Journal of Environmental Biology
©Triveni Enterprises, Lucknow (India)

April 2007, 28(2) 367-376 (2007)

Review Paper

Phytoremediation of toxic metals from soil and waste water

Vinita Hooda*

Department of Botany, Government College for Women, Rohtak-124 001, India

(Received: October 28, 2005 ; Revised received: July 20, 2006 ; Accepted: August 29, 2006)

Abstract: *Phytoremediation is an emerging technology, which uses plants and their associated rhizospheric microorganisms to remove pollutants from contaminated sites. This plant based technology has gained acceptance in the past ten years as a cheap, efficient and environment friendly technology especially for removing toxic metals. Plant based technologies for metal decontamination are extraction, volatilization, stabilization and rhizofiltration. Various soil and plant factors such as soil's physical and chemical properties, plant and microbial exudates, metal bioavailability, plant's ability to uptake, accumulate, translocate, sequester and detoxify metal amounts for phytoremediation efficiency. Use of transgenics to enhance phytoremediation potential seems promising. Despite several advantages, phytoremediation has not yet become a commercially available technology. Progress in the field is hindered by lack of understanding of complex interactions in the rhizosphere and plant based mechanisms which allow metal translocation and accumulation in plants. The review concludes with suggestions for future phytoremediation research.*

Key words: *Phytoremediation, Metals, Plants*

Introduction

Natural processes such as volcanic eruptions, continental dusts and anthropogenic activities like mining, combustion of fossil fuel, phosphate fertilizers, military activities, metal working industries *etc.* lead to emission of heavy metals and accumulation of these chemicals in ecosystem. These heavy metals are toxic because they cause DNA damage and their carcinogenic effects in animals and humans are probably caused by their mutagenic ability (Knasmuller *et al.*, 1998; Baudouin *et al.*, 2002). Potential threat is that heavy metals are not degradable and without intervention stay in soil for centuries. The cleanup of most of the contaminated sites is mandatory in order to reclaim the area and to minimize the entry of toxic elements into the food chain. Various engineering – based methods such as soil excavation, soil washing or burning or pump and treat systems are already being used to remediate metal contaminated soils. However, these non biological techniques are not fully acceptable as they destroy the biotic components of soil and are technically difficult and expensive to implement. Since last decade phytoremediation has emerged out as a new low-tech cost effective technology that uses plants and their associated microbial flora for environmental clean up (Raskin *et al.*, 1994; Salt *et al.*, 1995a; Salt *et al.*, 1998).

Phytoremediation takes advantage of the unique, selective and naturally occurring uptake capabilities of plant root systems, together with the translocation, bioaccumulation and pollutant storage/degradation abilities of the entire plant body. Besides being aesthetically pleasing, phytoremediation is on average tenfold cheaper than other physical, chemical or thermal remediation methods since it is performed *in situ*, is solar driven and can function with minimal maintenance once established. Phytoremediation market is growing rapidly in U.S. comprising ~$ 100-150 million per year. Although there is no significant commercial use of phytoremediation in developing countries, it may become a technology of choice for remediation projects due to its cost efficiency and ease of implementation. Phytoremediation of soil metals has been successfully carried out at military sites, agricultural fields, industrial sites and mine trailings (Bañuelos, 2000; Winter Sydnor and Redente, 2002). Inorganic pollutants that can be phytoremediated include plant macronutrients such as nitrate and phosphate (Horne, 2000), plant trace elements such as Cr, Cu, Fe, Mn, Mo and Zn (Lytle, *et al.,* 1998), nonessential elements such as Cd, Co, Fe, Hg, Se, Pb, V and W (Horne, 2000; Blaylock and Huang, 2000) and radioactive isotopes such as ^{238}U, ^{137}Cs and ^{90}Sr (Dushenkov, 2003; Dushenkov and Kapulnik, 2000).

Limitations of the technology include the potential for introducing the contaminant into food chain, long clean up times required, bioavailability of contaminant and toxicity encountered in establishing and maintaining vegetation at waste sites. The use of phytoremediation is also limited by the climatic and geologic conditions of the site to be cleaned, the temperature, soil type and the accessibility for agriculture equipment (Salt and Kramer, 2000; Schmoger *et al.*, 2000). Moreover, mechanisms of most of the biological processes underlying phytoremediation such as plant metal uptake, translocation, accumulation and /or degradation and plant microbe interactions, are poorly understood and need further research.

