BIOLOGIA PLANTARUM 51 (4): 618-634, 2007

REVIEW

Metal hyperaccumulation and bioremediation

K. SHAH* and J.M. NONGKYNRIH**

*Department of Zoology, Mahila Mahavidyalaya, Banaras Hindu University, Varanasi-221005, U.P., India**
*Department of Biochemistry, North Eastern Hill University, Shillong-793022, Meghalaya, India***

Abstract

The phytoremediation is an environment friendly, green technology that is cost effective and energetically inexpensive. Metal hyperaccumulator plants are used to remove metal from terrestrial as well as aquatic ecosystems. The technique makes use of the intrinsic capacity of plants to accumulate metal and transport them to shoots, ability to form phytochelatins in roots and sequester the metal ions. Harbouring the genes that are considered as signatures for the tolerance and hyperaccumulation from identified hyperaccumulator plant species into the transgenic plants provide a platform to develop the technology with the help of genetic engineering. This would result in transgenics that may have large biomass and fast growth a quality essential for removal of metal from soil quickly and in large quantities. Despite so much of a potential, the progress in the field of developing transgenic phytoremediator plant species is rather slow. This can be attributed to the lack of our understanding of complex interactions in the soil and indigenous mechanisms in the plants that allow metal translocation, accumulation and removal from a site. The review focuses on the work carried out in the field of metal phytoremediation from contaminated soil. The paper concludes with an assessment of the current status of technology development and its future prospects with emphasis on a combinatorial approach.

Additional key words: chaperones, phytoextraction, phytofiltration, phytomining, phytostabilization, phytovolitization, transporter.

Introduction

With an increase in anthropological practices, more and more toxic metal ions are being added to the natural environment disrupting the ecosystem. Metals like Cd, Pb, Zn, Cr, *etc*. when present in high concentration in soil show potential toxic effects on overall growth and metabolism of plants (Shah and Dubey 1998, Agrawal and Sharma 2006). Bioaccumulation of such toxic metals in the plants poses a risk to human and animal health (Wang *et al.* 2003). Removal of excess of metal ions, from the contaminated site is brought about by chemical as well as biological means. Chemical remediation involves the use of chemicals to clean the natural environment but is not universal *i.e.* one chemical cannot be used for all metal ions (Chaney *et al.* 1997). Moreover, the existence of many classes and type of chemical species make the removal of the toxic metals from the environment very complicated.

The use of biological means to clean the natural environment includes bioremediation techniques. The technology is based on the use of naturally occurring or genetically engineered microorganisms (GEMS) to restore contaminated sites and protect the environment (Baker *et al.* 2000). Other than microorganisms, certain plant species that accumulate high concentrations of heavy metals also have a potential towards restoration of environment. The rate of bioremediation is directly proportional to plant growth rate and the total amount of bioremediation is correlated with a plant total biomass, making the process very slow. This necessitates the identification of a fast growing (largest potential biomass and greatest nutrient responses) and more strongly metal-accumulating genotypes.

The present review gives an update on the cellular and molecular detoxification of metal ions, identified metal-hyperaccumulators and their possible role in the bioremediation process.

Received 18 November 2005, *accepted* 19 May 2006.
Abbreviations: ACC deaminase - 1-aminocyclopropane-1-carboxylicaciddeaminase; d.m. - dry mass; EDTA - ethylenediaminetetraacetic acid; MT - metallothionein; PC - phytochelatin.
Acknowledgements: Authors are grateful for the project grant no. SR/FT/L-75/2003 from the Department of Science and Technology, Government of India, New Delhi under FTYS program.
* Author for correspondence; fax: (+91)-542-2369695, e-mail: kavitashah@bhu.ac.in

Cellular balance, tolerance and detoxification of metal ions

Heavy metals such as Cu, Zn, *etc*. are essential for normal plant growth and development, as they form part of many enzymes and proteins. Elevated contents of both essential and non-essential metals in the soil lead to toxicity symptoms and growth inhibition in plants which might result from binding of metal to SH-group of proteins resulting in the inhibition of their activities or disruption of structure (Shah and Dubey 1998), displacement of an essential element resulting in deficiency effects (Van Assche and Clijster 1990), and stimulation of the formation of free radicals and reactive oxygen species, causing oxidative stress (Dietz *et al.* 1999, Shah *et al.* 2001).

Tolerance to heavy metal in plants may be defined as the ability to survive in a soil that is toxic to other plants and is manifested by an interaction between a genotype and its environment (McNair *et al.* 2000). The term tolerance is however, more widely used in the literature to include changes that may occur experimentally in the sensitive response to heavy metals. In a number of thorough genetic studies, the adaptive metal tolerance has been shown to be governed by a small number of major genes and perhaps contribution of some minor modifier genes (McNair 1993, McNair *et al.* 2000, Schat *et al.* 2002). Perhaps it is this adaptive metal tolerance that gears a plant species for hyperaccumulation. A genetic analysis of copper tolerance with Cu-tolerant and susceptible lines of *Mimulus guttatus* showed that a modifier gene that is active only in presence of the tolerance gene is responsible for the difference in Cu-tolerance in this species (Smith and McNair 1998). Similar studies with Zn-hyperaccumulator *Arabidopsis halleri* and the non-accumulator *Arabidopsis petrea* suggested that Zn-tolerance is also controlled by a single major gene (McNair *et al.* 2000).

To protect themselves from metal poisoning, plants must have developed a mechanism by which the heavy metal entering the cytosol of the cell, is either immediately excluded or complexed and inactivated, thus preventing the metal from inactivating catalytically active or structural proteins, presumably by adapting mechanism that may also be involved in the general homeostasis of essential mineral ions, and tolerate them. In this section of the review the transporters, chelators and proteins involved in metal homeostasis at the cellular level is discussed.

Transporter mediated uptake of metal ions

In recent years the molecular understanding of entry of both essential and non-essential metal ions in plant cells have been greatly advanced. Several plant metal transporters are known and more remain to be identified. The transporters identified so far include ZIP1-4, ZNT1, IRT1, COPT1, AtVramp1/3/4 and LCT1 on the plasma membrane-cytosol interface; ZAT, ABC type, AtMRP, HMT1, CAX2 seen in vacuoles; RAN1 seen in Golgi bodies (Table 1). Manipulations of these transporters to achieve removal of metal ions from the cell holds great potential (Tong *et al.* 2004).

Fe and Zn uptake is mediated by a group of transporters belonging to the ZIP family *i.e. Z*RT, IRT related proteins (Fox and Guerinot 1998, Saier 2000). IRT1 is isolated from *Arabidopsis* and its transcription is induced in *Arabidopsis* roots by iron starvation, which makes this transporter a likely candidate for mediating Fe(II) uptake from soil. IRT1 shows a broad substrate range and also transport Mn^{2+}, Zn^{2+}, and possibly Cd^{2+} (Korshunova *et al.* 1999). ZIP-transporters 1-3 confer Zn^{2+} uptake activity (Grotz *et al.* 1988, Guerinot and Eide 1999). Characterization of new metal transporters in *Medicago truncatula* with high similarities with the ZIP family (MtZIP) revealed their functions as metal transporters (Lopez-Millan *et al.* 2004). All six proteins contained conserved ZIP signature motifs. MtZIP4 and MtZIP7 protein restored yeast growth in Mn-limited media however, the transcript levels of MtZIP3 and MtZIP4 were down-regulated. MtZIP5 expression was up-regulated under Mn-limiting conditions in leaves but in roots it appeared to be down-regulated. MtZIP6 and MtZIP7 expression, remained unaffected by metal concentration (Lopez-Millan *et al.* 2004).

Table 1. Metal ion transporters in plants (data from S. Clemens 2001)

Localization	Transporter	Metal ions
Cytosol	ZIP1-4	Zn^{2+}, Cd^{2+}
	ZNT1	Zn^{2+}, Cd^{2+}
	IRT1	Fe^{2+}, Mn^{2+}, Zn^{2+}, Cd^{2+}
	COPT1	Cu
	AtVramp1/3/4	Fe^{2+}, Cd^{2+}
	LCT1	Cd^{2+}, Ca^{2+}
Vacuole	ZAT1	Zn^{2+}
	At MRP	Cd-PC
	HMT1	Cd-PC
	CAX2	Cd^{2+}
Golgi	RAN1	Cu complex

The Zn transporter gene *ZTP1* is highly similar to the *Arabidopsis ZAT* gene. It probably is an allele of the recently cloned *ZNT1* and its close homologue *ZnT2* gene from *Thlaspi caerulescens* (Pence *et al*. 2000). All three zinc transporter genes show increased expression in *T. caerulescens* compared with the non-hyperaccumulator congener *T. arvense*, suggesting an important role in heavy metal hyperaccumulation. *ZNT1* and *ZNT2* are predominantly expressed in roots and *ZTP1* is mainly expressed in leaves but also in roots. In *T. arvense*, *ZNT1*

and *ZNT2* are exclusively expressed under conditions of Zn deficiency. Their expression in *T. caerulescens* is barely Zn-responsive, suggesting that Zn hyperaccumulation might rely on a decreased Zn-induced transcriptional down-regulation of these genes. *ZTP1* expression was higher in plants from calamine soil than in plants from serpentine or normal soil. The calamine plants were also the most Zn tolerant, suggesting that high *ZTP1* expression might contribute towards Zn tolerance (Pence *et al.* 2000).

Kampfenkel *et al.* (1995) hypothesized that *Arabidopsis* COPT 1 is a Cu transporter as it rescues the Cu uptake deficiency of *Saccharomyces cerevisiae* mutants ctr1-3, however the mRNA for COPT 1 are not detectable in the roots, indicating the possibility of an unidentified transporter for Cu in roots, yet to be known.

The natural resistance associated macrophage proteins (Nramp) family of transporters has been recently characterized from rice and *Arabidopsis*. Based on sequence comparison the family is divided into two classes of transporters. One comprising of AtNramp1 and OsNramp1 and the other of AtNramp2-5 and OsNramp2. AtNramp3 is involved in Cd^{2+} uptake. Disruption of this gene enhanced Cd tolerance whereas its over-expression led to Cd hypersensitivity in the above plants (Curie *et al.* 2001). The expression of another transporter LCT1 that enhances uptake of Cd, elevates the protective action of calcium against cadmium toxicity in tobacco (Clemens *et al.* 1998, Antosiewicz and Hennig 2004). Another member of Nramp family of divalent metal ion transporter, SMF1 is shown to transport Mn in yeast (Celliar *et al.* 1995).

The HMT1 is a protein with similarity to ABC type transporters in *Schizosaccharomyces pombe* which is localized in the vacuolar membrane and mediates Mg-ATP, vanadate-inhibitable transport of PC-Cd complexes and apo-PC (Ortiz *et al.* 1995). Similar activities have been observed in the tonoplast of the oat roots, indicating the operation of an HMT1 like mechanism in plant cells (Salt and Rauser 1995). Since very few HMT1 sequences are known in plants, AtMRPs are considered as probable transporters of PC-Cd across the tonoplast (Rea *et al.* 1998).

YCF1 is a MgATP-energized vacuolar transporter responsible for sequestration of compounds after their *S*-conjugation with glutathione from *S. cerevisiae* (Tommasini *et al.* 1998). Over-expression of the *YCF1* gene in *A. thaliana* exhibited a 4-fold higher rate of glutathione-Cd uptake in YCF-1 transgenics than those of wild-type plants, indicating that its expression strongly increases Cd transport. Tolerance and resistance in transgenics improved both for Cd and Pb as desired for effective phytoremediation (Song *et al.* 2003).

The members of the cation diffusion facilitator (CDF) family of transporters have been reported in plants (Paulsen and Saier 1997, Van der Zaal *et al.* 1999) and their substrates include Zn and Cd ions. The CzcD from *Ralstonia eutropha* mediates Zn^{2+} efflux (Anton *et al.* 1999) and the proteins COT1 and ZRC1 from *S. cerevisiae* when over-expressed, confer Co and Zn/Cd tolerance in these plants, respectively (Kamizono *et al.* 1989, Conklin *et al.* 1992). Both these proteins are located in the tonoplast suggesting their role in metal sequestration (Li and Kaplan 1998).

CAX2 transporter characterized from *Arabidopsis* is considered to be a high affinity, high capacity H^+/heavy metal cation antiporter (Hirschi *et al.* 1996, 2000).

Metal chelation and detoxification

Chelators contribute to metal detoxification by buffering cytosolic metal concentrations, whereas chaperones specifically deliver metal ions to organelles and metal-requiring proteins (Fig. 1). In plants the principal classes of metal chelators include phytochelatins, metallothioneins, organic acids and amino acids.

Phytochelatins (PCs) are small metal-binding peptides found in plants and are well documented in literature (Grill *et al.* 1986a, Mehra and Winge 1988, Meuwley *et al.*1995, Klapheck *et al.* 1994, Chen *et al.* 1997). PC formation uses glutathione (Grill *et al.* 1989), homoglutathione, hydroxymethyl-glutathione (Klapheck *et al.* 1995) or γ-glutamylcysteine (Hayashi *et al.* 1991). It is catalyzed by phytochelatin synthase (PCS), a constitutive enzyme requiring post-translational activation by heavy metals and/or metalloids, in particular Cd, Ag, Pb, Cu, Hg, Zn, Sn, As and Au both *in vivo* and *in vitro* (Grill *et al.* 1987, Grill *et al.* 1989, DeKnecht *et al.* 1995, Klapheck *et al.*1995, Maitani *et al.* 1996, Chen *et al.* 1997, Wojcik and Tukiendorf 2005).

