Chapter 11
Perspective on Phytoremediation for Improving Heavy Metal-Contaminated Soils

Hong-Bo Shao, Li-Ye Chu, Fu-Tai Ni, Dong-Gang Guo, Hua Li, and Wei-Xiang Li

Abstract Heavy metal pollution of soil is a significant environmental problem and has its negative potential impact on human health and agriculture. Phytoremediation strategies with appropriate heavy metal-adapted rhizobacteria (for example, mycorrhizae) have received more and more attention. Some plants possess a range of potential mechanisms that may be involved in the detoxification of heavy metals, and they manage to survive under metal stresses. High tolerance to heavy metal toxicity could rely either on reduced uptake or increased plant internal sequestration, which is manifested by an interaction between a genotype and its environment. A coordinated network of molecular processes provides plants with multiple metal-detoxifying mechanisms and repair capabilities, which allow plants to survive under metal-containing soil environments. The growing application of

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molecular genetic technologies has led to an increased understanding of mechanisms of heavy metal tolerance/accumulation in plants and, subsequently, many transgenic plants with increased heavy metal resistance, as well as increased uptake of heavy metals, have been developed for the purpose of phytoremediation. This article reviews advantages, disadvantages, possible mechanisms, current status and future directions of phytoremediation for heavy metal contaminated soils and environments.

Keywords Phytoremediation · Heavy metals · Soil · Mechanisms · Signal transduction · Phytohormones · Transcription factors · Biotechnology · Hyperaccumulator · Gene expression

Contents

1 Introduction

Phytoremediation of metals is being developed as an effectiveand environment-friendly solution for heavy-metal–contaminatedsoils (Barceló and Poschenrieder 2003; Banuelos et al. 2007; Aina et al. 2007). In recent years, major scientific strides have been takenin understanding the soil chemical and plant molecular-geneticmechanisms that drive metal hyperaccumulation in plants. Becausehyperaccumulators are mostly low biomass and slow-growing plants,current research is focused mainly on designing transgenic plantsthat can overcome this deficiency. The complexity of plant-metal interactions and influences of the environment, andspecific matrix factors that control the chemical speciationof the metal, and interactions of other toxicants that may bepresent at the site all add to the strategy of phytoremediation (Bassirirad 2000; Bauer and Bereczky 2003). Extensive progress has been made in characterizingand modifying the soil chemistry of the contaminated sites topromote/accelerate metal phytoremediation. However, extensivefield deployment of this technique on a large scale is stillbeing hampered by a lack of specific understanding of the complex interactions between metal, soil, and plant systems that are instrumental in metal uptake, translocation, and storage in plants. A multidisciplinary research effort that integrates the work of plant biologists, soil chemists, microbiologists, and environmental engineers is essential for the success of phytoremediation as a viable soil cleanup technique in metal-contaminated sites (Brewer et al. 1999; Bennett et al. 2003).

Phytoremediation is the use of a plant's natural ability to contain, degrade, or remove toxic chemicals and pollutants from soil or water. It can be used to clean up metals, pesticides, solvents, explosives, crude oil, and contaminants that may leak from landfill sites. The term phytoremediation is a combination of two words – phyto, which means plants, and remediation, which means to remedy (Clemens 2006; Denton 2007; Shao et al. 2008a, b, c, d, e).

Researchers are investigating phytoremediation potential by using plants such as sunflower, ragweed, cabbage, geranium, *Thlaspi caerulescens*, *Arabidopsis thaliana*, *Lycopersicon esculentum*, *Zea mays*, *Hordeum vulgare*, *Oryza sativa*, *Pisum sativum*, *Lotus japonicas*, *Brassica*, *Sedum alfredii*, *Cannabis sativa*, as well as other less known species. The plants are often used in combination with other traditional technologies for cleaning up contaminated sites because of the phytoremediation limitations (Cobbett 2002; Curie and Briat 2003; Citterio et al. 2003; Czako et al. 2006) There are many advantages of phytoremediation for heavy metal-contaminated soils (Table 11.1).