The present review presents an overview of phytoremediation of metals from polluted soils and water. Various phytoremediation technologies (Phytoextraction, Phyto-volatilization, Phytostabilization and Rhizofiltration and soil and plant factors affecting phytoremediation have been reviewed in

---

Corresponding author: E-Mail: vinitahooda@yahoo.co.in, Tel.: 01262-256699, 9416515444

the context of phytoremediation. Before concluding phytoremediation potential of transgenics and future requirements have been discussed.

Phytoremediation technologies

Phytoremediation exploits plant's innate biological mechanisms for human benefit. Four subsets of this technology as applicable to toxic metal remediation from soil and water are: (i) Phytoextraction – the use of metal – accumulating plants to remove toxic metals from soil, (ii) Phytovolatilization – evaporation of certain metals from aerial parts of the plant, (iii) Phytostabilitzation the use of plants to eliminate the bioavailability of toxic metals in soils and (iv) Rhizofiltration – the use of plant roots to remove toxic metals from polluted waters.

Phytoextraction:

Metal phytoextraction relies on metal-accumulating plants to transport and concentrate polluting metals from soil into the harvestable aboveground shoots (Salt *et al.*, 1998; Vassil *et al.*, 1998). The plant material can subsequently be used for nonfood purposes (*e.g.* wood, cardboard) or ashed, followed by disposal in a landfill or, in the case of valuable metals, the accumulated element can be recycled. The latter is termed phytomining (Chaney *et al.*, 2000). Popular species for phytoextraction are Indian mustard and sunflower because of their fast growth, high biomass, and high tolerance and accumulation of metals and other inorganics (Blaylock and Huang, 2000; Salt *et al.*, 1995b).

Although, it has been known since the late 1800s that a special category of plants, the so called hyperaccumulators can accumulate extraordinary levels of metals, the idea of using these plants for phytoextraction only appeared in the literature in the last 20 years. At present, at least 45 plant families are known to contain metal-accumulating species (Reeves and Baker, 2000). Such plants can accumulate As, Cu, Co, Cd, Mn, Ni, Se, Pb or Zn upto levels that are 100 to 1,000 times of those normally accumulated by plants grown under the same conditions (Baker *et al.*, 2000; Ma *et al.*, 2001; Brooks, 1998). A number of these species are members of Brassicaceae, including a species of *Arabidopsis, A. halleri*, which can hyperaccumulate Zn in its shoots (Reeves and Backer, 2000). Recently, *Sonchus asper* and *Corydalis pterygopetata* grown on lead – zinc mining area in China have been identified as heavy metal hyperaccumulators (Yanqun *et al.*, 2005). Environment Canada has developed a database (PHYTOREM) of 775 plants with capabilities to accumulate or hyperaccumulate one or several key metallic elements. Table 1 lists some important hyperaccumulators including the recently discovered ones. Despite these properties hyperaccumulators are of limited use for large scale applications because they are often slow growing and attain low biomass. So far only one hyperaccumulator species, the Ni hyperaccumulator. *Alyssum bertolonii*, has been used for phytoremediation in the field (Chaney *et al.*, 2000; Li *et al.*, 2003). *Pteris vittata*, an As hyperaccumulating fern may also show promise for phytoextraction of As (Ma *et al.*, 2001). However, in the coming years, mining of the genomic sequences from *Arabidopsis thaliana* and rice and availability of new genomic technologies should lead to identification of novel genes important for heavy metal remediation. The relevant genes from these hyperaccumulators may then be introduced into higher biomass producing non-accumulators for an improved phytoremediation potential, making it a commercially viable technology.

Table - 1: Several metal hyperaccumulator species with respective metal accumulated