Iso-PCs, a series of PC-like homologous chelating peptides are reported with varying terminal amino acids like alanine (Grill *et al.* 1986b), serine (Klapheck *et al.* 1992), glutamic acid (Meuwly *et al.* 1995), glutamine (glu; Maitani *et al.* 1999) and have a C-terminal modified residue other than glycine (gly; Gekeler *et al.* 1989).

Peptides lacking the C-terminal amino acid are reported by Bernhard and Kagi (1987) in *Zea mays.* These have the formula $(\gamma\text{-glu-cysteine})_n$ and are called desGly-PC. The PC and iso-PC molecules form complexes with heavy metals like Cd. Low molecular mass cytosolic PC-metal complex are then transported into the vacuole where high molecular complexes are formed with incorporation of S^{2-}. In addition to PC-Cd complex other PC-metal-complexes include Ag, Cu (Maitani *et al.* 1996) and As (Schmoger *et al.* 2000).

In vitro experiments have shown that a series of metal-sensitive plant enzymes can tolerate a 10- to 1000-fold concentration of Cd in the form of a PC complex than as free radical ion (Kneer and Zenk 1992). PC reactivate metal poisoned plant enzymes such as nitrate reductase up to 1000-fold better than chelators

such as glutathione (GSH) or citrate, showing again the extraordinary sequestering potential of these peptides. Howden *et al.* (1995) succeeded in isolating a *cad*1-mutant of *Arabidopsis thaliana* sensitive to Cd ions and deficient in its ability to form Cd-PC complexes. In view of the fact that *Arabidopsis* has only single pathway for PC synthesis the finding of the Cd-sensitive mutant (impaired in PC synthesis) when challenged to Cd confirmed the role of PC in Cd detoxification and the authors concluded that *cad1* gene is likely the structural gene for PC synthase (Howden *et al.* 1995).

As-tolerant *Holcus lanatus* L is shown to synthesize high concentrations of PCs than As-intolerant species (Hartley-Whitaker *et al.* 2002). Studies on the effect of As on phosphorous content in soybean revealed a decrease in level of P, however no test for PC synthesis was done in these plants (Milivojević *et al.* 2006).

Aside from detoxification, PCs also play a role in homeostasis of heavy metal in plants. On one hand, they complex the metal ions to inactivate and store them in the vacuole and on the other, they transfer the essential metal to the newly synthesized apoenzymes that require Cu^{2+} or Zn^{2+} for catalytic activity or to nucleic acid structures such as Zn-fingers (Thumann *et al.* 1991).

On screening of plants for genes of metal tolerance a wheat cDNA, TaPCS1, was identified which stimulated the tolerance to Cd, in *S. cerevisiae* (Clemens *et al*. 1999, Vatamaniuk *et al.* 1999).

Metallothioneins (MTs) are ubiquitous low molecular mass cysteine (cys)-rich proteins, that bind metal ions in metal-thiolate clusters identified in mature embryos of wheat plants as early cys-labeled protein (Ec; Hamer 1986). It was shown to bind to Zn^{2+}. More than 50 MTs are reported in different plants categorized in four classes of MT proteins (Cobbett and Goldsbrough 2002). In plants, a wide range of MT genes from various sources have been over-expressed including those from human (Misra and Gedamu 1989), mouse (Maiti *et al.* 1991, Pan *et al.* 1994), Chinese hamster (Hattori *et al.* 1994) and yeast (Hasegawa *et al.* 1997, Thomas *et al.* 2003). The over-expression of copper inducible MT *cup 1* enhances Cu tolerance in plants (Hamer *et al.* 1986).

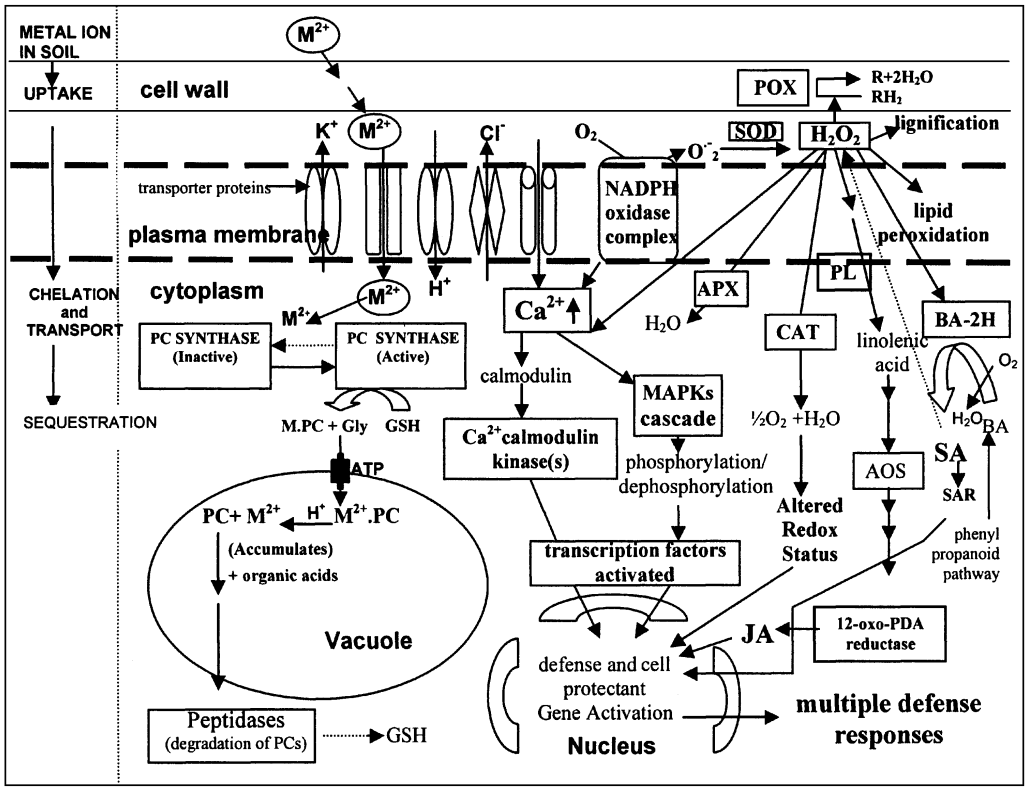

Fig. 1. A model of the mechanisms that occur in plant cell upon exposure to metals: metal ion uptake, chelation, transport, sequestration, signalling and signal transduction. The diagram shows the uptake of metal ions by K^+ efflux and transporter proteins, their sequestration by formation of PCs by enzyme PC synthase and GSH in vacuoles, the subsequent degradation of PC-peptides by peptidases to release GSH, the generation of ROI species, the contribution of Ca^{2+} towards activation of Ca^{2+}/calmodulin kinase(s) and MAP kinase(s) cascade leading to defense gene activation in nucleus, the effect of ROI on natural plant defense pathways like octadecanoid pathway (JA) and phenyl propanoid pathway (SA) biosynthesis that lead to defense and cell protectant gene activation is also included to correlate the induced metal stress defense with natural plant defense mechanism. AOS - allene oxide synthase; APX - ascorbate peroxidase; BA - benzoic acid; BA-2H - benzoic acid 2-hydroxylase; CAT - catalase; GSH - glutathione; JA - jasmonic acid; M^{2+} - metal ions; MAPK - mitogen activated protein kinase; 12-oxo PDA reductase - 12-oxo-cis-10,15-phytodienoic acid reductase; PC - phyochelatin; PL - phospholipase; POX - peroxidase; SA - salicylic acid; SOD - superoxide dismutase.

Organic acids and amino acids are suggestive potential ligands for chelation, owing to the capacity of metal ions to react with S, N and O. Citrate, malate, and oxalate have been implicated in a range of processes, including differential metal tolerance, metal transport through xylem and vacuolar metal sequestration (reviewed in Rauser 1995). Citric acid has been hypothesized to be a major Cd^{2+} ligand at low Cd^{2+} concentrations (Wagner 1993), has been shown to form complexes with Ni^{2+} in Ni-accumulating plants (Sagner *et al.* 1998) and suggested to contribute to Zn accumulation and tolerance (Goldbold *et al*. 1984). Similarly malate is proposed as a cytosolic Zn chelator in Zn tolerant plants (Mathys 1997). Salicylic acid is also reported to have a role in maintainence of ionic homeostasis in *Medicago sativa* seedlings treated with Cd or Fe (Dražić *et al.* 2006). Oven *et al.* (2002) reported an increase in the level of citric acid upon exposure to cobalt ions. The role of histidine in metal chelation was also studied and the histidine content of the xylem sap on exposure to Ni in Ni-hyperaccumulator *Alyssum lesbiacum* was reported to increase 36-fold (Kramer *et al.* 1996). Supplying His to a non-accumulating species greatly increased both its Ni tolerance and the capacity to transport Ni to the shoot. However, the His response seem to be a species specific mechanism of Ni tolerance as it was not observed in another Ni-hyperaccumulator, *Thlaspi geosingenses* (Persans *et al.* 1999). Recently, Nakazawa and coworkers (2004) selected and characterized nickel-tolerant cells from tobacco and suggested the correlation of Ni tolerance to the concentration of histidine in these cells.

Copper chaperones are essential in providing the copper atoms for several copper-requiring proteins. They can compete for Cu with other chelators and metal sequestering processes at extremely low cytosolic concentrations of free Cu ions. With the recent identification of Cu chaperones and Cu transporters from *S. cerevisiae* and human, intracellular metal trafficking pathways are emerging (reviewed by O'Halloran and Cullota 2000). The cytosolic Cu chaperones COX17 delivers Cu to cytochrome c oxidase complex (Glerum *et al.* 1996). ATX1 is involved in Cu transfer to post-Golgi vesicles *via* interaction with a Cu-pumping P-type ATPase, a class of copper chaperone protein CCC2, residing in the membrane of the post-Golgi vesicles (Fu *et al.* 1995, Pufahl *et al.* 1997). Thus it is quite evident that metal trafficking is mediated by specific metal ion chaperons, however still more remains to be known about them in plants.

Metal hyperaccumulators

The term hyperaccumulation was first described by Jaffre *et al.* (1976) when they observed the accumulation of Ni in *Sebertia accumulata*. Metal-hyperaccumulation is a phenomenon defined as uptake and sequestration of exceptional concentrations of heavy metal in the aboveground parts of a plant under field conditions (Pollard 2000). The definition is based on comparative surveys, indicating that on metalliferous soils most plants accumulate low concentrations of metal ions in their shoots while a few species, endemic to metalliferous sites accumulate distinctly high amounts (Baker and Brooks 1989). For a plant to be identified as a hyperaccumulator, the species can accumulate 0.1 % of dry mass [%(d.m.)] of elements such as Ni, Co or Pb; 1 %(d.m.) of Zn, Mn and 0.01 %(d.m.) of Cd. Such plants are resistant to certain metal ions suggesting their potential use for cleaning of contaminated soil (Chaney *et al.* 1997). Use of hyperaccumulators open a new branch of bioremediation technology termed as phytoremediation – an eco-friendly and scientific approach to remove, extract or inactivate metal ions in the soil using plants (Chaney 1983, 1993, Cunningham and Berti 1993, Baker *et al.* 1994, Raskin 1996, Cunningham *et al.* 1995, Lasat 2002, McGrath 1998, Salt *et al.* 1998). Metal hyperaccumulators are natural or purposely engineered for hyperaccumulation.

Natural hyperaccumulators

Nearly 450 hyperaccumulator plants also known as metallophytes have been described belonging to a wide range of taxa, ranging from annual herbs to perennial, geographically distributed in all continents, both in temperate and tropical environments (Table 2). Notable centres of distribution are for Ni: New Caledonia, Cuba, SE Asia, Brazil, Southern Europe and Asia Minor; Zn and Pb: NW Europe; Co and Cu: South-central Africa. Some families and genera are particularly well represented; *e.g*., for Ni: *Brassicaceae* (*Alyssum* and *Thlaspi*), *Euphorbiaceae* (*Phyllanthus*), *Leucocroton* and *Asteraceae* (*Senecio, Pentacalia*); Zn: *Brassicaceae* (*Thlaspi*); Cu and Co: *Lamiaceae*, *Scrophulariaceae* (Baker and Brooks 1989, Chaney *et al.* 1997, Brooks 1998, Clemens 2001, Broadhurst *et al.* 2004, Gratao *et al.* 2005, Prasad 2005, Vinterhalter and Vinterhalter 2005). Interestingly 75 % of the identified hyperaccumu-lators accumulate Ni and are termed as nickelophilous plants (Baker and Brooks 1989, Prasad 2005). Of the wide range of families of vascular plants the natural hyperaccumulating plant species are well represented by the members of *Brassicaceae* (Reeves and Baker 2000, Prasad and Freitas 2003, Gratao *et al.* 2005). Natural hyperaccumulators can grow in their natural habitat alone, have slow growth, low-biomass and very often are selective for an individual metal (Kamnev and

Table 2. Few natural plant metal-hyperaccumulator species and their bioaccumulation potential.

