the rate of pollution increase has become indispensable. The commonly used technologies for in situ and ex situ remediation of HM-contaminated soil are pneumatic fracturing, soil flushing, solidification, vitrification, electrophoresis, chemical reduction, soil washing and excavation. All these conventional methods, colloquially termed as "pump and treat" and "dig and dump" techniques, are limited in their applicability to small areas and have their own limitations.

Over the last one decade, a fast emerging, low-cost and eco-friendly alternative to the conventional remediation techniques has gained ground both in public and private sectors. Termed as "phytoremediation", this technique engages plants to cleanse the nature, as plants can absorb, accumulate and detoxify contaminants of their substrates (soil, water and air) through physical, chemical or biological processes. Various soil and plant factors such as the physical and chemical properties of the soil, the plant and microbial exudates, bioavailability of metals, and the ability of plants to uptake, accumulate, translocate, sequester and detoxify metals account for phytoremediation efficiency (Hooda, 2007). Several microbes including the mycorrhizal and non-mycorrhizal fungi, and the cultivated and wild metal-hyperaccumulating plants are being tested in labs and fields for decontaminating the metalliferrous substrates present in the environment. According to Salt et al. (1995), to clean up one acre of soil to a depth of 50 cm, phytoextraction (a type of phytoremediation) costs US $60,000–1,000,000, whereas soil excavation (a type of physical remediation) requires at least US $4,000,000. Understanding of the mechanisms of plant tolerance to a particular metal is important for developing plants that are suitable for phytoremediation of the contaminated sites. This review focuses on the physiological and molecular aspects of phytoremediation of the HM-infested soil and water.

Types of Phytoremediation

Plants utilize several methods to remediate the polluted sites. Phytoremediation technology can be subdivided, on the basis of its underlying process and applicability, as follows:

Phytoextraction

Phytoextraction, a common process of phytoremediation, involves uptake of the contaminant by plant roots with subsequent accumulation in the aerial plant parts,

followed generally by harvest and then disposal of plant biomass. The metal-accumulating plants are seeded or transplanted into the metal-contaminated soil and then cultivated with established agricultural practices. The roots of these plants absorb metal elements from the soil and translocate them to the aerial shoots, where they accumulate. After a sufficient plant growth and metal accumulation, the aerial plant parts are harvested and removed, thus ensuring a permanent removal of metals, such as Pb, Cd, Ni, Cu, Cr, and V, from the contaminated soils. However, it is applicable only to those sites containing low to moderate levels of metal pollution, because plant growth does not sustain in heavily polluted sites (Padmavathiamma & Loretta, 2007). Chelating agents are added to solubilize metals that have a low solubility in the soil solution (Prasad, 2003). EDDS, a chelating agent, increased Cu accumulation in *Cannabis sativa* (Meers et al., 2005). Plants to be used for phytoextraction should have: (a) tolerance to high concentrations metals, (b) high metal-accumulation capability, (c) heavy biomass, (d) ability to grow fast and a (e) profuse root system. The success of phytoextraction depends especially on the plant's ability (a) to accumulate biomass rapidly, and (b) to store large quantities of the uptaken metals in the shoot tissue (Blaylock et al., 1997; McGrath, 1998; Blaylock & Huang, 2000). Ability of plants to withstand difficult soil conditions (i.e. soil pH, salinity, soil structure, water content) and produce a dense root system are also important.

The strategies used in developing a phytoremediation plan are (a) screening of hyperaccumulator candidate plants, (b) plant breeding, and (c) development of improved hyperaccumulators using genetic tools. Applicability of phytoextraction technology in terms of cost and required time must shift the current paradigm of remedial targets, which is based on total metal concentrations, towards the "bioavailable contaminant stripping (BCS)", as proposed initially by Hamon and McLaughlin (1999). With this strategy, the clean up time can be shortened by targeting extraction of only the most liable and bioavailable metal pools (Schnept et al., 2002; Sommer et al., 2002).

Rhizofiltration

This technique relies on the ability of plant roots to take up and sequester metal contaminants or excess nutrients from the aqueous growth substrates (waste-water streams, nutrient–recycling systems). It remediates metals like Pb, Cd, Ni, Cu, Cr, V and radionucliides (U, Cs, Sr). The ideal plants should produce significant amounts of root biomass or root surface area, be able to accumulate and tolerate significant amounts of target metals, involve easy handling and a low maintenance cost, and have a minimum of secondary waste that requires disposal (Dushenkov & Kapulnik, 2000). Terrestrial plants more suitable for rhizofiltration because they produce longer, more substantial and often fibrous root systems with large surface areas for metal sorption. Indian mustard (*Brassica juncea*) and sunflower (*Helianthus annuus*) are most promising for metal removal from water. Indian mustard effectively removes Cd, Cr, Cu, Ni, Pb, and Zn (Dushenkov et al., 1995) whereas sunflower absorbs Pb (Dushenkov et al., 1995) and U (Dushenkov et al., 1997) from hydroponic solutions. Indian mustard could effectively remove a wide range (4–500 mg/l) of Pb concentration (Raskin & Ensley, 2000).

Rhizofiltration can be conducted in situ to remediate the contaminated surface-water bodies, *or ex-situ* where an engineered system of tanks is used to hold the introduced plants and the contaminated water. Commercialization of this technology is

driven by economics as well as by technical advantages like applicability to many problem metals, ability to treat high volumes of water, minimum requirement of chemicals and likelihood of regulatory and a public acceptance (Dushenkov et al., 1995).

Phytostabilization

In this technique, plants are used to transform toxic soil metals to less toxic forms (Eapen & Dsouza, 2005), which are not removed from the soil. Phytostabilization stabilizes wastes, prevents exposure pathways via wind and water erosion, provides a hydraulic control that suppresses vertical migration of contaminants into ground water and immobilizes the contaminants physically and chemically by root sorption and chemical fixation with various soil amendments (Cunningham et al., 1995; Berti & Cunningham, 2000). It requires plants that are able to grow in contaminated soil with their roots growing into the contamination zone, and alter the biological, chemical or physical conditions in the soil that convert the toxic forms of metal to less toxic ones. Immobilization of toxic metals by plants may be enhanced by soil amendments that increase the soil organic matter and pH (using lime), or by binding certain constituents with phosphate or carbonate without using soil amendments (Schnoor, 2000).