Table 11.1 Advantages of phytoremediation

Advantages	Disadvantages
1. Environment friendly, cost-effective, and aesthetically pleasing 2. Metals absorbed by the plants may be extracted from harvested plant biomass and then recycled 3. Phytoremediation can be used to clean up a large variety of contaminants; 4. May reduce the entry of contaminants into the environment by preventing their leakage into the groundwater systems	1. Relies on natural cycle of plants and therefore takes time 2. Phytoremediation works best when the contamination is within reach of the plant roots, typically three to six feet underground for herbaceous plants and 10 to 15 feet for trees 3. Some plants absorb a lot of poisonous metals, making them a potential risk to the food chain if animals feed upon them

2 Understanding Mechanisms of Phytoremediation for Improving Heavy Metal Contaminated Soils

2.1 Heavy Metal Accumulation in Plants

Heavy metals can be accumulated in various plant organs, which belong to the long-term effects of heavy metal action (Cunningham et al. 1995; Datta and Sarkar 2004). Their presence was detected in roots, stems, leaves, seeds and fruits. The cell wall is suggested to be the main accumulation site of Cd and other heavy metals. A similar accumulation site was found in vacuoles, especially in the case of Zn. In stems, Zn accumulated along the walls of vascular bundles, and in roots along cell walls. Its deposition occurred either in the form of simple Zn salts or proteins and carbohydrates complexes with Zn. Irons of heavy metals are detoxificated in the cytosol by high-affinity ligands like amino acids, organic acids and two types of peptides: PCs (phytochelins) and MTs (metallothioneins) (Deckert 2008; Doty 2008). It is generally assumed that the major sites of metal sequestration are vacuoles of root cells. PC-Cd complexes are transported into the vacuole, where heavy metal complexes are formed. Accumulation of heavy metals in chloroplasts is still controversial (Eide et al. 1996; Dhankher et al. 2002).

Ni was found to accumulate in seeds of *Raphanus sativus*, its level being maximal after 10 h of treatment (Elizabeth 2005). In wheat leaves, most of Ni accumulated up to the 3rd day after the application because of a fast and long distance transport of this metal (Fox and Guerinot 1998; Fayiga et al. 2004). Roots and shoots of *Pisum sativum* showed different metal accumulation capabilities. Ni amount in roots increased as a function of metal supply and was markedly higher than in shoots. In maize, Ni accumulated in chloroplasts of the bundle sheath cells and in the root apex. In chloroplasts, Ni was found to be more associated with their lamellar fraction than with the stroma and envelope (Gleba et al. 1999; Ghosh and Singh 2005; Huang and Cunningham 1996).

The content of Hg in tomato seedlings increased concurrently with Hg concentration and exposure time. More Hg was accumulated in roots than in above ground plant parts. Mature tomato leaves contained the greatest, whereas younger ones the smallest Hg content (Savenstrad and Strid 2004).

In rice seedlings growing at increasing lead concentration, Pb was distributed in an organ-dependent specific manner, which was greater in roots than in shoots. Pb was unevenly distributed in roots, where different tissues act as barriers to apoplastic and symplastic Pb transport, restricting its transport to shoots (Rugh et al. 1998; Hartley-Whitaker et al. 2001, 2002; Kramer 2005; Haydon and Cobbett 2007).

2.2 Genes Involved in Heavy Metal Perception and Signal Transduction

2.2.1 Heavy Metal Sensors

There are limited data on metal perception and signal transduction pathways in plants. The perception of extracellular signals is thought to be mediated by receptor-like protein kinases. The receptor-like kinase involved in heavy metal stress in plants has been reported very recently. The gene coding for lysine motif receptor-like kinase in barley was shown to be induced by Cr, Cd, Cu during leaf senescence (Fusco et al. 2006). The proteomic study on Cd-treated rice roots indicated the induction of putative receptor protein kinase. However, more detailed study on the function of this putative receptor has not been published so far.