Metals	Plant species	Amount [g kg^{-1}(d.m.)]	Reference
As	*Pteris vittata*	22.6	Ma *et al*. 2001
Cd	*Thlaspi caerulescens*	10.0	Lombi *et al.* 2001
Cr	*Salsola kali*	2.9	Gardea-Torresdey *et al.* 2005
Co	*Haumaniastrum robertii*	10.2	Brooks 1977
Cu	*Ipomea alpina*	12.3	Baker and Walker 1990
Pb	*Thlaspi rotundifolium*	0.13-8.2	Reeves and Brook 1983
Mn	*Phytolacca acinosa*	19.3	Xue *et al*. 2004
Ni	*Alyssum betolonni*	>10.0	Morrison *et al.* 1980
Se	*Brassica juncea*	2.0	Orser *et al.* 1999
Zn	*Thlaspi caerulescens*	30.0	Baker and Walker 1990

Van der Lelie 2000, Clemens *et al.* 2002*)*.

Among the earliest known natural metal hyper-accumulators are a group of small, weedy alpine flowers called Alpine pennycress (*Thlaspi* spp.) which lack the standard pathogen defense mechanism. *Thlaspi* spp. exhibit large interspecific and intraspecific variations which make it an important plant to study hyperaccumulation. Predominantly *Thlaspi* grow on nickel contaminated sites and accumulates ≈ 3 % of its d.m. as metal. Other than *T. arvense* (non-accumulator species) various species of *Thlaspi* are known to hyperaccumulate more than one metal. *T. caerulescens* accumulate Cd, Ni, Pb and Zn; *T. goesingense* and *T. ochroleucum* accumulate Ni and Zn and *T. rotundifolium* accumulates Ni, Pb and Zn (Baker and Brooks 1989, Baker and Walker 1990, Kramer *et al.* 1996, Prasad 2005). *Thlaspi caerulescens* has a remarkable capacity to accumulate extremely high levels of nonlabile zinc and cadmium in its shoots, 39.6 g(Zn) kg^{-1}(d.m.) and 10.0 g(Cd) kg^{-1}(d.m.), and has been the subject of intense research to gain a better understanding of heavy metal hyperaccumulation and tolerance mechanisms (Baker and Walker 1990, Lasat 2002). It has also been used as a source of genes for developing plant species better suited for the phyto-remediation of metal-contaminated soils (Lombi *et al.* 2001). Kramer (2005) demonstrated that an altered tonoplast Zn transport in root cells stimulated Zn uptake in leaf thus playing a role in Zn hyperaccumulation in *T. caerulescens*.

Pistia stratiotes is used for phytoremediation of wastewater or natural water bodies polluted with heavy metals. The species exhibit different patterns of response to Ag, Cd, Cr, Cu, Hg, Ni, Pb and Zn. A 5 mM concentration of each of these metals, resulted in distinct levels of growth inhibition and biomass production in *P. stratiotes*, with almost all the elements being accumulated at high concentrations in the root system. The plant species exhibited highest tolerance index (the ratio of the d.m. of plant in polluted soil at a particular level of metal to the d.m. of the same plant in non-polluted soil at zero level of metal) to Zn and lowest to Hg (Odjegba and Fasidi 2004, Rabie 2005).

According to Bennicelli *et al.* (2003), the water hyperaccumulator fern *Azolla caroliniana* Wild. (*Azollaceae*) accumulates high amounts of Hg and Cr with the capacity to purify waters polluted by these metals. *Spartina* are 3-fold more tolerant to Hg than tobacco plants, owing to their ability to absorb organic Hg and transform it into an inorganic form (Hg^{+}, Hg^{2+}). The inorganic Hg then accumulates in the underground parts of the plant and is re-transferred to the soil by diffusion and permeation, indicating that this natural hyper-tolerance towards Hg could be used in the phytoremediation of Hg polluted environment (Tian *et al.* 2004). A Cr-hyperaccumulator plant species *Salsola kali* has recently been reported to accumulate 0.6 - 2.9 g(Cr) kg^{-1}(d.m.) of hexavalent Cr in aerial parts of the plant suggesting its potential as a new option for phyto-remediation of Cr-contaminated soil (Gardea-Torresdey *et al.* 2005).

The oldest natural hyperaccumulator reported for Pb is *Thlaspi rotundifolium*. It can accumulate 0.13 - 8.2 g(Pb) kg^{-1}(d.m.) in leaves (Reeves and Brook 1983). *Helianthus annuus* has been known to concentrate Pb in its leaf and stem and can be used as a hyperaccumulator to restore abandoned mines and factory sites contaminated with elevated Pb levels (Boonyapookana *et al.* 2005). Recently another Pb hyperaccumulating species *Hemidesmus indicus* has been reported to accumulate Pb in roots and shoots proving itself to be a potential candidate for Pb removal from soil (Chandrashekhar *et al.* 2005). The hyperaccumulator *Sesbania drummondii* is shown to accumulate Pb as lead acetate in roots and leaves, lead sulfate in leaves and lead sulfide in root and shoot both (Sharma *et al.* 2004) indicating its ability to biotransform lead nitrate to lead acetate and lead sulfate in its tissues. This complexation of Pb with acetate and sulfate perhaps forms a part of Pb-detoxification strategy in these plants (Sharma *et al.* 2004).

Arsenic accumulation has been demonstrated in *Lemna gibba* and the species is warranted to be a preliminary bioindicator for As. It is used to monitor removal of As in mine tailing waters because of its high accumulation capacity (Mkandawire and Dudel 2005). Pickering *et al.* (2000) studied accumulation of As in *B. juncea*. These workers reported that As^{5+} is transported as a phosphate analogue in roots, which subsequently is

reduced to As^{3+} in shoots and stored as As^{3+}-tris-thiolate. *Pteris vittata* can also hyperaccumulate As from naturally contaminated soils, but is suitable for phytoremediation only in the moderately contaminated soils (Ma *et al*. 2001, Bondada and Ma 2003, Caille *et al.* 2004). In addition to *P. vittata*, *P. cretica*, *P. longifolia* and *P. umbrosa* are also able to hyperaccumulate As to a similar extent (Zhao *et al.* 2002).

Astragalus bisulcatus and the perennial *Stanleya pinnata* (*Brassicaceae*) grow on seleniferous soils and hyperaccumulate Se in their shoots. Parker *et al.* (2003) determined that 16 diverse populations of *S. pinnata* each absorb selenate preferentially over sulfate. Most of the Se in *S. pinnata* shoots occur in the form of soluble amino acids that may serve as direct precursors of volatile forms of Se such as dimethylselenide. The most widely studied Se-hyperaccumulator is *Brassica juncea* which concentrates nearly 2.0 g(Se) kg^{-1}(d.m.) in leaves (Orser *et al.* 1999).

Plant species differ considerably in their normal Mn leaf concentration: 0.30 - 5.0 g(Mn) kg^{-1}(d.m.), Clarkson 1988, Ducic and Polle 2005). Recently a new Mn-hyper-accumulator plant *Phytolacca acinosa* has been identified (Xue *et al.* 2004). This perennial herb can accumulate 19.3 g(Mn) kg^{-1}(d.m.) when grown on Mn-rich soil. Authors warranted that *P. acinosa* grows rapidly, has substantial biomass, is widely distributed with a broad ecological amplitude and has potential for use in phyto-remediation of Mn-contaminated soils (Xue *et al.* 2004).

Brassica oleracea var. *acephala* and *Iberis intermedia* are natural hyperaccumulators for thallium. These two species have been studied for mobility of Tl and its uptake (Al-Najar *et al.* 2005). Unusually high accumulation of Tl, 0.4 and 1.5 %(d.m.) in *Iberis intermedia* and *Biscutella laevigata,* respectively, are reported (Anderson *et al.* 1999).

Transgenic hyperaccumulators

Though several natural hyperaccumulators are known, the plants ideal for green technology to clean up soil should posses multiple traits. They must have deep roots, rapid growth, high biomass, be easily harvested, and must tolerate and accumulate a large range of heavy metals in their above-ground parts. No plant is known to have all of the above traits. The development of engineered plants (transgenic) harboring the required traits for bioremediation is perhaps the only alternative.

Over-expression and introduction of hyperaccumulating genes into a non-hyperaccumulator plant could be a possible way to enhance metal uptake and accumulation, tolerance and detoxification process (Clemens *et al.* 2002). The over-expression of a gene encoding a rate-limiting gene product would be expected to lead a faster overall rate of the pathway and to more efficient phytoremediation (Pilon-Smits and Pilon 2001). Besides this, the repression of an endogenous gene, by inserting a gene of reverse orientation (antisense technology) can also result in enhanced metal uptake by plants. Several reports on bioengineered plant tolerant to the presence of toxic levels of metals like Se (Berken *et al.* 2002), Cd (Kawashima *et al.* 2004), As (Lee *et al.* 2003a), Zn, Cr, Cu and Pb (Bennett *et al*. 2003), *etc.* have appeared in the literature in the recent years. In most of the studies the over-expression of the genes encoding for the enzymes of S-metabolism, glutathione, phytochelatin synthase, ACC deaminase, Hg^{2+}-reductase, arsenate reductase, aldolase/ aldehyde reductase, enzymes of histidine biosynthesis and metallothionein (MT)-genes have been carried out. The engineering of transporter genes to manipulate the transport of metal ions inside the cell has also been exploited effectively and a combination of some of these genes in rapidly growing plant species have led to a few promising results (Lee *et al.* 2003a,b, Song *et al.* 2004, Verret *et al*. 2004).

A well known example of transgenic metal hyperaccumulator is *Brassica juncea*, which over-express ATP sulphurylase. It shows higher uptake of Se and enhanced Se tolerance compared to wild type when grown in the presence of selenate in either hydroponic conditions or in soil (Pilon-Smits *et al.* 1999, Van Huysen *et al.* 2004). These transgenic plants can also tolerate Cd, Zn, Cu, Hg, As (III, IV). Transgenic Indian mustard over-expresssing cystathione- γ-synthase (CGS) had low shoot Se concentration with enhanced Se volatilization rate as well as Se tolerance than the wild type plants grown either hydroponically or in soil (Van Huysen *et al.* 2003, 2004). *Astralagus bisulcatus* is a seleniferous plant and accumulates Se, but it has a slow growth rate. Se-Cys, the form in which Se has deleterious effects that results from the coupling of selenide with *O*-acetyl-Ser in a reaction catalyzed by the action of Cys-synthase (Terry *et al.* 2000). Since Se toxicity stems mostly from the incorporation of Se-Cys into proteins in the place of Cys, it has been shown that over-expressing the enzyme selenocysteine methyltransferase (SMT) that specifically methylates selenocysteine (SeCys) to produce the non-protein amino acid methylselenocysteine (MetSeCys) in *A. bisculatus*, led to a reduction in the intracellular concentrations of SeCys and selenomethio-nine (SeMet), thus preventing their incorrect insertion into protein, thereby increasing tolerance to Se compounds, in particular selenite (Le Duc *et al.* 2004).

In an attempt to improve the potential for removal of metals using plants, Brewer *et al.* (1999) created somatic hybrids between *Thlaspi caerulescens* (Zn-hyper-accumulator) and *Brassica napus*. Accumulation of high levels of Zn was observed in hybrids, which otherwise are toxic for *B. napus.* Somatic hybrids obtained from *T. caerulescens* and *B. juncea* have been shown to remove significant amounts of Pb (Gleba *et al.* 1999).

Arabidopsis plants transformed with an *E. coli* gene *Znt A* that encodes for Pb^{2+}, Cd^{2+} and Zn^{2+} transport had

improved resistance to Pb^{2+} and Cd^{2+} (Lee *et al.* 2003b) and the ZntA was located at the plasma membrane (Lee *et al.* 2003b). Expression of a pea (*Pisum sativum*) MT gene *PsMTA* in *Arabidopsis thaliana*, accumulated more Cu (several-fold in some plants) in the roots of transformed plants as compared to controls (Evans *et al.* 1992). Similarly when a type 2 MT gene, *tyMT*, cloned from *Typha latifolia*, a wetland plant with constitutional tolerance, was introduced into *A. thaliana*, the transgenic plant showed an increased tolerance to both Cu^{2+} and Cd^{2+} (Zhang *et al.* 2004). The introduction of metallothionien proteins AtMT2a and AtMT3 from *A. thaliana* as fluorescent protein-fused forms into the guard cells of *Vicia faba* resulted in transgenic plants which had guard cells protected from degradation upon exposure to Cd brought about by reducing the reactive oxygen species. The authors concluded that the Cd stays bound to the MT in the cytoplasm and is not sequestered into the vacuole instead it gets detoxified by phytochelatins (PCs) in *V. faba* cells (Lee *et al.* 2003b).