S.No.	Plant species	Metal	References
1.	*Thlaspi caerulescens*	Zn, Cd	Reeves and Brooks (1983); Baker and Walker (1990)
2.	*Ipomea alpina*	Cu	Baker and Walker (1990)
3.	*Sebertia acuminata*	Ni	Jaffre *et al.* (1976)
4.	*Haumaniastrum robertii*	Co	Brooks (1977)
5.	*Astragalus racemosus*	Se	Beath *et al.* (2002)
6.	*Arabidopsis thaliana*	Zn, Cu, Pb, Mn, P	Lasat (2002b)
7.	*Thlaspi goesingens*	Ni	Kramer *et al.* (2000)
8.	*Brassica oleracea*	Cd	Salt *et al.* (1995b)
9.	*Arabidopsis halleri*	Zn, Cd	Reeves and Baker (2000); Cosio *et al.* (2004)
10.	*Sonchus asper*	Pb, Zn	Yanqun *et al.* (2005)
11.	*Corydalis pterygopetala*	Zn, Cd	Yanqun *et al.* (2005)
12.	*Alyssum bertolonii*	Ni	Li *et al.* (2003); Chaney *et al.* (2000)
13.	*Astragalus bisulcatus*	Se	Vallini *et al.* (2005)
14.	*Stackhousia tryonii*	Ni	Bhatia *et al.* (2005)
15.	*Hemidesmus indicus*	Pb	Chandra Sekhar *et al.* (2005)
16.	*Salsola kali*	Cd	de la Rosa *et al.* (2004)
17.	*Sedum alfredii*	Pb, Zn	Li *et al.* (2005)
18.	*Pteris vittata*	As	Ma *et al.* (2001); Zhang *et al.* (2004); Tu and Ma (2005)
19.	*Helianthus anus*	Cd, Cr, Ni	Turgut *et al.* (2004)

Phytovolatilization:

Phytovolatilization is the release of pollutants from the plant to the atmosphere as a gas. Although it works well for organics, this can be used for a few inorganics that can exist in volatile form *i.e.* Se, Hg and As (Hansen *et al.*, 1998; Rugh *et al.*, 1996). Members of the *Brassica* genus and some microorganisms are particularly good volatilizers of Se (Terry *et al.*, 1992). Among the aquatic species, rice, rabbit foot grass, *Azolla* and pickle weed are the best Se volatilizers (Hansen *et al.*, 1998; Lin *et al.*, 2000; Pilon-Smits *et al.*, 1999; Zayad *et al.*, 2000). Volatilization of Se involves assimilation of inorganic Se into the organic selenoaminoacids selenocysteine (SeCys) and selenomethionine (SeMet). The latter can be methylated to form dimethylselenide (DMSe), which is volatile (Terry *et al.*, 2000). Volatilization of As and Hg has been demonstrated for microorganisms, but these elements do not appear to be volatilized to significant levels by nontransgenic plants (Rugh *et al.*, 1996). In Hg-contaminated soils and sediments, microbial activity converts the highly toxic Hg (II) into organomercurials and, under optimum conditions, elemental Hg (which is far less toxic) enters the global biogeochemical cycle upon volatilization (Bizily *et al.*, 2000). Because volatilization completely removes the pollutant from the site as a gas, without need for plant harvesting and disposal, this is an attractive technology. A risk assessment study for volatile Se and Hg reported that the pollutant was dispersed and diluted to such an extent that it did not pose a threat (Lin *et al.*, 2000; Meagher *et al.*, 2000). Although phytovolatilization is a passive process, it may be maximized by using plant species with high transpiration rates, by overexpression of enzymes such as cystathionine-V-synthase that mediates S/Se volatilization (Van Huysen *et al.,* 2003) and by transferring gene for Se volatilization from hyperaccumulators to nonaccumulators (Le Duc *et al.*, 2004).

Phytostabilization:

The term denotes the use of plants to stabilize pollutants in soil (Berti and Cunningham, 2000). Phytostabilization of metals may employ plants to reduce leaching, runoff, and erosion *via* stabilization of soil by plant roots or root exudates may cause metals to precipitate, converting them to less bioavailable form (Berti and Cunningham, 2000; Burken *et al.*, 2000; Kramer and Chardonnens, 2001). For phytostabilization of metals a combination of trees and grasses work best. Fast-transpiring trees such as 'Poplar' maintain an upward flow to prevent downward leaching, while grasses prevent wind erosion and lateral runoff with thin dense root system. Further, grasses do not accumulate as much metals in their shoots as dicot species, minimizing exposure of wildlife to toxic elements (Pilon Smits, 2005).