Smith and Bradshaw (1992) developed two cultivars of *Agrostis tenius* and one of *Festuca rubra*, which are now commonly available for phytoremediation of the Pb-, Zn- and Cu-contaminated soils. Phytostabilization, though most effective at sites having fine-textured soils with high organic matter content, can treat a wide range of surface contamination (Cunningham et al., 1995; Berti & Cunningham, 2000). Deep-rooting plants could reduce the highly toxic Cr VI to Cr III, which is much less soluble and therefore, less bioavailable (James, 2001). Soil amendments should fix metals rapidly, followed by their incorporation, whereas chemical alterations should be long lasting if not permanent. The most important soil amendments are phosphate fertilizers, organic matter or bio-solids, iron or manganese oxyhydroxides, natural or artificial clay minerals or mixtures of these amendments. Phytostabilization does not require soil removal and/or disposal of the hazardous material or the biomass.

Phytovolatization

Use of plants to absorb HM contaminants and make convert them to volatile, less toxic chemical species through transpiration is called phytovolatization. Some metals, like As, Hg and Se, may exist as gaseous species in the environment. Some naturally occurring or genetically modified plants, like *Chara canescens* (musk-grass), *Brassica juncea* (Indian mustard) and Arabidopsis *thaliana*, are reported to possess capability to absorb heavy metals and convert them to gaseous species within the plant and subsequently release them into the atmosphere (Ghosh & Singh, 2005). Phytovolatization has been used primarily for the removal of mercury wherein mercuric ion is transformed into the less toxic gaseous elemental mercury (Ghosh & Singh, 2005). Some plants growing in high Se media e.g, *Arabidopsis thaliana* and *Brassica juncea*, produce volatile Se in the form of dimethylselenide and dimethyldiselenide (Banuelos, 2000). Overexpression of CGS in *Brassica* promoted selenium volatilization and the CGS seedlings were more tolerant to selenite than wild type. The CGS plants contained Se levels that were 20–40% lower in

shoots and 50–70% lower in roots than in wild type when supplied with selenite (Van Huysen et al., 2003). Phytovolatization has been successful also in removing Tritium (^{3}H), a radioactive isotope of hydrogen, which is decayed to stable helium with a half-life of about 12 years (Dushenkov, 2003). Effort has been made to insert the bacterial Hg ion reductase genes into plants for purpose of Hg phytovolatization (Rugh et al., 1996; Rugh et al., 1998). *Nicotiana tabacum* and *Arabidopsis thaliana*, which include a gene for mercuric reductase to convert ionic mercury (Hg II) to less toxic metallic mercury (Hg 0) and volatilize it, have been genetically modified (Meagher et al., 2000). Volatile Se compounds, such as dimethylselenide are 500 to 600 times less toxic than the inorganic forms of Se found in the soil (Deesouza et al., 2000). Furthermore, phytovolatization involves minimal site disturbance, little erosion and no disposal of contaminated plant material (Rugh et al., 2000). A gene, encoding the enzyme SMT, has been cloned from the Se-hyperaccumulator, *Astragulus bisculatus* (Neuhieral et al., 1999). When overexpressed in A*rabidopsis* and Indian mustard, it increased selenium tolerance, accumulation and volatilization. The SMT transgenic seedlings tolerated Se, particularly selenite, significantly better than the wild type, producing a 3–7 fold higher biomass and 3-fold longer roots (Le Duc et al., 2004). However, unlike other remediation techniques, once the contaminants have been removed via volatilization, one has no control over their migration to other areas.

Phytodegradation (Phytotransformation)

In this method, plants degrade organic pollutants by metabolic processes and using the rhizospheric associations between plants and soil microorganisms. Plant enzymes that metabolize contaminants may be released into the rhizosphere, where they may play active role in transformation of contaminants. Enzymes, like dehalogenase, nitroreductase, peroxidase, laccase and nitrilase, have been discovered in plant sediments and soils. Organic compounds such as munitions, chlorinated solvents, herbicides and insecticides and the inorganic nutrients can be degraded by this technology (Schnoor et al., 1995). The dissolved TNT (trinitrotoluene) concentrations in flooded soil decreased from 128 ppm to 10 ppm within one weak in the presence of the aquatic plant, *Myriophyllum aquaticum*, which produces nitroreductase enzyme that can partially degrade TNT (Schnoor et al., 1995). To engineer plant tolerance to TNT, two bacterial enzymes (PETN reductase and nitroreductase), able to reduce TNT into less harmful compounds, were overexpressed in tobacco plants. The two genes o*nr* and *nfs,* under the control of a constitutive promotor, provided the transgenic plants with increased tolerance to TNT at a concentration that severely affected the development of wild type plants (Hannink et al., 2001).

Various plants tested for different phytoremediation technologies are enlisted in Table 1.

Mechanisms of Metal Sequestration

Metal Uptake Mechanism

Metal uptake depends primarily on metal bioavailability. In soils, metals exist as a variety of chemical species in a dynamic equilibrium governed by the physical,

Table 1 Important Plants Used for Phytoremediation of Heavy Metals

Contaminant	Medium	Process	Plant	References
Arsenic	Soil	Phytoextraction	*Pteris vittata* L.	Gonzaga et al. (2006)
		Phytostabilization	*Piricum sativum* L.	Jonnalagadda and Nenzou (1997)
Boron	Soil	Phytoextraction	*Gypophila sphaerocephala* Fenzel	Babaoglu et al. (2004)
Cadmium	Soil	Phytoextraction	*Oryza sativa* L.	Murakami et al. (2007)
		Phytostabilization	*Vettiveria zizanioides* L.	Lai and Chen (2004)
	Water	Rhizofiltration	*Lemna minor* L.	Hou et al. (2007)
Chromium	Soil	Phytoextraction	*Brassica juncea* L.	Zhang et al. (2007)
		Phytostabilization	*Brassica juncea* L.	Salt et al. (1995)
	Water	Rhizofiltration	*Brassica juncea* L.	Diwan et al. (2008)
Cobalt	Soil	Phytoextraction	*Berkheya cod*dii Roessler	Keeling et al. (2003)
Copper	Soil	Phytoextraction	*Elsholtzia splendens* Nakai ex Maekawa	Jiang et al. (2004)
		Phytostabilization	*Festuca rubra* L.	Smith and Bradshaw (1979)
	Water	Rhizofiltration	*Lemna minor* L.	Hou et al. (2007)
Lead	Soil	Phytoextraction	*Chenopodium album* L.	Celestino et al. (2006)
		Phytostabilization	*Vetiveria zizanioides* L.	Rotkittikhun et al. (2007)
	Water	Rhizofiltration	*Hemidesmus indicus* L.	Chandrashekhar et al. (2004)
Manganese	Soil	Phytoextraction	*Phytolacca americana* L.	Min et al. (2007)
Mercury	Soil	Phytoextraction	*Marrubium vulgare* L.	Jimenez et al. (2006)
	Water	Rhizofiltration	*Pistia stratiotes* L.	Skinner et al. (2007)
Nickel	Soil	Phytoextraction	*Alyssum lesbiacum* (Candargy) Rech. f.	Singer et al. (2007)
		Phytostabilization	*Agropyron elongatum* (Host.)P. Beauv.	Chen and Wong (2006)
	Water	Rhizofiltration	*Lemna minor* L.	Axtell et al. (2003)
Selenium	Soil	Phytoextraction	*Brassica rapa* L.	Moreno et al. (2005)
		Phytovolatization	*Brassica* spp (Wild type)	Banuelos et al. (2005)
	Water	Rhizofiltration	*Lemna minor* L.	Zayed et al. (1998)
Uranium	Soil	Phytoextraction	*Lolium perenne* L.	Vadenhov and Heese (2004)
	Water	Rhizofiltration	*Chenopodium amaranticolor* H.J.Coste & Reyn	Eapen et al. (2003)
Zinc	Soil	Phytoextraction	*Cynodon dactylon* (L.)Pers.	Celestino et al. (2006)
		Phytostabilization	*Cynodon dactylon* (L.) Pers.	Pierzynski et al. (1994)
	Water	Rhizofiltration	*Brassica juncea* L.	Dushenkov et al. (1995)