Transgenic tomato plants expressing the bacterial gene 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase showed enhanced metal accumulation and tolerance levels for a range of heavy metals (Cd, Cu, Ni, Mg, Pb and Zn) than untransformed plants (Grichko *et al.* 2000). The expression of partial peptides from the C terminus of the TcHMA4 (the *Thlaspi* heavy metal ATPase) protein, which contains numerous possible heavy metal-binding His and Cys repeats residues, confer an extremely high level of Cd tolerance and hyperaccumulation in yeast. The possibilities for enhancing the metal tolerance and phytoremediation potential of higher plants via expression of TcHMA4 hold great potential in metal remediation studies (Papoyan and Kochian 2004).

Arsenic poisoning is a serious problem (Dondon *et al.* 2005) and Dhankher *et al.* (2002) examined the effects of co-expressing two bacterial genes for arsenate reductase (arsC) and γ-glutamylcysteine synthetase (γ-ECS) in *Arabidopsis* plants. They observed that plants expressing SRS1p/ArsC and ACT2p/γ-ECS together showed substantially greater arsenic tolerance than wild-type plants or plants expressing γ-ECS alone. In addition, when grown on arsenic, these plants accumulated 4 to 17-fold greater fresh shoot mass and accumulated 2 to 3-fold more arsenic per gram of tissue than wild-type plants or plants expressing γ-ECS or ArsC alone.

Other than plants the potential model phytoremediators for As now include various genotypes of transgenic trees, *e.g.*, *Austromyrtus bidwilli* (Sjaan *et al.* 2002). Trees are ideal in the remediation of heavy metals as they can withstand and accumulate higher concentration of pollutants owing to their large biomass and size, can reach a huge area and great depths for their extensive rootings and can stabilize an area. They prevent erosion, and the spread of the contaminant, because of their perennial presence (Sykes *et al.* 1999). Researchers are now trying to extend this technology to cottonwood trees, which are potential effective remediators (Dondon *et al.* 2005). Once the trees accumulate sufficient quantities of arsenic they could be harvested and removed from the site, taking with them large quantities of As (Sykes *et al.* 1999, Che *et al.* 2003, Dondon *et al.* 2005).

Phytoremediation techniques

The phytoremediation technology involves several techniques like phytoextraction, phytomining, phytovolatilization, phytofiltration, *etc*. all of which employ plants for cleaning of contaminants from the soil.

Phytoextraction is the extraction of metals from soil and is used to clean up heavy metals, pesticides and xenobiotics (Suresh and Ravishankar 2004), organic compounds (Newman and Reynolds 2004), toxic aromatic pollutants (Singh and Jain 2003), radionuclides (Soudek *et al.* 2004) and acid mine drainage (Archer and Caldwell 2004). A plant ability to phytoextract certain metals is a result of its dependence upon the absorption of metals such as zinc, manganese, nickel, and copper to maintain natural function (Lasat 2000, 2002). Research show that the hyperaccumulators often do not exclude non-essential metals in the absorption process, thus resulting in plants that can extract high contents of pollutants (1 - 2 % of their biomass) from contaminated soil (Lasat 2000, 2002). It is believed that plants initially developed the ability to hyperaccumulate non-essential metallic compounds as a means of self-protection from herbivorous predators, who would experience toxic side effects from ingestion of the hyperaccumulator's foliage (Pollard and Baker 1997).

Two approaches proposed for phytoextraction are continuous or natural phytoextraction and chemically enhanced phytoextraction (Salt *et al.* 1998). The former is based on the use of natural hyperaccumulator plants with exceptional metal-accumulating capacity, but have slow growth and take years or decades to clean up a contaminated site. McGrath *et al.* (1993), using field data, calculated that nine croppings of *T. caerulescens* would be required to decrease Zn concentration in the soil from 0.30 to 0.44 g(Zn) kg^{-1}. Similarly, Brown *et al.* (1994) estimated that 28 years of *T. caerulescens* cultivation would be necessary to remove all the Zn from a soil containing 2.1 g(Zn) kg^{-1}.

Phytoextraction has attracted attention in recent years for the low cost of implementation and environmental benefits. Metal as Pb are largely immobile in soil and their extraction rate is limited by solubility and diffusion to root surface. Chemically enhanced phytoextraction has been developed to overcome these problems (Huang and Cunningham 1996, Blaylock *et al.* 1997, Huang *et al.* 1998, Blaylock 2000). Sites are treated by acid leaching, physical separation of the contaminants or electrochemical processes (Alshawabkeh and Bricka 2000). This approach makes use of high-biomass crops that are

induced to take up large amount of metals when their mobility in soil is enhanced by chemical treatments. Several chelating agents, such as citric acid, ethylene-diamine-tetraacetic acid (EDTA), diethylene-triamine-pentaacetic acid (DTPA), ethylene-bis[oxyethylene-trinitrilo] tetraacetic acid (EGTA), nitriloacetate (NTA) and other synthetic chelators, have been studied for their ability to mobilize metals and increase accumulation of metal as Pb (Blaylock *et al.* 1997, Huang *et al*. 1997), U (Huang *et al.* 1998), ^{137}Cs (Lasat *et al.* 1998), and Au (Anderson *et al.* 1998) in different plant species. An increase in the accumulation of gold was observed in Indian mustard grown in soil treated with ammonium thiocyanate (Anderson *et al.* 1999). At present, the most promising application of phytoextraction is the remediation of Pb-contaminated soils using Indian mustard in combination with EDTA (Blaylock 2000). Despite the success of this technology, concerns have been expressed regarding the enhanced metal to soil mobility and their potential risk of leaching to ground water (Cooper *et al.* 1999). However, no detailed studies regarding the persistence of metal-EDTA complexes in contaminated soils have been conducted.

Soils contaminated with multiple heavy metals can present a difficult challenge for both approaches to phytoextraction. Although some hyperaccumulators appear to be capable of accumulating elevated concentrations of several heavy metals simultaneously, there still remains considerable specificity in metal hyperaccumulation (Baker *et al.* 2000). For chemically enhanced phytoextraction, establishment of a high-biomass crop is required before chelate application. This is difficult to achieve if the soil is heavily contaminated with metals like Zn, Cd, and Cu, which are usually much more bioavailable, and thus more phytotoxic, than Pb.

Phytofiltration is similar to phytoextraction, and plants are essentially used to clean-up contaminated groundwater rather than soil. For cleanup the plants are raised in greenhouses with their roots in water. Contaminated water is either collected from a waste site and brought to the plants or the plants are planted in the contaminated area. As the roots are saturated with contaminants, they are harvested. Sunflowers were successfully used to remove radioactive contaminants from pond water in a test at Chernobyl, Ukraine. Effective removal of Cr by water-plant *Spirodela polyrhiza* has been reported by Appenroth *et al.* (2000).

Phytostabilization or phytorestoration is the use of certain plant species to immobilize contaminants in the soil and groundwater through absorption and accumulation by roots, adsorption onto roots, or precipitation within the root zone of plants. The plants that exclude metal ions have a low potential for metal chelation, but they can be used for stabilizing the soil and preventing further contamination spread due to erosion. *Agrostis tenius* has been reported to avoid Cd, Cu, Zn and Pb uptake by precipitating the metal in the rhizosphere (Dahmani-Muller *et al.* 2000). The technology does not achieve a clean up of the soil, but changes mobility of the contaminant and prevents migration to the groundwater or air, and also reduces bioavailability for entry into the food chain. It can be used to re-establish a vegetative cover at sites where natural vegetation is lacking due to high metal concentrations in surface soil or physical disturbances to surficial materials. Phytoremediation of Pb contaminated soils involve both phytostabilization and phytoextraction techniques (Lasat 2000, 2002).

Phytomining is the harvesting of plant tissues from hyperaccumulators that have accumulated high amount of toxic metals, for metal recovery and reuse. This demands a proper selection of hyperaccumulator species and cultivars suitable for a particular metal or region (Alkorta *et al.* 2004). The technique is in demand with investors for recovery of precious metals as ≈ 0.57g(Au) kg^{-1}(d.m.) was observed to accumulate in Indian mustard (Anderson *et al.* 1999).

Phytovolitization is the uptake and vaporization of a solid or liquid contaminant to an airborne vapour by a plant either as the pure pollutant, or as the metabolized pollutant before it is vaporized, as in the case of mercury, lead and selenium (Black 1999, Boyajian and Carriera, 1997, Saxena *et al.* 1999, LeDuc *et al.* 2004). The metal can be rendered harmless by either enzymatic reduction or by incorporation into less toxic organic/metal compounds which occur naturally. This can be exploited for genetic manipulation by introducing genes coding for the enzymes responsible for the underlying biochemical reactions. A well-known example of such manipulation is the transfer and expression of a modified *E. coli* Hg^{2+}-reductase gene *(merA9pe)* in transgenic *Arabidopsis thaliana* plants (Rugh *et al.* 1996, 1998). Bacteria possessing *merA* are capable of converting highly toxic, Hg^{2+}, to less toxic elemental Hg. Thus, expression of *merA* in transgenic plants helps the removal of elemental Hg as vapours through natural mechanisms of respiration. Rugh *et al.* (1998) also examined the ability of yellow poplar (*Liriodendron tulipifera*) tissue cultures and plantlets to express modified mercuric reductase (*merA*) gene constructs. Certain plants can also volatilize Se in a similar way, with the help of Se-methylation enzymes (LeDuc *et al.* 2004). Tagmount *et al.* (2002) identified *S*-adenosyl-L-Met:L-Met *S*-methyltransferase (MMT), an enzyme involved in the methylation of Se-Met to Se-methyl-met. This enzymatic step is an important rate-limiting step in the Se phytovolatization process. *Arabidopsis* T-DNA knockout mutants lacking MMT activity exhibited almost no capability to volatilize Se. Phytovolatization potentially offers a low-cost alternative for Se removal from soil and water. During the process of Se phytovolatization, plants metabolize various inorganic or organic species of Se (*e.g.* selenate, selenite, and Se-Met [Met]) into a gaseous form (Berken *et al.* 2002). Dimethyl selenide, the major volatile form of Se, is more than 600 times less toxic than inorganic forms (Berken *et al.* 2002). Indian mustard (*Brassica juncea*) has a high rate of Se accumulation and volatilization, and a fast growth rate, making it a promising species for Se remediation (Pilon-Smits *et al.* 1999, Pilon-Smits 2005).

Phytodegradation is metabolization of pollutants by plants after the contaminant has been drawn into the plant. The pollutant assimilates into plant tissue and is degraded by plant-derived enzymes such as nitrate-reductase, laccase, dehalogenase, and nitrilase, as demonstrated in field studies (Boyajian and Carriera 1997). The daughter compounds can be either volatized or stored in the plant. If the daughter compounds are relatively benign, the plants can still be used in traditional applications. If the daughter compounds are less harmful than the parent compound, but not benign, then the plants can be burned or used in alternate applications (Boyajian and Carriera 1997).

Advantages and disadvantages of bioremediation

The biggest advantage of using plants for cleaning the environment is the utilization of their inherent agronomic traits and benefits of plants like high biomass, extensive root systems, ability to withstand environmental stress, *etc.* (Bizily *et al.* 1999). Plant-facilitated bioremediation is aesthetically pleasing and makes the environment green and clean. As the entire process is solar energy driven, no artificial source of energy is required to drive the bioremediation process, making it cost-effective and environmental friendly (Bizily *et al.* 1999). Plants offer a permanent, *in situ*, non-intrusive, self sustaining method of removal of soil contaminant. Planting vegetation on a contaminated site also reduces erosion by wind and water. Phytoextraction enables to reclaim and recycle precious metals and other useful materials from the soil making the process economically beneficial for investors (Moffat 1995). In addition, plants used in bioremediation do not disturb the topsoil thus conserving its utility (Sykes *et al.* 1999).

Few concerns regarding the phytoremediation technology are the slow speed of the process when compared to mechanical methods. Plants can take many growing seasons to clean up a site due to slow growth pertaining to climatic restrictions and species variations. Hyperaccumulator plants with short roots can clean up soil or groundwater near the surface *in situ*, but cannot remediate deep aquifers without further design work (Sykes *et al.*1999). Plants that absorb toxic materials may contaminate the food chain, as animals inhabiting the contaminated area might consume these plants (Moffat 1995). Phytoremediation technique is less efficient for hydrophobic contaminants, which bind tightly to soil (Bizily *et al.* 1999). Also the volitization of compounds can transform a groundwater pollution problem to an air pollution problem (Raskin 1996). The greatest problem lies in the fact that after phytoremediation of one site what would eventually be the fate of the plants used for the purpose and which are now rich in that particular contaminant? Their biodegradation or recycling makes the contaminants either fully or partially re-enter the soil (Gratao *et al.* 2005). The answer to this question is yet to be resolved.

The public opposition to developing genetically modified plant species or crops however, remain the most crucial hindrance in the advancement of phyto-remediation technique and development of transgenic hyperaccumulator plant species.