2.2.2 Signaling Involved in Calcium, Reactive Oxygen Species (ROS) and Mitogen-Activated Protein Kinases (MAPK)

The heavy metal stress signaling in plants involves calcium changes, MAPK cascades and transcriptional activation of the stress-responsive genes (Gasic and Korban 2007; Li et al. 2005; 2006). The expression of metal-induced barley receptor-like kinase is also mediated by Ca level. It was suggested that certain metals (Cd, Ni, Co) may cause perturbation in intracellular calcium level and interfere with calcium signaling by substituting Ca in calmodulin regulation (Kim et al. 2007). By using calcium indicator, it was recently proved that metals such as Cd and Cu induce calcium accumulation in rice roots (Yeh et al. 2007). The treatment of tobacco cells and Scots pine roots with Cd and lupine roots with Pb caused the generation of H_2O_2(Meda et al. 2007). The Cd-producing oxidative burst in tobacco is mediated by calmodulin and/or calmodulin-dependent proteins. Thus, available data suggest the involvement of Ca/calmodulin pathway in signaling of metal response in plants (Sunkar and Zhu 2004).

MAPK pathway is involved in the transduction of extracellular signals to intracellular targets in all eukaryotes (McCully 1999; Pence et al. 2000; Shao et al. 2008). It was recently indicated that Cd and Cu activate four different MAPKs (SIMK, MMK2, MMK3 and SAMK) in alfalfa, whereas Cd induces one such kinase (ATMEKK1) in *Arabidopsis* and one (OsMAPK2) in rice (Persans et al. 2001; Sasaki et al. 2006; Kassis et al. 2007). However, it is not clear if activation of MAPKs occurs by direct action of these metals or through ROS, which also activates MAPK cascade in *Arabidopsis* or it occurs via action of other mediators (Wawrzynski et al. 2006). Recent information shows that Cd- and Cu-induced MAPK activation requires the involvement of calcium-dependent protein kinase (CDPK) and phosphatidyl-inositol 3-kinase (PI3 kinase) (Yazaki et al. 2006). Therefore, the current model for Cd and Cu signal transduction pathway states that both metals induce ROS production and calcium accumulation. The CDPK and PI13 kinase may be involved in metal-induced MAPK activities. However, both of these metals induce MAPK activation via distinct ROS-generating systems, therefore the MAP responsiveness may differ depending on the type of metals and ROS involved. MAPKs usually link the cytoplasmic signal to nucleus, where they activate other protein kinases, specific transcription factors and regulatory proteins (Sunkar et al. 2006; Shao et al. 2008).

2.2.3 Phytohormone Signaling

The signaling pathways involving abscisic acid (ABA), salicylic acid (SA) and auxin (IAA) also participate in the response to heavy metals, as respective *cis*-DNA regulatory elements were detected in heavy metal-induced genes. The auxin-responsive mRNA was detected in Cd-treated *Brassica juncea* plants (Lindblom et al. 2006). Proteomic analysis of Cd-treated *Arabidopsis thaliana* showed the induction of nitrilase protein, which is involved in auxin biosynthesis (Roth et al. 2006). The transcription activation of the gene (*SAMT)* involved in biosynthesis of SA was detected in pea treated with Hg. It is known that Cd induces the biosynthesis of ABA and ethylene, which in turn evoke various stress responses. All these data confirm that phytohormones play a role in plant responses to heavy metals. However, it is not clear if they play the signaling role in activation of heavy metal-responsive genes, or serve as effectors of certain heavy metal-imposed reactions to participate in both processes.