chemical and biological processes of the soil (Chaney, 1988). Bioavailability of soil pollutants, a primary basis of remediation efficacy, refers to a fraction of the total pollutant mass in the soil and sediment available to plants. Uptake of metals by plants involves root interception of metal ions, entry of metal ions into roots and

their translocation to the shoot through mass flow and diffusion. The uptake is achieved by mobilizing metals bound to soil particles through the metal-chelating molecules (mugenic and aveic acids) secreted into the rhizosphere, specific plasma-membrane-bound metal reductase and the proton extrusion from roots (Salt et al., 1995).

Another type of exudate produced by grasses are phytosiderophores, which bind Fe and facilitate its uptake. Phytosiderophores are biosynthesized from nicotinamide, which is composed of three methionines coupled via non-peptide bonds (Higuchi et al., 1999). Chelation of phytosiderophores can help in the transport of metal ions across the plasma membrane as a metal-siderophore complex via specialized transporters. By reducing the chelated Fe (III) with a root ferric chelate reductase, plants are able to release soluble Fe (II) for uptake by roots (Salt et al., 1994). Plants can also solubilize iron and other metals by exuding protons from roots to acidify the rhizosphere (Salt et al., 1994). It is therefore possible to enhance the bioavailability of metal pollutants by manipulating the root processes. Once the metal is bioavailable to the plant, the entry of metal ions inside the plant, either through symplast (intercellular) or apoplast (extracellular), depends on the type of metal and the plant species. The apoplast continuum of root epidermis and cortex is readily permeable for solutes. Apoplastic pathway is relatively unregulated, because water and dissolved substance can flow and diffuse without crossing the membrane. The cell walls of the endodermal layer act as a barrier for apoplastic diffusion into the vascular system (Ghosh & Singh, 2005). Apoplastic transport is limited by high cation exchange capacity of the cell wall (Raskin et al., 1997). In the symplastic transport, metal ions move across the plasma membrane, which usually has a large negative resting potential of approximately 170 mV (negative inside the membrane). This membrane potential provides a strong electrochemical gradient for the inward movement of the metal ions (Ghosh & Singh, 2005). Most metal ions enter plant cells by an energy-dependent process via specific or generic metal-ion carriers or channels (Bubb & Lester, 1991). Cutler and Rains (1974) found that a large fraction of Cd was taken up by barley tissues through exchange absorption, and through diffusion coupled with sequestration, without any concomitant active metabolic uptake.

Although there is no direct evidence for a role of plasma membrane efflux transporters in the HM tolerance in plants, recent research has revealed that plants possess several classes of metal transporters such as heavy metal (or CPX-type) ATPases that are involved in the overall metal-ion homeostasis and tolerance in plants (Williams et al., 2000), the natural resistance-associated macrophage-protein (Nramp) family, cation-diffusion facilitator (CDF) proteins family and the zinc-iron permease (ZIP) family (Guerinot, 2000). Yang et al. (2005) found a correlation between uptake capacity and hyperaccumulation of ZIP family members in the plant, *Thlaspi caerulescens*. Under Zn-replete conditions, two ZIP cDNA (ZNT1 and ZNT2) are expressed at significantly higher levels in the roots of different *T. caerulescens* accessions than those of the non-hyperaccumulating, *T. arvense* (Pence et al., 2000; Assuncao et al., 2001). Thus, overexpression of the uptake systems may result in enhanced accumulation of the metals.

Metal Translocation to Shoots

Once the metal ions have entered the roots, they can either be stored in the root or forwarded to the shoot, primarily through the xylem. The rate of metal translocation

to the shoot may depend on metal concentration in the root (Hardiman et al., 1984). Prezemeck and Haase (1991) suggested a phytochelatin-mediated metal binding in the xylem sap as a possible mechanism for metal translocation. Low molecular weight chelators such as citrate (Lee et al., 1977) and free histidine as in *Alyssum lesbiacum* (Kramer et al., 1996) were associated with this process. Other chelating compounds like malate, citrate, and histidine may also have a role in the metal-ion-mobility in plants (Von Wiren et al., 1999). Membrane transport systems are likely to play a central role in the translocation process. Many gene families that are involved in metal transport have been identified. Some of them are heavy-metal ATPases, natural resistance-associated macrophage proteins (NRAMPs), cation diffusion facilitators, the Zrt- and Irt- like proteins family, and the cation antiporters (Hall & Williams, 2003).

Tolerance Mechanism

Plant tolerance to a particular metal is governed by an inter-related network of physiological and molecular mechanisms, to be understood essentially for developing plants suitable for phytoremediation of the contaminated sites. The apparent tolerance to increasing levels of toxic elements can result from the exclusion of toxic elements or the metabolic tolerance of plants to specific elements. (Singh et al., 2003).

Exclusion

Transport across the root-cell membrane initiates the process of metal absorption by plant tissues. The electrical charge prevents metal ions from diffusing freely across the lipophilic cellular membranes into the cytosol (Horst et al., 2002). Therefore, ion transport into cells must be mediated by membrane proteins with transport functions. Root-uptake kinetics has been investigated for a variety of metal ions including Cd^{2+} (Cohen et al., 1998; Hart et al., 1998), Cu^{2+} (Thornton, 1991) and Zn^{2+} (Santa Maria & Cogliatti, 1988; Vazquez et al., 1994). Entry of Cd in the cells of radish seeds through Ca channels was proposed by Rivetta et al. (1997).