Concluding remarks and future outlook

The present situation of metal pollution affects the whole ecosystem. Using genetic modifications, scientists have been able to expand the role of plants in the environment. In order to restore environmental balance the bioremediation technique evidently does indicate several benefits and is one of the most preferred methods to deal with this problem. However, the efficiency of the method lies in the fact that to implement a specific bioremediation method, a scientific and well formulated strategy must be adopted taking into consideration the type of metal ions, geographical location, biomass of the hyperaccumulator plant, *etc*. Detailed studies in the field of bioremediation have improved the methods and practice, however, further improvements are required to reduce the limitations of the existing protocols so that they may be utilized with less negative output.

The various studies of metal-ion homeostasis in human, yeast and plants suggest that there exists a complex regulated network for metal ion transport, chelation and sequestration. Although some information regarding genes controlling the synthesis of peptides that sequester metals, like phytochelatins (*e.g.* the *Arabidopsis cad1* gene, Howden *et al.* 1995), genes encoding transport proteins, such as the *Arabidopsis IRT1* gene that encodes a protein that regulates the uptake of iron and other metals (Eide *et al.* 1996) or genes encoding enzymes that change the oxidation state of heavy metals, like the bacterial *merA* gene encoding mercuric oxide reductase (Rugh *et al.* 1996) are currently being used to improve metal hyperaccumulation in plants, further identification of plant genes encoding metal-ion/metal-complex transporters and their molecular components could be of immense use for bioremediation studies. Further manipulations of these genes would prove useful to determine plant metal hypertolerance and hyper-accumulation. The strategy could be thus used as a tool to specifically select several more plant species fit for phytoremediation studies (Fig. 2).

Screening of mutagenized *Arabidopsis* populations identified mutants tolerant to Mn (Delhaize *et al.* 1993),

P (Delhaize *et al.* 1995) and Pb (Chen *et al.* 1997). Further identification of other mutants for biochemical, molecular and physiological analysis and important molecules involved in tolerance and metal hyper-accumulation in plants is required to develop ideal transgenic hyper-accumulators.

Isolation of metal-sensors that sense the metal status of the cell and to elucidate the subsequent regulatory steps in terms of up-regulation and down-regulation of metal-responsive genes, the signal transduction pathway and the metal responsive transcription factors would also be of immense use for developing effective phyto-remediators.

Strategies for enhancing phytoremediation of organics are potentially more straightforward. Genes encoding biodegradative enzymes can be introduced and/or over-expressed in transgenic plants, leading to enhanced biodegradative abilities. Such genes can be of any origin (bacterial, plant or even animal) or can even be synthetic, perhaps making use of advanced techniques of protein engineering or directed mutagenesis to create optimized catalytic proteins.

Metal hyperaccumulator plants are relatively rare, often occurring in remote areas geographically and threatened by devastation from mining activities. Population sizes can be extremely small. There is thus an urgent need to collect these materials, bring them into cultivation and establish a germplasm facility for large-scale production of hyperaccumulator species for future research, development and trial work.

The cleaning of the metal-contaminated environment might be feasible with a combinatorial approach, *i.e.*, integrate and use different bioremediation methods simultaneously and target the technology both qualitatively as well as statistically. Plant biologists, microbiologists, agronomists and engineers will have to integrate their efforts for the bioremediation of a specific site contaminated with specific heavy metal(s). It is important to proceed with caution and implement the technology at the larger scale only after longitudinal studies are conducted.

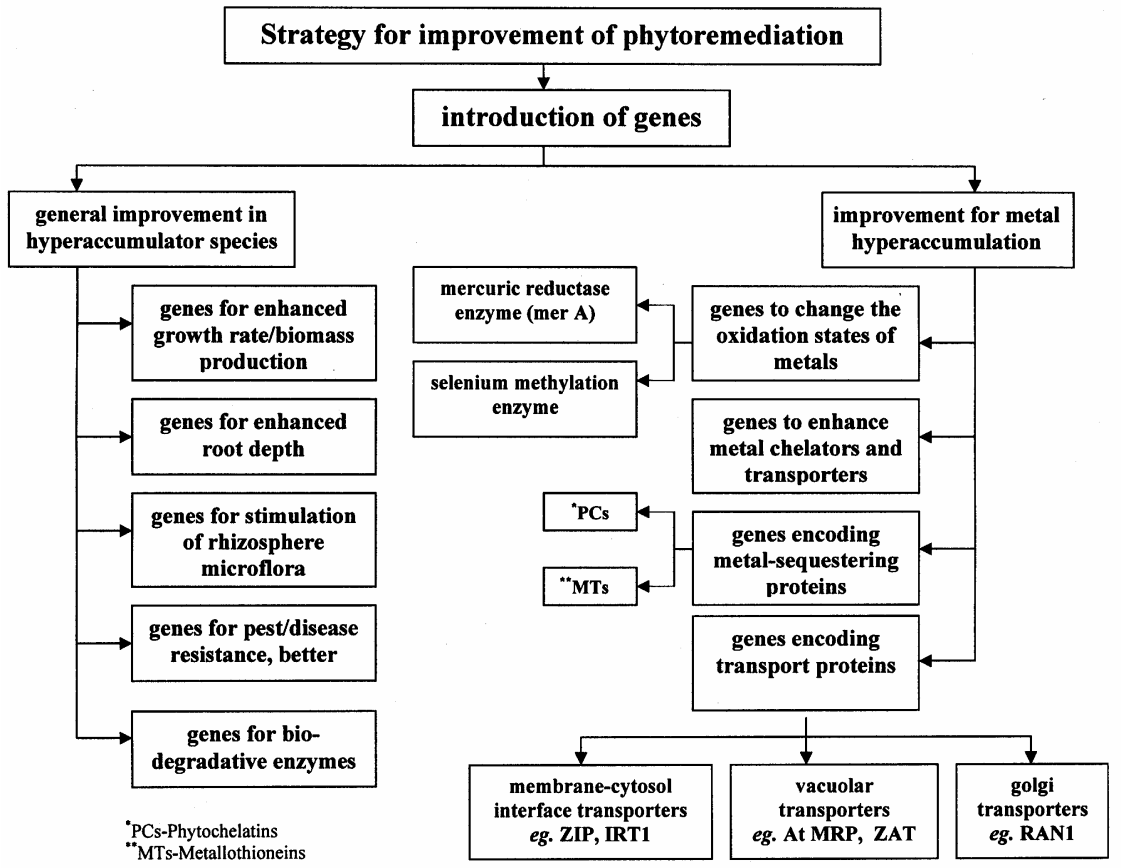

Fig. 2. Strategies for improvement of hyperaccumulators using genetic engineering.

References

Agrawal, V., Sharma, K.: Phytotoxic effects of Cu, Zn, Cd and Pb on *in vitro* regeneration and concomitant protein changes in *Holarrhena antidysentrica*. - Biol. Plant. **50**: 307-310, 2006.

Alkorta, I., Hernandez-Allica, J., Becerril, J.M., Amezaga, I., Albizu, I., Garbisu, I.: Recent findings on the phyto-remediation of soils contaminated with environmentally toxic heavy metals and metalloids such as zinc, cadmium, lead and arsenic. - Environ. Sci. Biotechnol. **3**: 71-90, 2004.

Al-Najar, H., Kaschl, A., Schulz, R., Romheld, V.: Effects of thallium fractions in the soil and pollution origin in thallium uptake by hyperaccumulator plants: a key factor for assessment of phytoextraction. - Int. J. Phytorem. **7**: 55-67, 2005.

Alshawabkeh, A.N., Bricka, R.M.: Basics and applications of electrokinetic remediation. - In: Pessarakli, M. (ed.): Remediation Engineering of Contaminated Soils. Pp. 95-111. Marcel Dekker, New York 2000.

Anderson, C.W.N., Brooks, R.R., Steward, R.B., Simack, R.: Harvesting a crop of gold in plants. - Nature **395**: 553-554, 1998.

Anderson, C.W.N., Brooks, R.R., Chiarucci, A., Lacoste, C.J., Leblanc, M., Robinson, B.H., Simack, R., Steward, R.B.: Phytomining for nickel, thallium and gold. - J. Geochem. Explor. **67**: 407-415, 1999.

Anton, A., Grosse, C., Reissmann, J., Prebyl, T., Nies, D.H.: CxcD is a heavy metal ion transporter involved in regulation of heavy metal resistance in *Ralstonia* sp strain CH34. - J. Bacteriol. **181**: 6876-6881, 1999.

Antosiewicz, D.M., Hennig, J.: Over-expression of LCT1 in tobacco enhances the protective action of calcium against cadmium toxicity. - Environ. Pollut. **129**: 237-245, 2004.

Appenroth, K.J., Bischoff, M., Gabrys, H., Stoeckel, J., Walckzak, T.: Kinetics of chromium (V) formation and reduction in fronds of duckweed *Spirodela polyrhiza* – a low frequency EPR study. - J. Inorg. Biochem. **78**: 235-242, 2000.

Archer, M.J.G., Caldwell, R.A.: Response of six Australian plants species to heavy metal contamination at an abandoned mine site. - Water Air Soil Pollut. **157**: 257-267, 2004.

Baker, A.J.M., Brooks, R.R.: Terrestrial higher plants which hyperaccumulate metallic elements – a review of their distribution, ecology and phytochemistry. - Biorecovery **1**: 81-126, 1989.

Baker, A.J.M., McGrath, S.P., Reeves, R.D., Smith, J.A.C.: Metal Hyperaccumulator plants: A review of the ecology and physiology of a biochemical resource for phytoremediaton of metal polluted soil. - In: Terry, N., Baneulos, G. (ed.): Phytoremediation of Contaminated Soil and Water. Pp. 85-107. Lewis Publications, Boca Raton 2000.

Baker, A.J.M., Reves, R.D., Hajar, A.S.M.: Heavy metal accumulation and tolerance in British population of the metallophytes *Thlaspi caerulescens* J&C Presl. (Brassicaceae). - New Phytol. **127**: 61-68, 1994.

Baker, A.J.M., Walker, P.L.: Ecophysiology of metal uptake by tolerant plants. - In: Shaw, A.J. (ed.): Heavy Metal Tolerance in Plants: Evolutionary Aspects. Pp. 155-177. CRC Press, Boca Raton 1990.

Bennett, L.E., Burkhead, J.L., Hale, K.L., Terry, N., Pilon, M., Pilon-Smits, E.A.H.: Analysis of transgenic Indian mustard plants for phytoremediation of metal-contaminated mine tailings. - J. Environ. Qual. **32**: 432-440, 2003.

Bennicelli, R., Steniewska, Z., Banach, A., Szajnocha, K., Ostrowski, J.: The ability of *Azolla caroliniana* to remove heavy metals {Hg (II), Cr (III), Cr (VI)} from municipal waste waters. - Plant Physiol. **133**: 14-15, 2003.

Berken, A., Mulholland, M.M., LeDuc, D.L., Terry, N.: Genetic engineering of plants to enhance selenium phytoremediation. - Crit. Rev. Plant Sci. **21**: 567-582, 2002.

Bernhard, W.R., Kagi, H.R.: Purification and characterization of a typical cadmium-binding peptides from *Zea mays*. - Experientia. **52** (Suppl.): 309-315, 1987.

Bizily, S.P., Rugh, C.L., Summers, A.O., Meagher, R.B.: Phytoremediation of methylmercury pollution: merB expression in *Arabidopsis thaliana* confers resistance to organomercurials. - Proc. nat. Acad. Sci. USA **96**: 6808-6813, 1999.

Black, H.: Phytoremediation: a growing field with some concerns. - Scientist **13**: 1-3, **1999**.

Blaylock, M.J., Salt, D.E., Duschenkov, S., Zakarova, O., Gussmann, C., Kapulnik, Y., Ensley, B.D., Raskin, I.: Enhanced accumulation of lead in Indian mustard by soil applied chelating agents. - Environ. Sci. Technol. **31**: 860-865, 1997.

Blaylock, M.J.: Field demonstration of phytoremediation of lead contaminated soils. - In: Terry, N., Banuelos, G. (ed.): Phytoremediation of Contaminated Soil and Water. Pp. 1-12. Lewis Publications, Boca Raton 2000.

Bondada, B.R., Ma, L.Q.: Tolerance of heavy metals in vascular plants: arsenic hyperaccumulations in Chinese brake fern (*Pteris vittata*). - In: Chandra, S., Shrivastava, M. (ed.): Pteridology in the New Millennium. Pp. 397-420. Kluwer Academic Publishers, Dordecht - Boston - London 2003.

Boonyapookana, B., Parkplan, P., Techapinyawat, S., DeLaune, R.D., Jugsujinda, A.: Phytoaccumulation of lead by sunflower (*Helianthus annuus*), tobacco (*Nicotiana tabaccum*), and vetiver (*Vetiveria zizanioides*). - J. environ. Sci. Heal. A. **40**: 117-137, 2005.

Boyajian, G., Carriera, L.H.: Phytoremediation: a clean transition from laboratory to marketplace. - Natur. Biotechnol. **15**: 127-128, 1997.

Brewer, E.P., Saunders, A.J., Angle, J.S., Chaney, R.L., Macintosh, M.S.: Somatic hybridization between the zinc accumulator *Thlaspi caerulescens* and B*rassica napus.* - Theor. appl. Genet. **99**: 761-771, 1999.