2.2.4 Heavy Metal – Induced Transcription Factors and Heavy Metal Responsive Elements

Little is known about transcriptional processes in plants in response to heavy metals as well as functional link between signaling pathways and responses at transcription level. The transcriptional profiling of plants treated with various heavy metals indicated that they can induce into heavy metal-induced transcription factors (LeDuc et al. 2006). The Cd-induction of transcripts for basic region leucine zipper (bZIP) and zinc finger transcription factors has been detected in *Arabidopsis thanliana* and *Brassica juncea* (Ramos et al. 2007). Screening of Cd-responsive genes in *Arabidopsis thanliana* indicated that *DREB2A* gene is up-regulated by Cd. The DREB proteins bind to dehydration response element and in Cd-treated *Arabidopsis thaliana*, *DREB2A* preferentially activates the *rd29A* gene, which is thought to play an important role under cold, high-salt and dehydration (Rosen 2002; Srivastava et al. 2005; Shao et al. 2008). On the other hand, one of the Cd-induced bZIP transcription factor (OBF5) in *Arabidopsis thaliana* binds to promoter region of glutathione transferase gene (*GST6*), which is known to be induced by auxin, SA and oxidative stress (Qi et al. 2007). The Zn treatment of *Arabidopsis thaliana* caused the induction of one type of transcription factor (bHLH), whereas the expression of two others (WRKY and zinc-finger, GATA-type) was decreased in the presence of excess of Zn (Ouelhadj et al. 2007). Despite existing data on the heavy metal-induction of different transcription factors, it is still not clear if these activations are specific to particular heavy metal ,common to most of the metals, related to oxidative stress (caused directly or indirectly by most of the heavy metals), mediated by phytohormones or connected with the general plant stress response (Sun and Zhou 2005). The process of ROS-mediated transcription activation of factors is thought to be a common link in different stress responses in plants. Therefore, among all possible pathways, ROS seems to play a key, but not the only one, role in activation of heavy metal-induced transcription factors in plants. Other organisms, such as yeast and animals, contain specific heavy metal-induced transcription factors which bind to heavy metal responsive element present in promoters of heavy metal-responsive genes (Cobbett 2002). The *cis*-acting elements related to heavy metal responsive elements have been found within promoters of a few plant genes, including metallothionein-like genes, however there is no evidence that these sequences confer heavy metal responsiveness of these genes. So far only two types of *cis*-DNA elements, which may be functional in heavy metal response, have been described in plants (Deckert 2008). One type is iron-dependent regulatory sequences (IDRS), which are responsible for the iron-regulated transcription of genes involved in Fe acquisition. The second one has been recently identified within the promoter region of *PvSR2* gene from *Phaseolus vulgaris*. *PvSR2* gene encodes a heavy metal stress related protein, whose expression is strongly stimulated by Hg, Cd, As and Cu, but not by other environmental stresses such as UV radiation, high temperature or pathogens. The heavy metal-responsive elements were localized within two regions of *PvSR2* gene promoter. Region I contains a motif similar to the consensus metal-regulatory element of the animal metallothionein genes, whereas the region II represents a novel heavy metal-responsive element in plants and has no similarity to previously identified *cis*-acting DNA elements involved in heavy metal induction.

According to the above concerning the activation of various transcription factors, which also confer the response to other stimuli, the lack of specific heavy metal-induced transcription factors and very limited data on the function of *cis*-acting and metal-specific DNA elements indicate that plants employ a wide array of mechanisms to activate the genes required to cope with the excess of heavy metals in their environment (Rocovich and West 1975; Ma et al. 2001; Rupali and Sarkar 2004). Possible molecular mechanisms of phytoremediation for heavy metal-contaminated soils, in combination with signaling pathways and transcription regulation, has been summarized in Fig. 11.1.

2.2.5 Phospholipid Signaling

Phospholipid signaling plays a crucial role in serving as a second messenger in plant responses to heavy metal stress (Shao et al. 2008). Phospholipds are rapidly produced in response to a variety of stimuli by the activation of lipid kinases or phosphatases. The expression of phospholipase D was shown to be induced by ABA, cold, drought, high salinity, wound and pathogen interactions (Bergmann and Munnik 2006). Some results indicate that this pathway may also be involved in plant response to heavy metals as the increased level of phospholipases transcripts were observed in cadmium-treated plants and phosphatidyl-inositol 3-kinase was shown to take part in cadmium and copper activation of MAPKs in rice roots (Yeh et al. 2007). The growing evidence suggests that plant signaling consists of network of pathways operating during various stress situations and that the crosstalk exists among stress responses, phytohormones and ROS signaling (see Fig. 11.1) (Sunkar and Zhu 2004; Sunkar et al. 2006; Fujita et al. 2006; Shao et al. 2008).