Vacuolar Compartmentalization

Metal compartmentalization in the vacuole is well documented (Vazquez et al., 1994; Kupper et al., 1999). Significant for metal detoxification and plant tolerance, this process prevents free concentration of metal ions in the cytosol and forces them into a limited space (Tong et al., 2004). Several transporters have been shown to mediate Zn fluxes across the cellular membranes (Paulsen and Saier, 1997) including the tonoplast (Macdiarmid et al., 2000). In yeast, over-expression of membrane MTPS (metal tolerance proteins) from *Thlaspi goesingense* confers resistance to Cd, Co, Ni and Zn possibly due to their transport into the vacuole (Persans et al., 2000). Cd-phytochelatin complexes as well as apo-phytochelatins are transported through specific carriers against concentration gradient across the tonoplast. They accumulate inside the tonoplast vesicles (Salt & Rauser, 1995). Intact vacuoles isolated from the tobacco and barley leaves exposed to Zn contained Zn ions (Brune et al., 1994;

Krotz et al., 1989). High-level expression of a vacuolar metal-ion transporter TgMTP1 in *T. goesingense* was proposed to account for the enhanced ability of plants to accumulate metal ions within shoot vacuoles (Persans et al., 2001). Within plant cells, PC-metal complexes bound by GSH or PCs are shuttled to the vacuole by an AB-type transporter protein in the tonoplast (Lu et al., 1997). Anthocyanins can also bind metals (Pilon-Smits & Pilon, 2002) and have a role in metal sequestration. Other molecules involved in metal complexation in the vacuole are the organic acids (Kramer et al., 2000). Vacuolar Zn accumulation has been confirmed in roots and shoots of *Thlaspi caerulescens* (Vazquez et al., 1992, 1994). Increase in the vacuolar volume fraction of meristematic cells in *Festuca rubra* during zinc exposure also confirms Zn accumulation within the vacuole as a detoxification mechanism (Davies et al., 1991). To date, the best characterized vacuolar transporter and channel involved in metal tolerance is YCF1 from *Saccharomyces cerevisiae*. This is an MgATP-energized glutathione-S-conjugate transporter responsible for vacuolar sequestration of organic compounds after their S-conjugation with glutathione and GSH-metal complexes. It catalyses transport of bis (glutathione) cadmium (Cd-GS_2) (Li et al., 1997), Ag-GS_3 (Ghosh et al., 1996) and Hg-GS_2 (Gueldry, 2003) into vacuoles. Overexpression of such vacuolar transporters can be used to engineer advanced phytoremediators with increased ability to pump heavy metals into a safe compartment.

Phytochelatins

HM accumulation in plants induces production of phytochelatins (PCs), a family of thiol–rich peptides of a general structure (γ-Glu-Cys) n-Gly, where n is normally in the range of 2–5 (Steffens, 1990; Rauser, 1995). Cd and As effectively induce phytochelatin synthesis, while Zn and Ni hardly do so (Grill et al., 1989). Glutathione, the substrate for PC synthase, is synthesized from its constituent amino acids in two steps; the first step is catalyzed by γ-glutamyl-cys synthase (γ-ECS) and the second one by glutathione synthase (GS). The γ -glutamyl-Cys-synthase activity is controlled through feedback regulation by glutathione and is dependent on the availability of cysteine (Mejare & Billow, 2001). The gene encoding this enzyme has been identified in *Arabidopsis thaliana* (Clemens et al., 1999; Ha et al., 1999; Vatamaniuk et al., 1999), *Triticum aestivum* (Clemens et al., 1999) and *Schizosaccharomyces pombe* (Clemens et al., 1999; Ha et al., 1999).

Although many earlier studies have suggested a role of PCs in metal detoxification, it was clearly demonstrated by the PC-deficient Cad1-3 mutant of *Arabidopsis* and the PC-synthase targeted deletion mutant *of S. pombe*. Both the mutants are highly sensitive to Cd and As but with a little or no sensitivity to metals like Cu, Hg, and Ni, showing that PCs are essential for the detoxification of Cd and As but have little involvement in that of Cu, Hg and Ni. (Ha et al., 1999). In such cases there may be a more effective alternative like metallothioneins and histidine. Lee et al. (2003) overexpressed an *Arabidopsis* PC synthase (AtPCS1) in transgenic *Arabidopsis* with the goal of increasing transgenic plants showing increased production of PCs (1.3–2.1 fold at 85 µM $CdCl_2$ stress for 3 days) as compared with wild type plants. However, PCs lines paradoxically showed hypersensitivity to cadmium and zinc when grown on agar medium containing a 50 or 85 µM $CdCl_2$.

GSH-dependent PC-synthase activity was identified in cultured cells of *Silense cucubalis* (Grill et al., 1989). The enzyme was active only in the presence of Cd, Cu, Zn, Ag, Hg and Pb. Similar activities have been identified, inter acia, in tomato (Howden et al., 1995) and pea (Klapheck et al., 1995). Despite the detection of PC-synthase activity a decade ago, identification of a corresponding gene remained elusive until Vatamaniuk et al. (1999) identified an *Arabidopsis* cDNA; named AtPCS1. Expression of AtPCS1 protein mediated increase in Cd accumulation, suggesting a possible role of AtPCS1 in Cd chelation or sequestration. Clemens et al. (1999) identified a wheat cDNA, TaPCS1, that increased Cd resistance in wild-type yeast. The resistance, associated with increase in Cd accumulation, was GSH-dependent. Occurrence of the AtPCS1 and TaPCS1 mediated tolerance in vacuole-deficient mutants suggests that PCs are localized in cytosol and play important role in tolerance mechanism. The significance of PC-Cd complex formation for detoxification of Cd^{2+} in plants was supported by the isolation of the Arabidopsis *cad 1* mutant, which contains wild-type levels of GSH, but is PC-deficient and Cd^{2+} hypersensitive (Howden et al., 1995). The CAD1 gene, also called AtPCS1, encodes a PC-synthase as evident by the detection of GSH- and metal-dependent PC synthesis in *Escherichia coli* cells expressing AtPCS1 (Ha et al., 1999). Independently, AtPCS1 and TaPCS1 from wheat were isolated in screens for plant cDNAs conferring Cd^{2+} tolerance (Vatamaniuk et al., 1999). The *S. cerevisiae* cells expressing these genes display a Cd^{2+}tolerance phenotype that is GSH-dependent and correlates with PC synthesis. Purified recombinant PCS proteins from *Arabidopsis* and *S. pombe* catalyze the formation of PCs from GSH (Clemens et al., 1999; Vatamaniuk et al., 1999). The Arabidopsis cad1-3 mutant is highly sensitive to Cd^{2+} and AsO_4^{2-}, compared with the wild type, and displays slightly elevated sensitivities towards Cu, Hg and Ag (Ha et al., 1999). Gisbert et al. (2003) reported that overexpression of wheat TaPCS1 gene encoding PC synthase in tobacco greatly increased its tolerance to Pb and Cd, causing the seedling roots to grow 160% longer than in the wild type. The transgenic seedlings grown in the mining soils containing 1,572 mg/Kg Pb accumulated double the amount of Pb and Cd accumulated by the wild type. Raab et al. (2004) have developed a method to ascertain the nature of As-PC complex in extracts of the As-tolerant grass (*Holeus lanatuss)* and As-hyperaccumulator fern (*Pteris cretica*), using the metal-specific (inductively coupled plasma-mass spectroscopy) and organ-specific (electro spray ionization-mass spectroscopy) detection systems.