Broadhurst, C.L., Chaney, R.L., Angle, J.S., Maugel, T.K., Erbe, E.F., Murphy, C.A.: Simultaneous hyperaccumulation of nickel, manganese, and calcium in *Alyssum* leaf trichomes. - Environ. Sci. Technol. **38**: 5797-5802, 2004.

Brooks, R.R.: Copper and cobalt uptake in *Hauminiastrum* species. - Plant Soil **48**: 541-544, 1977.

Brown S.L., Chaney, R.L., Angle, J.S., Baker, A.J.M.: Phytoremedaition potential of *Thalspi caerulescens* for zinc and cadmium contaminated soil. - J. environ. Qual. **23**: 1151-1157, 1994.

Caille, N., Swanwick, S., Zhao, F.J., McGrath, S.P.: Arsenic hyperaccumulation by *Pteris vittata* from arsenic contaminated soils and the effect of liming and phosphate fertilization. - Environ. Pollut. **132**: 113-120, 2004.

Celliar, M., Prive, G., Belouchi, A., Kwan, T., Rodriguez, V., Chia, W., Gros, P.: Nramp defines a family of membrane proteins. - Proc. nat. Acad. Sci. USA **91**: 10089-10093, 1995.

Chandra Sekhar, K., Kamala, C.T., Chary, N.S., Balaram, V.,

Garcia, G.: Potential of *Hemidesmus indicus* for phytoextraction of lead from industrially contaminated soils. - Chemosphere **58**: 507-514, 2005.

Chaney, R.L.: Plant uptake of inorganic waste constitutes. - In: Parr, J.F., Marsh, P.B., Kla, J.M. (ed.): Land Treatment of Hazardous Wastes. Pp. 50-76. Park Ridge Noyes Data Corp., London 1983.

Chaney, R.L.: Zinc phytotoxicity. - In: Robson, A.D. (ed.) Zinc in Soil and Plants. Pp.135-158. Kluwer Academic Publisher, Dordrecht - Amsterdam 1993.

Chaney, R.L., Malik, M., Li, Y.M., Brown, S.L., Brewer, E.P., Angle, J.S., Baker, A.J.M.: Phytoremediation of soil metals. - Curr. Opin. Biotechnol. **8**: 279-284, 1997.

Che, D.S., Meagher, R.B., Heaton, A.C.P., Lima, A., Rugh, C.L., Merkle, S.A.: Expression of mercuric ion reductase in eastern cottonwood (*Populus deltoides*) confers mercuric ion reduction and resistance. - J. Plant Biotechnol. **1**: 311-319, 2003.

Chen, J., Huang, J.W., Casper, T., Cunningham, S.D.: *Arabidopsis* as a model system for studying lead accumulation and tolerance in plants. - In: Kruger, E.L. (ed.): Phytoremediation of Soil and Water Contaminants. Pp 264-273. American Chemical Society, Washington 1997.

Chen, J., Zhao, J., Goldsbrough, P.B.: Characterization of phytochelatin-synthase from tomato. - Physiol. Plant. **101**: 165-172, 1997.

Clarkson, D.T.: The uptake and translocation of manganese by plant roots. - In: Graham, R.D., Hannam, R.J., Uren, N.C. (ed.): Manganese in Soil and Plants. Pp.101-111. Kluwer Academic Publisher, Dordrecht 1988.

Clemens, S.: Molecular mechanisms of plant metal hoemostatsis. - Planta. **212**: 475-486, 2001.

Clemens, S., Antonsiewz, D.M., Ward, J.M., Schachtman, D.P., Schroder, J.J.: The plant cDNA LCT1 mediates the uptake of calcium and cadmium in yeast. - Proc. nat. Sci. USA **95**: 12043-12048, 1998.

Clemens, S., Kim, E., Newmann, J, Schroeder, D.: Tolerance to toxic metals by a gene family of phytochelatin synthases from plants and yeast. - EMBO J. **18**: 3325-3333, 1999.

Clemens, S., Palmgren, M.G.. Kramer, U.: A long way ahead: understanding and engineering plant metal accumulation. - Trends Plant Sci. **7**: 309-314, 2002.

Cobbet, C., Goldsbrough, P.: Phytochelatins and metallo-thioneins: role in heavy metal detoxification and homeostasis. - Annu. Rev. Plant Biol. **53**: 159-182, 2002.

Conklin, D.S., MacMasters, J.A., Culbertson, M.R., Kung, C.: *COT1* genes involved in cobalt accumulation in *Saccharomyces cerevisiae*. - Mol. cell. Biol. **12**: 3678-3688, 1992.

Cooper, E.M., Sims, J.T., Cunningham, S.D., Huang, J.W., Berti, W.R.: Chelate-assisted phytoextraction of lead from contaminated soils. - J. environ. Qual. **28**: 1709-1719, 1999.

Cunningham, S.D., Berti, W.R.: Remediation of contaminated soils with green plants: An overview. - *In Vitro* cell. dev. Biol. Plant **29**: 207-212, 1993.

Cunningham, S.D., Berti, W.R., Huang, J.W.: Phytoremediation of contaminated soils. - TIBTECH **13**: 393-397, 1995.

Curie C., Panaviene Z., Loulerguech C., Delaporta S.L., Briat J. F., Walker E.L.: Maize yellow stripe encodes a membrane protein directly involved in Fe (III) uptake. - Nature **409**: 346-349, 2001.

Dahmani-Muller, Van Oort, F., Gelie, B., Balabane, M.: Strategies of heavy metal uptake by three plant species growing near a metal smelter. - Environ. Pollut. **109**: 231-238, 2000.

De Knecht, J.A., Van Baren, N., Ten Bookum, W.M., Wong, F., Sang, H.W., Koevoet, P.L.M., Schat, H., Verkleij, J.A.C.: Synthesis and degradation of phytochelatins in cadmium-sensitive and cadmium-tolerant *Silene vulgaris*. - Plant Sci. **106**: 9-18, 1995.

Delhaize, E., Randall, P.J., Wallace, P.A., Pinkerton, A.: Screening *Arabidopsis* for mutants in mineral nutrition. - Plant Soil **156**: 134-141, 1993.

Delhaize, E., Ryan, P.R.: Aluminium toxicity and and tolerance in plants. - Plant Physiol. **107**: 315-321, 1995.

Dhankher, O.P., Li, Y., Rosen, B.P., Shi, J., Salt, D., Senecoff, J.F., Sashti, N.A., Meagher, R.B.: Engineering tolerance and hyperaccumulation of arsenic in plants by combining arsenate reductase and γ-glutamylcysteine synthetase expression. - Natur. Biotechnol. **20**: 1140-1145, 2002.

Dietz, K.J, Baier, M., Kramer, U.: Free radicals and reactive oxygen species are mediators of heavy metal toxicity in plants. - In: Prasad, M.N.V., Hagemmeyer, J. (ed.): Heavy Metal Stress in Plants: from Molecules to Ecosystem. Pp. 79-97. Springer-Verlag, Berlin 1999.

Dondon, M.G., De Vathaire, F., Quenel, P., Frery, N.: Cancer mortality during the 1968-1994 period in a mining area in France. - Eur. J. Cancer Prev. **14**: 297-301, 2005.

Dražić, G., Mihalović, N., Lojić, M.: Cadmium concentration in *Medicago sativa* seedlings treated with salicylic acid. - Biol. Plant. **50**: 239-244, 2006.

Ducic, T., Polle, A.: Transport and detoxification of manganese and copper in plants. - Braz. J. Plant Physiol. **17**: 103-112, 2005.

Eide, D., Broderius, M., Fett, J., Guerinot, M.L.: A novel iron-regulated metal transporter from plants identified by functional expression in yeast. - Proc. nat. Acad. Sci. USA **93**: 5624-5628, 1996.

Evans, K.M., Gatehouse, J.A., Lindsay, W.P., Shi, J., Tommey, A.M., Robinson, N.J.: Expression of the pea metallothionein-like gene *PsMTA* in *Escherichia coli* and *Arabidopsis thaliana* and analysis of trace metal ion accumulation: implications for *PsMTA* function. - Plant mol. Biol. **20**: 1019-1028, 1992.

Fox, T.C., Guerinot, M.L.: Molecular biology of cation transport in plants. - Annu. Rev. Plant Physiol. Plant. mol. Biol. **49**: 669-696, 1998.

Fu, D., Beeler, T.J., Dunn, T.M.: Sequence, mapping and destruction of *CCC2*: a gene that cross complements the Ca^{2+}- sensitive phenotype of *csg*1 mutant and encodes a P-type ATPase belonging to the Cu^{2+}-ATPase subfamily. - Yeast **11**: 283-292, 1995.

Gardea-Torresday, J.L., De la Rosa, G., Peralta-Videa, J.R., Montes, M., Cruz-Jiminez, G., Cano-Aguilera: Differential uptake and transport of trivalent and hexavalent chromium by tumble wed (*Salsola kali*). - Arch. Environ. Contam. Toxicol. **48**: 225-232, 2005.

Gekeler, W., Grill, E., Winnacker, E-L., Zenk, M.H.: Survey of the plant kingdom for the ability to bind metals through phytochelatins. - Z. Naturforsch. **44**: 361-369, 1989.

Gleba, D., Borisjuk, N.V., Borisjuk, L.G., Kneer, R., Poulev, A., Skarzhinskaya, M., Dushenkov, S., Logendra, S., Gleba, Y.Y., Raskin, I.: Use of plant roots for phytoremediation and molecular farming. - Proc. nat. Acad. Sci. USA **96**: 5973-5977, 1999.

Glerum, D.M., Shtanko, A., Tzagoloff, A.: Characterization of *COX17*, a yeast gene involved in copper metabolism and assembly of cytochrome oxidase. - J. biol. Chem. **271**: 14504-14509, 1996.

Goldbold, D.L., Horst, W.J., Collins, J.C., Thumann, D.A.,

Marschner, H.: Accumulation of zinc and organic acids in the roots of zinc-tolerant and non-tolerant ecotypes of *Deschampia caespitosa*. - J. Plant. Physiol. **116**: 59-69, 1984.

Gratão, P.L., Prasad, M.N.V., Cardoso, P.F., Lea, P.J., Azevedo, R.A.: Phytoremediation: green technology for the clean up of toxic metals in the environment. - Braz. J. Plant Physiol. **17**: 53-64, 2005.

Grichko, V.P., Filby, B., Glick, B.R.: Increased ability of transgenic plants expressing the enzyme ACC deaminase to accumulate Cd, Co, Cu, Ni, Pb and Zn. - J. Biotechnol. **81**: 45-53, 2000.

Grill, E., Loffler, S., Winnacker, E.-L., Zenk, M.H.: Phytochelatins, the heavy metal binding proteins are synthesized from glutathione by a specific γ-glutamyl-cysteine dipeptidyl transpeptidase (phytochelatin synthase). - Proc. nat. Acad. Sci. USA **86**: 6838-6842, 1989.

Grill, E., Winnacker, E.-L., Zenk, M.H.: Homo-phytochelatins as heavy metal-binding peptides of homoglutathione-containing *Fabales*. - FEBS Lett. **205**: 47-50, 1986a.

Grill, E., Winnacker, E.-L., Zenk, M.H.: Synthesis of seven homologous phytochelatins in metal-exposed *Schizosaccharomyces cerevisiae* cells. - FEBS Lett. **197**: 115-120, 1986b.

Grill, E., Winnacker, E.-L., Zenk, M.H.: Phytochelatins the metal binding peptides from plants are functionally analogous to metallothioneins. - Proc. nat. Acad. Sci. USA **84**: 439-443, 1987.

Grotz, N., Fox, T., Cannoly, E., Park, W., Guerinot, M.L., Eide, D.: Identification of a family of zinc transporter from *Arabidopsis* that respond to zinc deficiency. - Proc. nat. Acad. Sci. USA **86**: 6838-6842, 1988.

Guerinot, M.L., Eide, D.: Zeroing in on zinc uptake in yeast and plants. - Curr. Opin. Plant Biol. **2**: 244-249, 1999.

Hamer, D.H.: Metallothioneins. - Annu. Rev. Biochem. **55**: 913-951, 1986.

Hartley-Whitaker, J., Woods, C., Meharg, A.A.: Is differential phytochelatin production related to decreased arsenate influx in arsenate tolerant *Holcus lanatus*? - New Phytol. **155**: 219-225. 2002

Hasegawa, I., Terada, E., Sunairi, M., Wakita, H., Schimachi, F., Nakajima, M., Yazaki, J.: Genetic improvement in heavy metal tolerance in plants by transfer of yeast metalllothionein gene (*CUP1*). - Plant Soil **196**: 277-281, 1997.

Hattori, J., Labbé, H., Miki, B.L.: Construction and expression of a metallothionein-γ-glucuronidase gene fusion. - Genome **37**: 508-512, 1994.

Hayashi, Y., Nakagawa, C.W., Mutoh, N., Isobe, M., Goto, T.: Two pathways in the biosynthesis of cadystins $(\gamma\ EC)_nG$ in the cell free system of fission yeast. - Biochem. Cell Biol. **69**: 115- 121, 1991.