2.2.6 Posttranscriptional Regulation of Heavy Metal-Dependent Genes By MicoRNAs

MicroRNAs (miRNA) and short interfering RNAs (siRNAs) are small noncoding RNAs that have recently come out as a global important regulator of mRNA degradation, translational repression and chromatin modification (Sunkar and Zhu 2004). MicroRNAs are small, 21–22 nucleotides long, RNA molecules that can contribute to the regulation of gene expression in plants by directing an endoribonuclease complex to degrade the target mRNAs.The involvement of miRNAs in regulation of gene expression is mostly known for various developmental processes

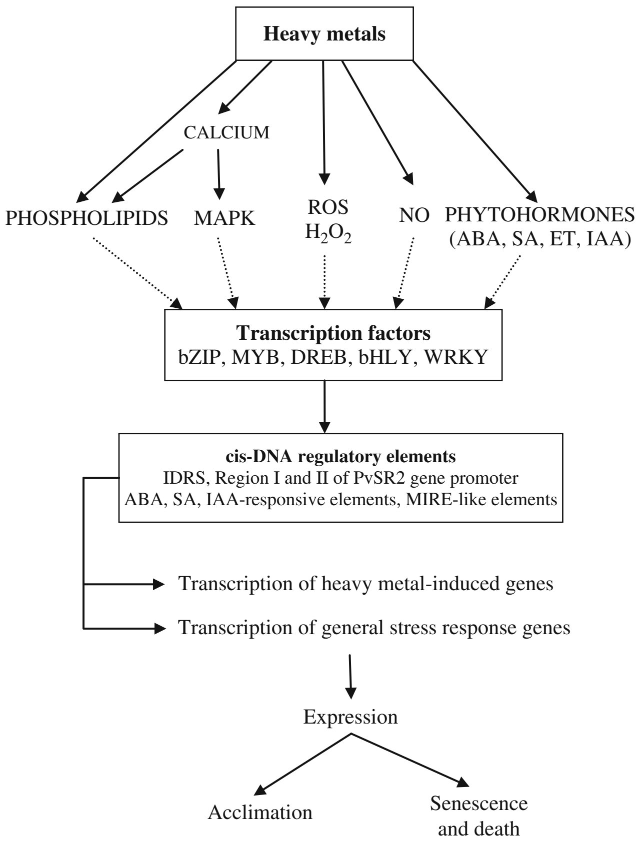

Fig. 11.1 Possible molecular mechanisms of phytoremediation for heavy metal-contaminated soils, in combination with signaling pathways and transcription regulation

(Dugas and Bartel 2004; Shao et al. 2008), but recently their participation in stress responses has been paid more attention (Sunkar and Zhu 2004; Shao et al. 2008). The predicted targets of number of *Arabidopsis thaliana* microRNA families, designated as miR398, are the mRNAs coding for cytoplasmic and chloroplast Cu-Zn-superoxide dismutase (Cu,Zn-SOD:CSD1 and CSD2) and a subunit of mitochondrial cytochrome C oxidase (COX5b-1). It was shown that miR398 expression is down-regulated transcriptionally by heavy metals, light and other oxidative stresses. This down-regulation of miR398 is important for up-regulation of mRNAs coding for Cu-Zu-SOD and oxidative stress response (Sunkar et al. 2006). Further studies indicated that the same microRNA (mir398) regulated copper homeostasis and mediated this regulation by controlling the degradation of Cu-Zn-SOD mRNA when Cu was limited (Yamasaki et al. 2007). It is clear that posttranscriptional processes involving microRNAs play important roles in regulating plant heavy metal dependent genes, which is a fine performance of acclimating mechanisms of higher