The HM-detoxification process is not limited to the chelation of the metal ions. After the activation of PC synthase by the metal ions and metal chelation by the PCs synthesized, the metal ion complex is transported to the vacuole and stabilized there by forming a complex with sulfides or organic acids (Rauser, 1990). The tonoplast transport and vacuolar compartmentation of PC-Cd complexes increase the metal-binding capacity of phytochelatins (Vogeli-Lange & Wagner, 1989; Salt & Rauser, 1995; Bae & Mehra, 1998).

Metallothioneins

Metallothioneins (MTs) is a group of low molecular weight, cysteine-rich, metal-binding proteins, which provide thiols for metal chelation. Overexpression of genes

involved in the synthesis of metal chelators may lead to enhanced or reduced metal uptake and enhanced metal translocation and or sequestration (Cherian & Oliveira, 2005). An MT- gene (CUP1), when overexpressd in cauliflower, resulted in a 16-fold higher cadmium tolerance (Hesegawa et al., 1997). Similarly, high Cu accumulation has been reported in *Arabidopsis thaliana* by the overexpression of a pea MT gene (Pan et al., 1994). Although PCs formally belong to this group of compounds (Class III MTs), the enzymatic synthesis of PCs distinguishes them from MT proteins. Since their discovery as the Cd-binding proteins in the equine kidney (Margoshes & Valte, 1957), MT proteins and genomes have been reported in animals as well as in eukaryotic microorganisms and plants. Plants have a family of metallothionein (MT) genes encoding peptides that generally consist of 60-80 amino acids and contain 9–16 cysteine residues (Chatthai et al., 1997). Metallothionein-metal complex can be glutathioned (Brouwer et al., 1993), suggesting that they may be transported into vacuoles for long-time sequestration. The MT-gene structure and expression are described and discussed extensively in some recent reviews (Rauser, 1999; Cobbett & Golsbrough, 2002). Although metals, including copper, either do not affect or repress MT-gene expression in some species, copper induces expression of a Type 1 MT gene (Murphy & Taiz, 1995). Since other stresses, like heat shock and aluminium, also induce this type of expression, it was suggested that these MTs might express as part of a general stress response (Cobbett & Golsbrough, 2002). There is, however, some evidence to suggest that MTs are involved in copper homeostasis and detoxification (Zhou & Goldsbrough, 1995). Given the wide range of factors that induce MT synthesis, Karin (1985) has suggested that metal detoxification is not the primary role of MTs. They are known to sequester metals in the cell and pass metal ions to the apoenzymes that require them. Reactivation of the Zn-requiring apoenzymes by transfer of zinc ions from MTs has been demonstrated (Udom & Brady, 1980); similar is the case of the transfer of copper from *Nuerospora crassa* MT to copper-requiring apoenzymes (Beltramini & Lerch, 1982).

Expression of plant MT genes is well studied. A detailed investigation of the *Arabidopsis* MT1a and MT2a expression using reverse transcription polymerase chain reaction (RT-PCR) and in situ hybridization has revealed distinct patterns for the two genes (Garcia-Hernandez et al., 1998). While both mRNA species were detected in root maturation zones and leaf trichomes, only MT1a expressed in the vascular tissue and the mesophyll cells. The MT2a induction by Cu treatment is correlated with Cu tolerance of the *Arabidopsis* ecotypes (Murphy & Taiz, 1995). A significant amount of evidence suggests that metal tolerance, an important feature of hyperaccumulator plants, is regulated by few major genes. This provides hope that biotechnology may be used to engineer more efficient hyperaccumulator plants. Transcriptional regulation of genes is affected by metal exposure and responds to metal deficiency. For instance, the cDNA-AFLP expression profiling (Bachem et al., 1996) in the metallophyte *Arabidopsis halleri* has shown that a large number of genes are either up-regulated or down-regulated upon metal treatment (Clemens, 2001). However, the respective signal-transduction pathway is not known in plants (Xiang & Oliver, 1998). The metal-responsive transcription factors (ACE1, MAC1, ZAP1) described in *S. cerevisiae* appear to bind directly and specifically to the respective metal and may function as metal sensors (Winge et al., 1997). The

growing interest in problems related to metal transport, trafficking and tolerance, the use of model systems such as *S. cerevisiae* and *S. pombe*, and the beginning of molecular analysis of model hyperaccumulators like *Arabidopsis halleri* and some *Thlaspi* species, are likely to elucidate further the molecular mechanism of metal tolerance and homeostasis in plants.

Improving Plants for Phytoremediation

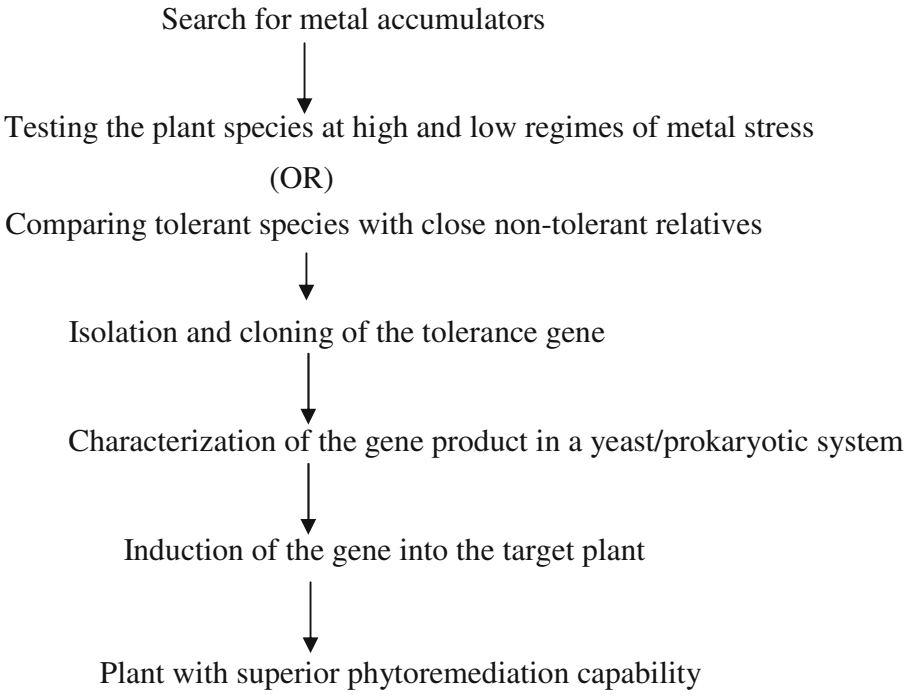
Plant capability for hyperaccumulation can be improved by genetic manipulations or the chelator-regulated strategies.