Hirschi. K.D., Zhen, R.G., Cunningham, K.W., Rea, P.A., Fink, G.R.: CAX1 and H^+/Ca^{2+} antiporter from *Arabidopsis*. - Proc. nat. Acad. Sci. USA **93**: 8782-8786, 1996.

Hirschi, E.D., Korenkey, V.D., Wilganewski, N.I., Wagner, G.I.: Expression of *Arabidopsis* CAX2 in tobacco. altered metal accumulation and increased manganese tolerance. - Plant Physiol. **124**: 128-133, 2000.

Howden, R., Goldsborough, P.B., Anderson, C.R., Cobbett, C.S.: Cadmium-sensitive, *cad1* mutants of *Arabidopsis thaliana* are phytochelatin deficient. - Plant Physiol. **107**: 1059-1066, 1995.

Huang, J.W., Blaylock, M.J., Kapulnik, Y., Ensley, B.D.: Phytoremediation of Uranium-contaminated soils: Role of organic acids in triggering Uranium hyperaccumulation in plants. - Environ. Sci. Technol. **32**: 2004-2008, 1998.

Huang, J.W., Chen, J.W., Berti, W.R., Cunningham, S.D.: Phytoremediation of lead contaminated soil: role of synthetic chelates in lead phytoextraction. - Environ. Sci. Technol. **31**: 800-805, 1997.

Huang, J.W., Cunningham, S.D.: Lead phytoextraction: species variation in lead uptake and translocation. - New Phytol. **134**: 75-84, 1996.

Jaffre, T., Brooks, R.R., Lee, J., Reeves, R.D.: *Sebertia acuminata*: a hyperaccumulator of nickel from New Caledonia. - Science **193**: 579-580, 1976.

Kamizono, A., Nishizawa, M., Teranishi, Y., Murata, K., Kimura, A.: Identification of a gene conferring resistance to zinc and cadmium in *Saccharomyces cerevisiae*. - Mol. gen. Genet. **219**: 161-167, 1989.

Kamnev, A.A., Van der Lelie, D.: Chemical and biological parameters as tools to evaluate and improve heavy metal phytoremediation. - Biosci. Rep. **20**: 239-258, 2000.

Kawashima, C.G., Noji, M., Nakamura, M., Ogra, Y., Suzuki, K.T., Saito, K.: Heavy metal tolerance of transgenic tobacco plants over-expressing cysteine synthase. - Biotech. Lett. **26**: 153-157, 2004.

Klapheck, S., Crost, B., Stark, J., Zimmermann, H.: γ-Glutamylcysteinylserine: a new homologue of glutathione in plants of the family *Poaceae*. - Bot. Acta **105**: 174-179, 1992.

Klapheck, S., Fleigner, W., Zimmer, I.: Hydroxymethyl phytochelatins [(γ-glutamylcystine)n serine] are metal induced peptides of *Poaceae*. - Plant Physiol. 104: 1325-1332, 1994.

Klapheck, S., Schlunz, S., Bergmann, L.: Phytochelatins and homo-phytochelatins in *Pisum sativum* L. - Plant Physiol. **107**: 515-521, 1995.

Kneer, R., Zenk, M.H.: Phytochelatins protect plant enzymes from heavy metal poisoning. - Phytochemistry **31**: 2663-2667, 1992.

Korshunova, Y.O., Eide, D., Clark, W.G., Guerinot, M.L., Pakrasi, H.B.: The IRT1 protein from *Arabidopsis thaliana*, is a metal transporter with a broad substrate range. - Plant mol. Biol. **40**: 37-44, 1999.

Kramer, U.: Phytoremediation: novel approaches to cleaning up polluted soils. - Curr. Opin. Biotechnol. **16**: 133-141, 2005.

Kramer, U., Cotter-Howells, J.D., Charnock, J.N., Baker, A.J.M., Smith, A.C.: Free histidine as a metal chelator in plants that accumulate nickel. - Nature **379**: 635-638, 1996.

Lasat, M.M.: Phytoextraction of metals from contaminated soil: a review of plant/soil/metal interaction and assessment of pertinent agronomic issues. - J. Hazard. Substr. Res. **2**: 1-25, 2000.

Lasat, M.M.: Phytoextraction of toxic metals: a review of biological mechanisms. - J. Environ. Qual. **31**: 109-120, 2002.

Lasat, M.M., Baker, A.J.M., Kochain, L.V.: Altered zinc compartmentation in the root symplasms and stimulated ainc absorption in the leaves and the mechanism involved in *Thalspi caerulescens*. - Plant Physiol. **118**: 875-883, 1998.

LeDuc, D.L., Tarun, A.S., Montes-Bayon, M., Meija, J., Malit, M.F., Wu, C.P., Abdel Samie, M., Chiang, C.Y., Tagmount, A., DeSouza, M., Neuhierl, B., Bock, A., Caruso, J., Terry, N.: Over-expression of selenocysteine methyltransferase in *Arabidopsis* and Indian mustard increases selenium tolerance and accumulation. - Plant Physiol. **135**: 377-383, 2004.

Lee, J., Bae, H., Jeong, J., Lee, J.Y., Yang, Y.Y., Hwang, I.,

Martinoia, E., Lee, Y.: Functional expression of a bacterial heavy metal transporter in *Arabidopsis* enhances resistance to and decrease uptake of heavy metals. - Plant Physiol. **133**: 589-596, 2003a.

Lee, S., Moon, J.S., Ko, T.S., Petros, D., Goldsbrough, P.B., Korban, S.S.: Over-expression of *Arabidopsis* phytochelatin synthase paradoxically leads to hypersensitivity to cadmium stress. - Plant Physiol. **131**: 656-663, 2003b.

Li, L., Kaplan, J.: Defect in yeast iron transport system results in increased metal hypersensitivity because of the increased expression of transporter with broad transition metal specificity. - J. biol. Chem. **271**: 22181-22187, 1998.

Lombi, E., Zhao, F.J., Dunham, S.J., McGrath, S.P.: Phytoremediation of heavy metal-contaminated soils: natural hyperaccumulation versus chemically enhanced phytoextraction. - J. Environ. Qual. **30**: 1919-1926, 2001.

Lopez-Millan, A.F., Ellis, D.R., Grusak, M.A.: Identification and characterization of several new members of the ZIP family of metal ion transporters in *Medicago truncatula.* - Plant mol. Biol. **54**: 583-596, 2004.

Ma, L.Q., Komar, K.M., Tu, C., Zhang, W., Cai, Y., Kenelly, E.D.: A fern that hyperaccumulates arsenic. - Nature **409**: 579-582, 2001.

Maiti, I.B., Hunt, A.G., Wagner, G.J., Yeargan, R., Hunt, A.G.: Light inducible and tissue specific expression of a chimeric mouse metallothionein cDNA gene in tobacco. - Plant Sci. **76**: 99-107, 1991.

Maitani, T., Kubota, H., Sato, K., Yamada, T.: The composition of metal bound to class III metallothioneins (phytochelatins and its desglycyl peptide) induced by various metals in root culture of *Rubia tinctorum*. - Plant Physiol. **110**: 1145-1150, 1996.

Maitani, T., Kubota, H., Sato, K., Yamada, T.: Phytochelatins (class III metallothioneins) and their desglycyl peptides induced by cadmium in root culture of *Rubia tinctorum* L. - In: Klassen, C. (ed.): Metallothionein. Vol. IV. Pp 201-205. Birkhauser Verlag, Basel 1999.

Mathys, W.: The role of malate, oxalate and mustard oil glucosides in the evolution of zinc resistance of herbage plants. - Physiol. Plant. **40**: 130-136, 1997.

McGrath, S.P.: Phytoextraction for soil remediation. - In: Brooks, R. (ed.): Plants that Hyperaccumulate Heavy Metals Their Role in Phytoremediation, Microbiology, Archaeology, Mineral Exploration and Phytomining. Pp. 261-287. CAB International, New York 1998.

McGrath, S.P., Sidoli, C.M.D., Baker, A.J.M., Reeves, R.D.: The potential for the use of metal-accumulating plants for the *in situ* decontamination of metal-polluted soils. - In: Eijsackrs, H.J.P., Hamer, T. (ed.): Integrated Soil and Sediment Research: a Basis for Proper Protection. Pp. 673-676. Kluwer Academic Publishers, Dordrecht 1993.

McNair, M.R.: The genetics of metal tolerance in vascular plants. - New Phytol. **124**: 541-559, 1993.

McNair, M.R., Tilstone, G.H., Smith, S.S.: The genetics of metal tolerance and accumulation in higher plants. - In: Terry, N., Bañuelos, G. (ed.): Phytoremediation of Contaminated Soil and Water. Pp. 235-250. Lewis Publishers, Boca Raton 2000.

Mehra, R.K., Winge, D.R.: Cu (I) binding to *Schizosaccaromyces pombe* γ-glutamyl transferase peptides varying in chain lengths. - Arch. Biochem. Biophys. **265**: 381-389, 1988.

Mehra, R.K., Winge, D.R.: Metal ion resistance in fungi. Molecular mechanism and their regulated expression. - J. Cell. Biochem. **45**: 30-40, 1991.

Meuwly, P., Thibault, P., Schwan, A.L., Rauser, W.E.: Three families of thiol peptides are induced by cadmium in maize. - Plant J. **7**: 391-400, 1995.

Milivojević, D.B., Nikolić, B.R., Drinić, G.: Effects of arsenic on phosphorous content in different organs and chlorophyll fluorescence in primary leaves of soybean. - Biol. Plant. **50**: 149-151, 2006

Misra, S., Gedamu, L.: Heavy metal tolerant transgenic *Brassica napus* L. and *Nicotiana tabaccum* L. plants. - Theor. appl. Genet. **78**: 161-168, 1989.

Mkandawire, M., Dudel, E.G.: Accumulation of arsenic in *Lemna gibba* L. (duckweed) in tailing waters of two abandoned uranium mining sites in Saxony, Germany. - Sci. total Environ. **336**: 81-89, 2005.

Moffat, A.S.: Plants proving their worth in toxic metal cleanup. - Science **269**: 302-303, 1995.

Morrison, R.S., Brooks, R.R., Reeves, R.D.: Nickel uptake by *Alyssum* species. - Plant Sci. Lett. **17**: 453-457, 1980.

Nakazawa, R., Kameda, Y., Ito, T., Ogita, Y., Michihata, R., Takenaga, H.: Selection and characterization of nickel-tolerant tobacco cells. - Biol. Plant. **48**: 497-502, 2004.

Newman, L.A., Reynolds, C.M.: Phytodegradation of organic compounds. - Curr. Opin. Biotechnol. **15**: 225-230, 2004.

Odjegba, V.J., Fasidi, I.O.: Accumulation of trace elements by *Pistia stratiotes*: implications for phytoremediation. - Ecotoxicology **13**: 637-646, 2004.

O'Halloran, T.V., Cullota, V.C.: Metallo-chaperones – an intra cellular shuttle service for metal ions. - J. biol. Chem. **275**: 25057-25060, 2000.

Orser, C.S., Salt, D.E., Pickering, I.I., Epstein, A., Ensley, B.D.: *Brassica* plants to provide enhanced mineral nutrition: Selenium phytoenrichment and metabolic transformation. - J. Med. Food **1**: 253-261, 1999.

Ortiz, D.F., Russcitti, T., McCuc, K.F., Ow, D.W.: Transport of metal binding peptides by HMT1, a fission yeast ABC type vacuolar membrane protein. - J. biol. Chem. **27**: 4721-4728, 1995.

Oven, M., Grill, E., Golan-Goldhirsh, A., Kutcxhan, T.M., Zenk, M.H.: Increase in free cysteine and citric acid in plant cells exposed to cobalt ions. - Phytochemistry **60**: 467-474, 2002.

Pan, A.H., Yang, M., Tie, F., Li. L., Che, Z., Ru, B.: Expression of mouse metallothionein-I gene confers cadmium resistance in transgenic tobacco plants. - Plant mol. Biol. **24**: 341-351, 1994.

Papoyan, A., Kochian, L.V.: Identification of *Thlaspi caerulescens* genes that may be involved in heavy metal hyperaccumulation and tolerance. Characterization of a novel heavy metal transporting ATPase. - Plant Physiol. **136**: 3814-3823, 2004.

Parker, D.R., Feist, L.J., Varvel, T.W., Thomason, D.N., Zhang, Y.Q.: Selenium phytoremediation potential of *Stanleya pinnata*. - Plant Soil **249**: 157-165, 2003.

Paulsen, I.T., Saier, M.H., Jr.: A novel family of ubiquitous heavy metal ion transport proteins. - J. Membr. Biol. **156**: 99-103, 1997.

Pence, N.S., Larsen, P.B., Ebbs, S.D., Letham, D.L., Lasat, M.M., Garvin, D.F., Eide, D., Kochian, L.V.: The molecular physiology of heavy metal transport in zinc/cadmium hyperaccumulator *Thlaspi caerulescens*. - Proc. nat. Acad. Sci. USA **97**: 4956-4960, 2000.

Persans, M.W., Yan, X., Patnoe, J.M., Kramer, U.: Molecular dissection of the role of histidine in hyperaccumulation in *Thlaspi geosingense*. - Plant Physiol. **121**: 1117-1126, 1999.