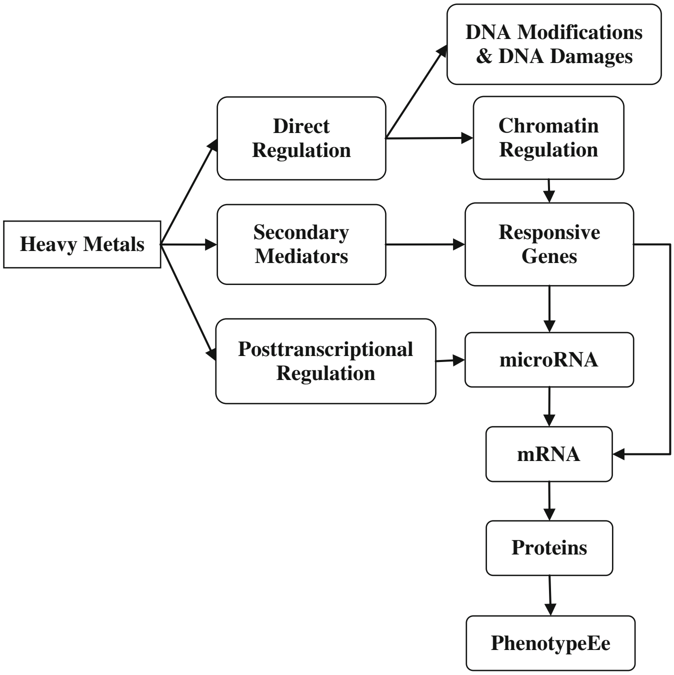

Fig. 11.2 A framework for the gene expression and regulation when plants are exposed to heavy metals

plants under the changing environment. A possible framework for the gene expression and regulation when plants are exposed to heavy metals is summarized in Fig. 11.2.

3 Important Standards for Heavy Metal Hyperaccumulator Plants

How do heavy metal hyperaccumulator plants achieve this remarkable bio-accumulation of soil heavy metals? Researchers have identified several characteristics that are important:

1. The plant must be able to tolerate high levels of the element in root and shoot cells; hypertolerance is the key property which makes hyperaccumulation possible. Such hypertolerance is believed to result from vacuolar compartmentalization and chelation. The most direct demonstration used isolated vacuoles from protoplasts of tobacco cells which had accumulated high levels of Cd and Zn. Whether hypertolerance in the known hyperaccumulators is due to an enhancement of these mechanisms is not yet known. However, electron

microprobe analysis supports vacuolar compartmentation for Zn in the leaves of the hyperaccumulator *Thlaspi caerulescens*.

2. A plant must have the ability to translocate an element from roots to shoots at high rates. Normally root Zn, Cd or Ni concentrations are 10 or more times higher than shoot concentrations, but in hyperaccumulators, shoot metal concentrations can exceed root levels. Researchers recently found that although the chemical forms of Ni found in extracts of leaves of Alyssum hyperaccumulators are the chelates with malate and citrate, in the xylem exudate histidine chelates about 40% of the total Ni present; nearly all of the histidine in exudate is chelated with Ni. Whether Ni^{2+} or a mixed chelate such as Ni (histidine, malate) is pumped into the xylem by a membrane transporter remains unknown. Additions of histidine to nutrient solution increased Ni tolerance and transport to shoots by *Alyssum montanum*, a non-hyperaccumulator species.
3. There must be a rapid uptake rate for the element at levels which occur in soil solution. Here quite different patterns have been observed in different groups of hyperaccumulators. Studies showed that *T. caerulescens* accumulated Zn and Cd from nutrient solution only about as well as tomato and *Silene vulgaris* did, but tomato was severely injured at 30 μM Zn, *S. vulgaris* at 320 μM Zn, and *T. caerulescens* only at 10,000 μM Zn. Because this species can keep tolerating and accumulating Zn and Cd at high soil solution levels, it is found in nature with 1–4% Zn while surrounding plants are <0.05% Zn (Zn excluders). Further, studies have shown that Zn hypertolerant genotypes of *T. caerulescens* require much higher solution Zn^{2+} (104-fold) and leaf Zn concentrations (100–300 mg kg^{-1} vs. 10−12 mg kg^{-1} in normal plants) to grow normally than do related non-hyperaccumulator species. By implication, the highly effective compartmentalization to reduce the toxicity of Zn and Cd appears to require the plant to accumulate much more Zn to have adequate supply. In contrast, the Ni-hyperaccumulator *Alyssum* species accumulate remarkably higher shoot Ni levels compared to other species grown at the same Ni^{2+} activity in solution. The Se-hyperaccumulating species similarly accumulate higher shoot Se levels and many can volatilize Se at high rates growing beside plants with more normal levels and slow volatilization.