Genetic Strategies

The growing knowledge of factors affecting phytoremediation can form a basis for genetic modification of plants for improved remediation performance. Identification of metal hyperaccumulators has shown that plants have a genetic potential to clean up the contaminated soil and water. According to Haque et al. (2007), a plant is a hyperaccumulator, it meets the following criteria: (1) concentrations of heavy metals in plant shoots should reach hyperaccumulating level, which is different for different heavy metals e.g, it is more than 1,000 mg Kg^{-1} for Pb and Cu (Baker & Walker, 1989, Baker et al., 1994), for As (Ma et al., 2001), for Ni and Co (Brooks, 1998) and for Cr (Lombi et al., 2001) and more than 10,000 mg Kg^{-1} for Zn (Brown et al., 1994) (2) concentrations of heavy metals in shoots should be 10–500 times greater than those in normal plant (Shen & Lui, 1998), (3)HM concentration in shoots should be invariably greater than in roots (Baker et al., 1994) and (4) an enrichment coefficient(the ratio of metal concentration in shoot to that in soil) should be greater than one (Brown et al., 1994, Wei et al., 2002). Metal hyperaccumulators are notorious for their small size and slow growth. These characteristics have an adverse impact on their potential for metal phytoextraction and severely restrict the employment of effective agronomic practices such as mechanical harvest (Tong et al., 2004). To overcome these disadvantages, conventional breeding has been used whereby slow-growing, low-biomass hyperaccumulator plants are bred into high-biomass varieties (Li et al., 2004a). Another approach aims at enhancing the capability of plants to detoxify HM ions in the cytoplasm through their inactivation via the chelation, or conversion of toxic ions into a less toxic or easier to handle form, and/or compartmentalization. Modification or overexpression of enzymes involved in the synthesis of GSH and PCs might be a good approach to enhance the HM tolerance and consequently the phytoremediation potential in plants. Zhu et al. (1999) overexpressed the *Escherichia coli* counterparts of γ -ECS and GSH synthetase in the Indian mustard plants that accumulate more Cd than the wild-type plants. Pilon-Smits et al. (1999) overexpressed the ATP-sulfurylase (APS) gene in Indian mustard; the transgenic plants had a four-fold higher APS activity and accumulated three times more Se than the wild-type plants.

Recently, Dhankher et al. (2002) have reported a genetics-based strategy to remediate As from contaminated soils. They overexpressed two bacterial genes in *Arabidopsis*, the *E. coli* AsrC gene encoding arsenate reductase coupled with a light-

induced soybean rubisco promoter, and the *E.coli* γ-ECS gene coupled with a strong constitutive actin promoter. The AsrC protein, which expresses strongly in the stem and leaves, catalyzes the reduction of arsenate to arsenite, whereas γ-ECS, which is the first enzyme in the PC-biosynthetic pathway, increases the pool of PCs in the plant. The transgenic plants expressing both AsrC and γ -ECS proteins have shown a substantially high As tolerance; when grown on As, these plants gained a 4–17 fold greater fresh shoot weight and accumulated 2–3 fold more As than the wild-type plants. The strategy for designing the metal-accumulating plants, as modified from Karenlampi et al. (2000), is summarized below:



Production of transgenic plant, search of microbes and field trials for remediation of heavy metals will make phytoremediation technology more applicable and effective. Bacteria can reduce several HM ions to less toxic states (Lovely, 1993). Mercury resistance in gram-negative bacteria is encoded by an operon, including a mercuric-ion reductase gene (merA). MerA is an enzyme that converts toxic Hg^{2+} to the less toxic mercury (Hg0) by the reaction:

$$Hg^{2+} + NADPH - mer\,A \rightarrow Hg(0) + NADP^{+} + H^{+}$$

Rugh et al. (1996) constructed a mutagenised merA sequence and transformed it to the *Arabidopsis thaliana* transgenic seedlings that evolved 2–3 times more Hg0 than in the control. Hg (0) can be volatized by the cell.

Transgenic *Populus deltoides* overexpressing merA9 and mer18 genes evolved 2–4 fold Hg (0) relative to wild plants when exposed to Hg (II) (Che et al., 2003). These transgenic trees when grown in soil with 40 ppm of Hg (II), developed larger

biomass. Sub-cellular targeting of methylmercury lyase may enhance plant potential for the organic mercury detoxification (Bizily et al., 2003). The transgenic *A. thaliana* plants expressing a selenocysteine methyltransferase (SMTA) isolated from the Se hyperaccumulator, *Astragalus bisculcatus,* accumulated methylselenocysteine and contained up to eight-fold higher Se concentrations than the wild–type plants, when grown on a soil supplemented with selenite (SeO_3^-) (Ellis et al., 2004).

Induction of various proteins by metals is another perspective of genetic strategies for phytoremediation. The modern proteome and DNA array technologies may be applied for searching candidate genes/proteins for phytoremediation; some of the metal-induced proteins may play a role in metal tolerance or accumulation. However, examples of a process correlation between protein induction and metal tolerance are not many. Xiang and Oliver (1998) have shown increased transcription of genes for synthesis of glutathione, γ-Glutamyl cysteine synthetase, glutathione synthetase and glutathione reductase under the influence of Cd and Cu. Glutathione S-transferase is known to mediate glutathione conjugation, which is followed by transport of the resulting complex to the vacuole (Marrs, 1996). Expression of citrate synthase gene (dela Fuente et al., 1997) resulted in plants with enhanced Al tolerance. These plants produced 10- fold citrate in their roots and released a greater amount than the control plants. Transfer of nicotinamide amino-transferase genes (NAAT) resulted in overproduction of iron chelator-deoxymuginic acid in rice (Takashi et al., 2001). The transgenic plants released phytosiderophores and grew bettering the Fe-deficient soils. Transfer of iron binding protein, ferritin, enhanced the level of iron in tobacco leaves (Goto et al., 1999). A comprehensive knowledge of the genetic basis for hyperaccumulation is essential for using biotechnology effectively to design transgenic plants capable of efficient phytoremediation. Critical analysis of enhanced metal acquisition, translocation, tolerance and accumulation abilities in natural metal hyperaccumulators will help in identifying genes responsible for synthesis of metal-binding proteins/peptides. Identification of genes encoding both MTs and enzymes involved in PC synthesis thus forms the first step towards elucidation of the molecular mechanism of phytoremediation.