Pickering, I.J., Prince, R.C., George, M.J., Smith, R.D., George,

D.N., Salt, D.E.: Reduction and co-ordination of arsenic in Indian mustard. - Plant Physiol. **122**: 1171-1177, 2000.

Pilon-Smits, E., Hwang, S., Lytle, M., Zhu, Y., Tai, J.C., Bravo, R.C., Chen, Y., Leustek, T., Terry, N.: Over-expression of ATP sulfurylase in *Brassica juncea* leads to increased selenate uptake, reduction and tolerance. - Plant Physiol. **119**: 123-132, 1999.

Pilon-Smits, E., Pilon M.: Phytoremediation of metals using transgenic plants. - Crit. Rev. Plant Sci. **6**: 91-95, 2001.

Pilon-Smits, E.: Phytoremediation. - Annu. Rev. Plant Biol. **56**: 15-39, 2005.

Pollard, A.J.: Metal hyperaccumulation. - New Phytol. **146**: 179-181, 2000.

Pollard, A.J., Baker, A.J.M.: Deterrence of herbivory by zinc hyperaccumulation in *Thlaspi caerulescens*. - New Phytol. **135**: 655-658, 1997.

Prasad, M.N.V.: Nickelophilous plants and their significance in phytotechnologies. - Braz. J. Plant. Physiol. **17**: 113-128, 2005.

Prasad, M.N.V., Freitas, H.: Metal hyperaccumulation in plants – biodiversity prospecting for phytoremediation technology. - Electronic J. Biotechnol. **6**: 275-321, 2003.

Pufahl, R.A., Singer, C.P., Peariso, K.L., Lin, S.J., Schmidt, P.J., Fahrni, C.J., Penner-Hahn, J.E., O'Halloran, T.V.: Metal ion chaperone function of the soluble copper (I) receptor ATX1. - Science **278**: 853-856, 1997.

Rabie, G.H.: Contribution of arbuscular mycorrhizal fungus to red kidney and wheat plants tolerance grown in heavy in metal polluted soil. - Afr. J. Biotechnol. **4**: 332-345, 2005

Raskin, I.: Plant genetic engineering may help with environmental cleanup. - Proc. nat. Acad. Sci. USA **93**: 3164-3166, 1996.

Rauser, W.E.: Phytochelatins and related peptides. - Plant Physiol. **109**: 1141-1149, 1995.

Rea, P.A., Li, Z-S., Lu, Y-P., Drosdowicz ,Y.M., Martinoia, E.: From vacuolar GS-X pumps to multi-specific transporters. - Annu. Rev. Plant Physiol. Plant mol. Biol. **49**: 727-760, 1998.

Reeves, R.D., Baker, A.J.M.: Metal-accumulating plants. - In: Raskin, I., Ensley, B.D. (ed.): Phytoremediation of Toxic Metals. Pp. 193-229. John Wiley, New York 2000.

Reeves, R.D., Brooks, R.R.: Hyperaccumulation of lead and zinc by two metallophytes from mining areas of Central Europe. - Environ. Pollut. Ser. A **31**: 277-285, 1983.

Rugh, C.L., Senecoff, J.F., Meagher, R.B., Merkle, S.A.: Development of transgenic yellow poplar for mercury phytoremediation. - Natur. Biotechnol. **16**: 925-**??**, 1998.

Rugh, C.L., Wilde, H.D., Stack, N.M., Thompson, M.D., Summers, A.O., Meagher, R.B.: Mercuric ion reduction and the resistance in transgenic *Arabidopsis thaliana* plants expressing a modified bacterial *mer A* gene. - Proc. nat. Acad. Sci. USA **93**: 3182-3187, 1996.

Sagner, S., Kneer, R., Warner, T., Cosson, J.P., Deus-Neumann, B., Zenk, M.H.: Hyperaccumulation, complexation, distribution of nickel in *Sibertia acuminata*. - Phytochemistry **47**: 339-347, 1998.

Saier, M.H., Jr.: A functional phylogenetic classification system for transmembrane solute transporters. - Microbiol. mol. Biol. Rev. **64**: 354-411, 2000.

Salt, D.E., Rauser, W.E.: Mg-ATP dependent transport of phytochelatins across the tonoplast of oat roots. - Plant Physiol. **107**: 1293-1301, 1995.

Saxena, P.K., Krishna Raj, S., Dan, T., Perras, M.R., Vettakkorumakankav, N.N.: Phytoremediation of metal contaminated and polluted soils - In: Prasad, M.N.V., Hagemeyer, J. (ed.):Heavy Metal Stress In Plants – From Molecules To Ecosystems. Pp. 305-329. Springer-Verlag, Heidelberg - Berlin - New York 1999.

Schat, H., Llugany, M., Voojis, R., Harley-Whitaker, J., Bleeker, P.M.: The role of phytochelatins in constitutive and adaptive heavy metal tolerances in hyperaccumulator and non-hyperaccumulator metallophytes. - J. exp. Bot. **53**: 2381-2392, 2002.

Schat, H., Vooijs, R.: Multiple tolerance and co tolerance to heavy metals in *Silene vulgaris*: a co-segregation analysis. - New Phytol. **136**: 489-496, **1997**.

Schmoger, M.C., Oven, M., Grill, E.: Detoxification of arsenic by phytochelatins in plants. - Plant Physiol. **128**: 793-801, 2000.

Shah, K., Dubey, R.S.: Effects of cadmium on RNA levels as well as activity and molecular form of ribonuclease in growing rice seedlings: role of proline as a possible enzyme protectant. - Plant Physiol. Biochem. **33**: 577-584, 1995.

Shah, K., Dubey, R.S.: A 18 kDa cadmium inducible protein complex: its isolation and characterization from rice (*Oryza sativa* L.) seedlings. - J. Plant Physiol. **152**: 448-454, 1998.

Shah, K., Kumar, R.G., Verma, S., Dubey, R.S.: Effects of cadmium on lipid peroxidation, superoxide anion generation and activities of antioxidant enzymes in growing rice seedlings. - Plant Sci. **161**: 1135-1144, 2001.

Sharma, N.C., Gardea-Torresday, J.L., Parson Sahi, S.V.: Chemical speciation of lead in *Sesbania drumondii*. - Environ. Toxicol. Chem. **23**: 2068-2073, 2004.

Singh, O.V., Jain, R.K.: Phytoremediation of toxic aromatic pollutants from soil. - Appl. Microbiol. Biotechnol. **63**: 128-135, 2003.

Sjaan, D.B., Woodrow, I.E., George, N. B., Sommer-Knudsen, J.: Hyperaccumulation of manganese in the rainforest tree *Austromyrtus bidwillii* (Myrtaceae) from Queensland, Australia. - Func. Plant Biol. **29**: 899-905, 2002.

Smith, S.E., McNair, M.R.: Hypostatic modifiers causes variation in degree of copper tolerance in *Mimulus guttatus*. - Heredity **80**: 760-768, 1998.

Song, W.-Y., Martinoia, E., Lee, J., Kim, D., Kim, D-Y, Vogt, E., Shim, D., Choi. K.S., Hwang, I., Lee, Y.: A novel family of cys-rich membrane proteins mediates cadmium resistance in *Arabidopsis*. - Plant Physiol. **135**: 1027-1039, 2004.

Song, W.-Y., Sohn, E.J., Martinoia, E., Lee, Y.J., Yang, Y.Y., Jasinski, M., Forestier, C., Hwang, I., Lee, Y.: Engineering tolerance and accumulation of lead and cadmium in transgenic plants. - Natur. Biotechnol. **21**: 914-919, 2003.

Soudek, P., Podračka, E., Vágner, M., Vaněk, T., Petřík, P., Tykva, R.: ^{226}Ra uptake from soils into different plant species. - J. Radioanalytical Nucl. Chem.. **262**: 187-189, 2004.

Suresh, B., Ravishankar, G.A.: Phytoremediation - a novel and promising approach for environmental clean up. - Crit. Rev. Biotechnol. **24**: 97-124, 2004.

Sykes, M., Yang, V., Blankenburg, J., Abu Bakr, S.: Biotechnology: working with nature to improve forest resources and products. - Int. environ. Conf. **29**: 631-637, 1999.

Tagmount, A., Berken, A., Terry, N.: An essential role of S-adenosyl-L-methionine:L-methionine S-methyltransferase in selenium volatilization by plants. Methylation of selenomethionine to selenium-methyl-L-selenium methionine, the precursor of volatile selenium. - Plant Physiol. **130**: 847-856, 2002.

Terry, N., Zayed, A.M., De Souza, M.P., Tarun, A.S.: Selenium in higher plants. - Annu Rev. Plant Physiol. Plant mol. Biol.

51: 401-432, 2000.

Thomas, J.C., Davies, E.C., Malick, F.K., Endreszl, C., Williams, C.R., Abbas, M., Petrella, S., Swisher, K., Perron, M., Edwards, R., Ostenkowski, P., Urbanczyk, N., Wiesend, W.N., Murray, K.S.: Yeast metallothionein in transgenic tobacco promotes copper uptake from contaminated soils. - Biotechnol. Progr. **19**: 273-280, 2003.

Thumann, J., Grill, E., Winnacker, E.L., Zenk, M.H.: Reactivation of metal-requiring enzymes by phytochelatin-metal complexes. - FEBS Lett. **284**: 66-69, 1991.

Tian, J.L., Zhu, H.T., Yang, Y.A., He, Y.K.: Organic mercury tolerance, absorption and transformation in *Spartina* plants. - J. Plant Physiol. mol. Biol. (China) **30**: 577-582, 2004.

Tommasini, R., Vogt, E., Fromenteau, M., Hortensteiner, S., Matile, P., Amrhein, N., Martinoia, E.: An ABC transporter of *Arabidopsis thaliana* has both glutathione conjugate and chlorophyll catabolite transport activity. - Plant J. **13**: 773-780, 1998.

Tong, Y.P., Kneer, R., Zhu, Y.G.: Vacuolar compartmentalization: a second-generation approach to engineering plants for phytoremediation. - Trends Plant Sci. **9**: 7-9, 2004.

Van Assche, F., Clijster, H.: Effects of metal in enzyme activity in plants. - Plant Cell Environ. **13**: 773-780, 1990.

Van der Zaal, B.J., Neutboom, L.W., Pinars, J.E., Chardonnens, A.N., Schat, H., Verkleij, J.A., Hooykaas, P.J.: Over-expression of a novel *Arabidopsis* gene related to putative zinc transport genes from animals can lead to enhanced zinc resistance and accumulation. - Plant Physiol. **119**: 1047-1056, 1999.

Van Huysen, T., Abdel-Ghany, S., Hale, K.L., LeDuc, D., Terry, N., Pilon-Smits, E.A.H.: Over-expression of cystathionine-gamma-synthase enhances selenium volatilization in *Brassica juncea.* - Planta **218**: 71-78, 2003.

Van Huysen, T., Terry, N., Pilon-Smits, E.A.H.: Exploring the selenium phytoremediation potential of transgenic Indian mustard over-expressing ATP sulfurylase or cystathionine-γ-synthase. - Int. J. Phytoremed. **6**: 111-118, 2004.

Vatamaniuk, O.K., Mari, S., Lu, Y.P., Rea, P.A.: AtPCS1, a phytochelatin synthase from *Arabidopsis*: isolation and *in vitro* construction. - Proc. nat. Acad. Sci. USA **96**: 7110-7115, 1999.

Verret, F., Gravot, A., Auroy, P., Leonhardt, N., David, P., Nussaume, L., Vavasseur, A., Richaud, P.: Over-expression of *AtHMA4* enhances root-to-shoot translocation of zinc and cadmium and plant metal tolerance. - FEBS Lett. **576**: 306-312, 2004.

Vinterhalter, B., Vinterhalter, D.: Nickel hyperaccumulation in shoot cultures of *Alyssum narkgrafii*. - Biol. Plant. **49**: 121-124, 2005.

Wagner, G.J.: Accumulation of cadmium in crop plants and its consequences to human health. - Adv. Agron. **51**: 173-212, 1993.

Wang, Q.R., Cui, Y.S., Liu, X. M., Dong, Y. T., Christie, P.: Soil contamination and uptake of heavy metals at polluted sites in China. - J. environ. Sci. Health. **38**: 823-838, 2003.

Wojcik, M., Tukiendorf, A.: Cadmium uptake, localization and detoxification in *Zea mays*. - Biol. Plant. **49**: 237-245, 2005.

Xue, S.G., Chen, Y.X., Reeves, R.D., Lin, Q., Fernando, D.R.: Manganese uptake and accumulation by the hyperaccumulator plant *Phytolacca acinosa* Roxb. (Phytolaccaeae). - Environ. Pollut. **131**: 393-399, 2004.

Zhang, Y.W., Tam, N.F.Y., Wong, Y.S.: Cloning and characterization of type 2 metallothionein-like gene from a wetland plant, *Typha latifolia*. - Plant Sci. **167**: 869-877, 2004.

Zhao, F.J., Dunham, S.J., McGrath, S.P.: Arsenic hyper-accumulation by different fern species. - New Phytol. **156**: 27-31, 2002.