Rhizofiltration:

In rhizofiltration plant roots grown in water absorb, concentrate and precipitate toxic metals and organic chemicals from polluted effluents (Schmoger *et al.*, 2000). The plants can be used as filters in constructed wetlands (Horne, 2000; Lytle *et al.,* 1998) or in a hydroponic setup (Raskin *et al.*, 1997), comprising a complex ecosystem of plants, microbes and sediment that together act as a biogeochemical filter, efficiently removing contaminants from wastewater. Constructed wetlands are useful for filtering large volumes of wastewater whereas hydroponic set up is an indoor, contained setup which is relatively expensive to implement and therefore most useful for relatively small volumes of wastewater containing hazardous inorganics such as radionuclides (Negri and Hinchman, 2000; Dushenkov and Kapulnik, 2000). Constructed wetlands have been used for a wide range of inorganics including metals, Se, perchlorate, cyanide, nitrate and phosphate (Hansen *et al.*, 1998; Horne, 2000; Nzengung and McCutcheon, 2003). Recently two bacterial strains, *Bacillus mycoides* and *Stenotrophomonas maltophilia* have shown potential to detoxify Se and a model system for Se rhizofiltration based on *Astragalus bisulcatus* – rhizobacteria interactions has also been proposed by Vallini *et al.* (2005).

In constructed wetlands water hyacinth, *Azolla* and duckweed are popular because they are good metal accumulators and can be harvested easily. Cattail and poplar are also used because they are tolerant, grow fast and attain large biomass. Hydroponic system involves aeration and therefore is not limited to aquatic species; it often makes use of terrestrial species with large roots and good capacity to accumulate inorganics, such as *Helianthus annuus* (Dushenkov and Kapulnik, 2000) or *Phaseolus vulgaris* (Piechalak *et al.*, 2002).

Different phytoremediation technologies make use of different plant properties and are suitable for different classes of pollutants. Finally all such processes, which either decontaminate the soil or stabilize the pollutant within it, are not mutually exclusive and occur simultaneously, in an effort to totally decontaminate the environment. For example accumulation, stabilization and volatilization of Se has been reported to occur simultaneously in a constructed wetland (Hansen *et al.*, 1998).

Factors affecting phytoremediation

The success of phytoremediation as an environmental clean up technology depends on several factors including bioavailability of metals in soil, plant's ability to uptake, translocate and accumulate metals in shoots and plant-microbe interactions. To dismay, the underlying biological mechanisms of plant decontamination processes are poorly understood and much still remains to be known.

Metal bioavailability:

For plants and their associated microbes to remediate pollutants, they must be in contact with them and able to act on them. Therefore, the bioavailability of a pollutant is important for its remediation. Pollutant bioavailability depends on the chemical properties of the pollutant, soil properties, environmental conditions and biological activity. Soils with small particle size (clay) hold more water than sandy soils, and have more binding sites for ions, especially cations (CEC) (Taiz and Zeiger, 2002). The concentration of humus in soil is also positively correlated with CEC because humus mainly consists of dead plant material, and plant cell walls have negatively charged groups that bind cations, as well as lignin that binds hydrophobic compounds (Burken, 2003).

Metals are usually present as charged cations or anions, and thus are hydrophilic. The bioavailability of cations is inversely correlated with soil CEC. At lower soil pH, the bioavailability of cations generally increases due to replacement of cations on soil CEC sites by H^+ ions (Taiz and Zeiger, 2002). The bioavailability of ions is also affected by the redox conditions. Most terrestrial soils have oxidizing conditions, and elements that can exist in different oxidation states will be in their most oxidized form. In aquatic habits more reducing conditions exist, which favour more reduced elemental forms. The oxidation state of an element may affect its bioavailability (*e.g.* its solubility), its ability to be taken up by plants, as well as its toxicity. Other physical conditions that affect metal bioavailability are temperature and moisture. Higher temperatures accelerate physical, chemical and biological processes in general. Precipitation stimulates general plant growth and higher soil moisture increases migration of water-soluble pollutants. Bioavailability of metals is also enhanced by metal chelators that are released in rhizosphere by plants and bacteria chelators such as siderophores, organic acids, and phenolics can release metal cations from soil particles (Taiz and Zeiger, 2002) which makes the metals more available for plant uptake. Furthermore, plants extrude H^+ *via* ATPases, which replace cations at soil CEC sites, making metal cations more bioavailable (Taiz and Zeiger, 2002).