Completion of the *Arabidopsis* genome project, followed eventually by genome sequencing for other plants, should lead to identification of a full range of genes that are potentially involved in HM homeostasis and accumulation (Dhankher et al., 2002). Recently "ionomics" screens have been initiated in the phytoremediation–related research involving unbiased multi-element profiling in the *A. thaliana* mutant populations in order to identify mutants with altered elemental composition of rosette leaves. (Lahner et al., 2003; Salt, 2004). These and other similar screens will serve to identify novel genes with a key role in metal accumulation. Table 2 enumerates transgenic plants used for phytoremediation. Comparison of amino acid sequences of metal transporters from several hyperaccumulator species might be a starting point in the identification of determinants of the differential metal specificities (Rogers et al., 2000). Efforts are on to understand the genetics and biochemistry of metal uptake, transport and storage in hyperaccumulator plants so as to be able to develop transgenic plants with improved phytoremediation capability (Salt & Kramer, 2000; Baker et al., 2000). In addition, manipulation of metal transporters and the vacuolar targeting of metals will find fruitful application in developing plants for phytoremediation.

Table 2 Various Examples of Transgenic Plants Used for Phytoremediation

Authors	Gene	Origin	Target	Effect
Misra and Gedamu (1989)	MT2 gene	Human	*Nicotiana tabacum* L.	Cd tolerance
Evans et al. (1992)	PsMTA	*Pisum sativum* L.	*Arabidopsis thaliana* L.	Cu accumulation
Pan et al. (1994)	MT-1 gene	Mouse	*Nicotiana tabacum* L.	Cd tolerance
Rugh et al. (1998)	MerAPe9, merA18	*Shigella*.	*Liriodendron tulipifera* L.	Maximum Hg evolution
Samuelson et al. (1998)	FRE1, FRE2	*Saccharomyces cerevisiae* L	*Nicotiana tabacum* L.	Elevated Fe-III reduction
Zhu et al. (1999)	γ-glutamylcysteine Synthase	. *Escherichia coli* L	*Brassica juncea* L.	Cd tolerance
Goto et al. (1999)	Ferretin	*Glycine max* L.	*Nicotiana tabacum* L.	Increased Fe accumulation
Goto et al. (1999)	Ferretin	*Glycine max* L.	*Oryza sativa* L.	Increased Fe uptake in seeds
Pilon-Smits et al. (1999)	APSI	*Arabidopsis thaliana* L.	*Brassica juncea* L.	Increased Se uptake
Van der Zaal et al. (1999)	ZAT	*Arabidopsis thaliana* L.	*Arabidopsis thaliana* L.	Increased Zn accumulation
Ezaki et al. (2000)	Glutathione-S-transferase	*Nicotiana tabacum* L.	*Arabidopsis thaliana* L.	Al, Cu, Na tolerance
Dhankher et al. (2002)	MerA	Gram-ve bacteria	*Arabidopsis thaliana* L.	Mercury volatization
Dhankher et al. (2003)	arsC	*Escherichia coli* (T. Escherich)	*Nicotiana tabacum* L.	Increased Cd tolerance
Gisbert et al. (2003)	Phytochelatin synthase (TaPCS)	*Triticum aestivum* L.	*Nicotiana glauca* Graham	Pb accumulation
Song et al. (2003)	YCF1	*Saccharomyces cerevisiae* L.	*Arabidopsis thaliana* L.	Cd and Pb tolerance
Ellis et al. (2004)	SMTA	*Arabidopsis bisculatus* L.	*Brassica juncea* L.	Increased Se volatization
Van Huysen et al. (2004)	Cystathionine-gamma synthase (CGS)	*Arabidopsis bisculatus* L.	*Brassica juncea* L.	Se volatization

Characterization of single genes involved in metal homeostasis has yielded important insights into their functions and potential use in phytoremediation (Song et al., 2004), the most important being the high affinity iron-uptake system, IRT1, of *A. thaliana* (Connolly et al., 2002; Vert et al., 2002; Verret et al., 2003) and the two P1B-type Zn^{2+}/Cu^{2+}-ATPases, HMA2 and HMA4 (Eren & Arguello, 2004; Hussein et al., 2004; Papoyan & Kochian, 2004; Verret et al., 2004), which function in the root-to-shoot transport of $Zn^{2+}$and Cu^{2+}. Somatic cell hybrids, both symmetric and asymmetric, have been produced between *Brassica juncea,* a high-biomass Pb accumulator and *Thlaspi caerulescens,* a known Zn and Ni hyperaccumulator (Gleba et al., 1999). The hybrid has shown increased resistance to Pb, Ni and Zn and the total amount of the phytoextracted Pb was much greater because of a huge amount of the biomass produced (Gleba et al., 1999; Dushenkov et al., 2002). So, attempting somatic cell hybridization between high-biomass plants and low-biomass metal hyperaccumulators can be helpful in obtaining hybrids with high-biomass and hyperaccumulation capabilities.

The problem of low-biomass phytoremediators can be overcome by increasing plant yield and metal uptake by engineering common plants with hyperaccumulating genes. If the non-native transgenic plants are used for phytoremediation, proper control of their dissemination has to be adopted to avoid introduction of new weed species. Development and implementation of "biological encapsulation" strategies will help in popularizing the transgenic phytoremediation. "Biological encapsulation" denotes procedures that dramatically decrease probability of the spread of a transgenic from a genetically modified crop to the natural plant populations, as for example, introduction of transgene into chloroplast genome instead of the nuclear genome (Ruiz et al., 2003).