4 Biotechnology and Phytoremediation of Heavy Metal Contaminated Soils

Biotechnology approaches to develop phytoremediation plants have been examined. Traditional plant breeding can only use available genetic diversity within a species to combine the characteristics needed for successful phytoremediation. Researchers expected that increasing the concentrations of metal binding proteins or peptides in plant cells would increase metal binding capacity and tolerance. Although plant cell cultures expressing mammalian metallothioneins (MTs) or phytochelatins (PCs) are more tolerant of acute Cd toxicity, the transfer of mammalian metallothionein genes to higher plants appears to provide no benefit for phytoremediation. Further, when natural metal hypertolerant plants were examined, the concentration of PCs showed no difference, suggesting that hypertolerance to Cd and Zn in these plants was not due to the hyperaccumulation of PC peptides. The evidence for the role of PCs is that their presence does correlate with normal levels of metal tolerance, since mutations that abolished PC production in *Arabidopsis* and fission yeast resulted in hypersensitivity to Cd. Cd-sensitive (hypotolerant) single gene mutants *cad1* and *cad2* of *Arabidopsis thaliana* have been identified and studied (e.g. PC synthesis). For a plant species with normal tolerance (*A. thaliana*), PCs were essential for the normal level of tolerance (Cunningham et al. 1995; Wu et al. 2006; Doty 2008; Shao et al. 2008).

Although these studies have allowed cloning of genes involved in acute Cd tolerance, and characterization or confirmation of metabolic pathways, the environmental relevance of findings from such acute Cd exposure has not been established. An alternative view of Cd-catalyzed PC biosynthesis is that chelation of PCs with Cd alleviates the feedback inhibition of the PC-synthase; as long as Cd activity in the cytoplasm is high, an enzyme supports more transfer to form more PCs and longer PCs. Because the level of Zn present in nearly all environments is 100 times higher than that of Cd, if an acute toxic Cd dose is provided, the plants would be killed by Zn. Even the formation of the sulfide-stabilized high molecular weight Cd-PC complex in vacuoles may result from the acute toxic Cd supply without Zn. Further, the finding that the hmt1 vacuolar membrane pump protein (which restored Cd hypertolerance to mutant fission yeast) transported both Cd-PCs and PCs without Cd, raises questions about how the pump works to induce Cd hypertolerance *in vivo*. Cadmium (Cd) phytotoxicity in soil is a recent anthropogenic effect, whereas Zn phytotoxicity and co-accumulation of trace levels of Cd are normal biogeochemical phenomena. It seems increasingly likely that the Cd hypertolerance mechanisms are incidental biochemical phenomena. Although Cd-PCs can be found at low levels in plants in the environment, they account for only a small fraction of the tissue Cd (Suzuki et al. 2001; Jonak et al. 2004).

Another goal of developing transgenic plants with increased metal binding capacity was to use these metal-binding factors to keep Cd in plant roots, thus reducing Cd movement to the food chain or into tobacco. Vacuolar compartmentation of Cd only in roots may reduce Cd translocation to shoots; expression in plants of the hmt1 vacuolar pump for Cd-PCs from fission yeast has not yet been successful, and modification of gene sequences may be required before its effectiveness can be tested (similar to the mercury reductase gene sequence changes). The expression of MT as the whole protein, the Cd binding '-domain' part of the protein, or a fusion protein with -glucuronidase, under several promoters increased Cd tolerance of tobacco and other plants, but had little effect on Cd transport to shoots(Pence et al. 2000). Recently use of the improved 35S2 promoter may have increased the ability of MT to keep Cd in roots, however, tests have not yet progressed to soil studies which must be the important measure of success. Some promising genes that are involved in phytoremediation of heavy metal–contaminated soils in plant roots are listed in Table 11.2.

Table 11.2 Some promising genes involved in phytoremediation of heavy metal–contaminated soils in plant roots

Gene	Functions of gene products	Plant species	Roles of gene products	Gene regulation
Atfro2	Ferric chelate reductase	*Arabidopsis thaliana*	iron reductase	Induction
Psfro1		*Pisum sativum*	Iron reductase	Induction
Atirt1	ZIP transporter	*Arabidopsis thaliana*	Fe II transport in root epidermis	Up-regulation
Atirt2		*Arabidopsis thaliana*	Iron (metal) transporter	Up-regulation
Leirt1		*Lycopersicon esculentum*	Iron (metal) transporter	Up-regulation
Leirt2		*Lycopersicon esculentum*	Iron (metal) transporter	Similar expression
Psrit1		*Pisum sativum*	Iron (metal) transporter	Induction
Osrit1		*Oryza sativa*	Iron (metal) transporter	Induction
Atnramp1	NRAMP	*Arabidopsis thaliana*	Iron (metal) transporter	Up-regulation
Atnramp3		*Arabidopsis thaliana*	Iron (metal) transporter	Up-regulation
Atnramp4		*Arabidopsis thaliana*	Iron (metal) transporter	Up-regulation
Lenramp1		*Lycopersicon esculentum*	Putative iron(metal)transporter	Up-regulation
Lenramp3		*Lycopersicon esculentum*	Putative iron(metal)transporter	Similar expression
Osnramp1		*Oryza sativa*	Iron (metal) transporter	Not analysed