Chelate-assisted Strategies

A chelate is a complex chemical compound composed of a central metal ion attached to a large molecule (ligand), forming a ring structure. A chelating agent, also called a chelator is a chemical that can form several bonds to a single metal ion. In other words, a chelating agent is a multi-dentate ligand. EDTA, NTA, citrate, oxalate, malate, succinate, tartrate, phthalate, salicylate and acetate etc have been used as chelators for rapid mobility and uptake of metals by plants from contaminated soils. Use of synthetic chelators significantly increased Pb and Cd uptake and translocation from roots to shoots, thus facilitating phytoextraction of metals from the low-grade ores (Raskin et al., 1997; Blaylock et al., 1997). Synthetic cross-linked poly-acrylates, hydrogels have protected plant roots from HM toxicity and prevented the entry of toxic metals into roots. The synthetic and natural zeolites are applied to the soil through irrigation at specific stages of plant growth in a bid to accelerate metal accumulation in plant tissues (Blaylock et al., 1997). Synthetic organic chelating agents are being used in agriculture; EDTA, DTPA (diethylene triamino- pentacetic acid), HEDTA (N- hydroxy ethylene triamine triacetic acid), EGTA (ethylene glycol-bis (β-amino-ethyl ether)-N, N, N', N'-tetraacetic acid) and NTA (nitrilo-triacetic acid) can enhance uptake of metals by increasing their availability to plants and their transport to shoots (Blaylock et al., 1997). These chemicals increase the amount of the bioavailable metal in the soil solution by either liberating or displacing the metal

from the solid phase of the soil or by making the precipitated metal species more soluble (Prasad, 2003). Research in this area has been moderately successful.

Addition of chelating materials, such as EDTA, HEDTA and EDDHA, to soils is most effective in liberating the labile metal-contaminants into the soil solution. Chelates complex the free metal ions in solution, allowing further dissolution of the sorbed or precipitated phases until equilibrium is reached between the complexed metal, free metal and insoluble metal fractions (Norvell, 1999). Chelates are used to enhance phytoextraction of a number of metal contaminants including Cd, Cu, Ni, Pb and Zn (Blaylock et al., 1997). The chelate-mediated accumulation of toxic metals in a non-accumulator species is termed as "chelate-assisted hyperaccumulation"(Huang et al., 1997). Metal-accumulation efficiency appears to be directly related to the affinity of the applied chelating agent (Salt et al., 1998). Thus, synthetic chelating agents with a high affinity for the metal of interest (e.g., EDTA for PB, EGTA for Cd) are preferred (Blaylock et al., 1997). When a chelate-induced hyperaccumulation is the goal, metals on the site are initially immobilized to allow for a rapid establishment and growth of agronomic crops such as corn. When the crop accumulates sufficient biomass, chelating materials are applied to the soil so as to liberate large quantities of metals into the soil solution. After their death, plants with accumulated metals in their roots are disposed (Prasad, 2003). A chelate-induced hyperaccumulation thus differs from the normal phytoextraction where plants are given a gradual exposure to non-toxic quantities of metal in the solution, and accumulation occurs gradually as the plants grow. The fate of the residual chelate in the soil after the metal absorption has taken place has caused controversies (Brooks, 1998). Application of synthetic chelating agents to the soil needs to be coupled with a system capable of containing leakage of water through the soil to avoid groundwater pollution by the metals that are mobilized by the chelating agents (Navari-izzo & Quartacci, 2001).

Future Prospects

In order to make phytoremediation a reliable clean-up method, accurate definitions of the range of applicability and potential profit margins for various applications are necessary. Furthermore, monitoring and testing for toxicity and bioaccumulation of transformation products and the pollutants involved will continue to be necessary until all significant pathways have been defined. This will completely eliminate the need for costly off-site disposal. Characterization of the functions of proteins (e.g., lignin and cellulose) involved in xenobiotic chemical transformation, transfer and conjugation (i.e. tolerance and detoxification) can be accomplished by classical isolation methods or reverse genetics, and by genetic transformation (Marmiroli & McCutcheon, 2003). There exists considerable information about the induction of different proteins by metals. Proteome and DNA array technology may be used for searching the suitable candidate genes/proteins for phytoremediation. Such efforts may lead to a better understanding of metal metabolism in plants, and open up important plant applications for environmental clean-up. A systemic screening of plant species and genotypes for metal accumulation and resistance will broaden the spectra of genetic material available for optimization of phytoremediation technology and its transfer to commercial scale.

According to Cherian and Oliveira (2005), research in the following areas appears to be worth pursuing in the future to gain further in phytoremediation research. Manipulation of metal transporters and their cellular targeting to specific cell organelles, such as vacuoles, to allow for safe compartmentation of heavy metals in locations that do not disturb other cellular functions. Genetic manipulations of the chloroplast genome, which may be an alternative approach for some plants to achieve high gene expression while avoiding the risk of transgene escape via pollen (Ruiz et al., 2003). (3) Identification of candidate plants with substances that may deter the herbivores from feeding and the subsequent transformation of such plants with altered or improved metal tolerance capabilities. Such a system will avoid the transfer of metals to the food chain (Li et al., 2004b). (4) Developing plants with ability to secrete metal-selective ligands capable of solubilising elements for phytoremediation (Eapen & Dsouza, 2005). (5) A multigene approach involving a simultaneous transfer of several genes into suitable candidate plant to remove contaminants of complex nature. (6) Establishment of data on the field performance of transgenes developed in phytoremediation studies.

Phytoremediation technology is still in a research and development phase, and many technical barriers need to be addressed. The complex interactions that take place under site-specific conditions necessitate a multi-disciplinary approach to metal phytoextraction. Success will ultimately depend upon employment of a holistic approach to integrate the efforts of plant biologists, soil microbiologists, agronomists and environmental engineers. Phytoremediation promises to be an integral waste management option for the current century.

Conclusions

Phytoremediation technology is specially beneficial in remediating the HM-contaminated soil and water as plants can grow in large areas, provide aesthetic value to the landscape of the contaminated sites, and may have potential of economic returns which would offset the cost involved, which is already low. Moreover, the process is environment-friendly because plants uptake and accumulate the environmental contaminants within their tissues.

However, phytoremediation has some technical limitations. Information needed to consolidate phytoextraction into a cost-effective method is at present deficient. Expectations from phytoremediation should also be revised and more appropriately managed. Phytoremediation requires a long time period to be effective. In one estimate, given the low growth rate and biomass production in hyperaccumulators, a complete remediation of metals may not be achieved even in 10–20 years (Ernest, 1996). Strategies to address this potential difficulty should include identification of fast-growing plants as hyperaccumulators, and harvesting of vegetation several times a year.

Since concentrations of the contaminants can be phytotoxic and prevent plant growth, the preliminary phytotoxicity studies are necessary to screen the candidate plants. Phytoremediation efficiency is strongly influenced by the ability of plants to escape deleterious concentrations of toxic form of pollutants and the active oxygen species that may be generated in the treated tissue. Extensive progress has been

made in characterizing soil-chemistry management needed for phytoremediation, and physiology of plants that hyperaccumulate and hypertolerate metals. It is increasingly clear that hypertolerance is fundamental to hyperaccumulation, and high rates of uptake and translocation characterize the hyperaccumulating plants.

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