Table 11.2 (continued)

Gene	Functions of gene products	Plant species	Roles of gene products	Gene regulation
Osnramp3		*Oryza sativa*	Putative iron(metal)transporter	Not analysed
Atfrd3	Transporter MATE	*Arabidopsis thaliana*	Putative transporter	Weak up-regulation
Lechln	Nicotianamine sysnthase	*Lycopersicon esculentum*	Nicotianamine synthase	Similar expression
Hvnas		*Hordeum vulgare*	nicotianamine synthase	Induction
Osnas		*Oryza sativa*	Nicotianamine synthase	Induction
Hvnaata *Hvnaatb*	Phytosiderophore enzyme	*Hordeum vulgare*	nicotianamine aminotransferase	Induction
Hvids2 *Hvids3*		*Hordeum vulgare*	Putative dioxygenase	Induction
Lefer	Regulator	*Lycopersicon esculentum*	regulator, putative transcription factor	Similar expression

5 Conclusion

Extensive progress has been made in characterizing soil chemistry management needed for phytoremediation, and physiology of plants which hyperaccumulate and hypertolerate metals. It is increasingly clear that hypertolerance is fundamental to hyperaccumulation, and high rates of uptake and translocation are observed in hyperaccumulator plants. Fundamental characterization of mechanisms and cloning of genes required for phytoremediation has begun with the mercuric ion reductase, and *hmt1* expression in higher plants is expected soon. Improved hyperaccumulator plants and agronomic technology to improve the annual rate of phytoextraction and to allow recycling of soil toxic metals accumulated in plant biomass is important to support commercial environmental remediation, which society can afford in contrast with present practices. Although most phytoremediation systems are still in development, or in plant breeding to improve the cultivars for field use, application for Se phytovolatilization has already begun. Many opportunities have been identified for research and development to improve the efficiency of phytoremediation. Progress had been hindered by limited funds for research and development for 15 years since the first report of the model for phytoremediation. New commercial firms are moving into this field and phytoremediation technologies will be increasingly applied commercially in the near future.

At the present time, phytoremediation is an emerging technology and there is still a significant need to pursue both fundamental and applied research to fully exploit the metabolic and growth habits of higher plants. It is precisely the purpose of the European COST Action 837 to stimulate the development and evaluate the potential of plant biotechnology for the removal of organic pollutants and toxic heavy metals from wastewater and contaminated soils.

Heavy metals affect plant gene expression at different scales. They can influence DNA directly and may act via modification of chromatin structure. The activation of heavy metal stress-responsive genes occurs by a complex array of signaling pathways, which is a dimensional network. The various secondary mediators participate in the activation of regulatory proteins that bind to promoter regions of target genes. Some of these processes constitute a general plant stress response and are not solely specific to the heavy metal stress. The regulation of plant genes by heavy metals also occurs post-translationally by microRNA silencing. The framework for the mechanism is referred to Figs. 11.1 and 11.2, although there are more details remained to be known.

Overall, the main limitations of heavy metal phytoextraction technology for soil remediation are related to low-deep penetrating roots, low yields of hyperaccumulator plants and the disposal of their metal-enriched biomass and the little knowledge about the detoxifying process in plants and soil. So, phytoremediation is very much dependent on plant and soil factors, such as soil suitability for plant growth, depth of the contamination, depth of the plant root system, level of contamination, and urgency in cleaning up. Furthermore, there is also need for a full understanding of the physiology, biochemistry, molecular biology, and uptake process of the plants employed. In combination with biotechnology, selection of new hyperaccumulators (including ferns) is also a challenge.

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