Understanding the process affecting pollutant bioavailability can help optimize phytoremediation efficiency. Aged soils are more difficult to phytoremediate as pollutants in aged polluted soils tend to be less bioavailable and more recalcitrant than pollutants in soil that is newly contaminated. In such cases, amendments may be added to soil that make metal cations more bioavailable for plant uptake. For instance, adding the natural organic acids citrate or malate will lower the pH of soil particles and consequently will enhance cation bioavailability. Recently malate has been identified as a ligand for Ni, supporting detoxification/transport and storage of this heavy metal in *Stackhousia tryonii* (Bhatia *et al.*, 2005). The synthetic metal chelator EDTA (Ethylene di-amine tetra acetic acid) is also extremely efficient at releasing metals from soil. This principle is used in chelate assisted phytoextraction where EDTA is added to soil shortly before plant harvesting, greatly increasing plant metal uptake (Salt *et al.*, 1998). A recent study indicated that EDTA addition can increase the potential and efficiency of Cu phytoextraction by *Elsholtzia splendens* in polluted soils (Jiang and Yang, 2004). EDTA and citric acid (Turgut *et al.*, 2004) as well as EDTA and HEDTA (Chen and Cutright, 2001) combination can also enhance Cd, Cr and Ni uptake in *Helianthus annus*. Before chelate assisted phytoextraction is used in the field, it is important to do a risk assessment study to determine possible effects of the chelator on metal leaching. In other situations it may be desirable to decrease metal bioavailability if metals are present at phytotoxic levels or in phytostabilization. In such cases lime may be mixed with the soil to increase the pH or organic matter to bind metals (Bennett *et al.*, 2003; Brown *et al.*, 2003).

Plant uptake and translocation:

Access of heavy metals to bare roots is confined to the first few millimeters of the root tip. Uptake and transport across root cellular membrane is an important process which initiates metal absorption into plant tissues. Two different uptake routes have been reported: (a) passive uptake driven only by the concentration gradient across the membrane, and (b) inducible substrate-specific and energy dependent uptake mediated by membrane protein with transport functions (Nies, 1999; Williams *et al.*, 2000). Either through passive or active uptake, root cells capture metals from soil that remain bound by their cell wall and then transported across the membrane. But the electrical charge on metal ions prevents their diffusion freely across the lipophilic cellular membranes into the cytosol. Therefore, metal transport into cells is also driven by ATP – dependent protein pumps that catalize H^+ extrusion across the membrane.

For most elements multiple transporters exist in plants. The model plant *Arabidopsis thaliana*, for instance, has 150 different cation transporters (Axelsen and Palmgren, 2001) and 14 transporters for sulfate alone (Hawkesford, 2003). Individual transporter proteins have unique properties with respect to transport rate, substrate specificity, substrate affinity (low affinity transporters tend to be more promiscuous) and follow Michaelis Menton kinetics (Marschner, 1995). These properties may be subjected to regulation by metabolic levels or regulatory proteins (*e.g.* Kinases). Furthermore, the abundance of each transporter varies with tissue type and environmental conditions, which may be regulated at the transcription level or *via* endocytosis. As a consequence, uptake and movement of inorganics in plants are complex species and conditions dependent processes (Williams *et al.*, 2000; Pilon-Smits, 2005). Table 2 list some important transport proteins along with their source and function.

Plant microbe interactions:

The limited bioavailability of various metallic ions, due to their low solubility in water and strong binding to soil particles, restricts their uptake/accumulation by plants. However, root colonizing bacteria and mycorrhiza can significantly increase the bioavailability of various heavy metal ions for uptake. Some bacteria are known to release biosurfactants (*e.g.* rhamnolipids) that make hydrophobic pollutants more water soluble (Volkering *et al.*, 1998). Soil microorganism's organic exudates stimulate bioavailability and facilitate root absorption of a variety of metal ions including Fe^{2+} (Crowley *et al.*, 1991; Burkal *et al.*, 2000), Mn^{2+} (Barber and Lee, 1974) and Possibly Cd^{2+} (Salt *et al.*, 1995a). Root exudates which feed the microorganisms by providing carbohydrates, also contain lipophilic compounds and natural chelating agents (citric, acetic and other organic acids) that

Table - 2: List of important transport proteins with their respective sources and functions

S.No.	Transport protein	Source	Function	References
1.	CPx – ATPases (PAA1, RAN1)	*Arabidopsis, Chlamyelomonas reinhardtii*	Heavy metal transport and trafficking	Williams *et al*., 2000
2.	CDF/CE	Bacteria, archea, Eukaryotes, *Chlamyelomonas reinhardtii*	Zn, Co, Cu transport, sequestracton, catablyzes metal efflux	Paulsen and Saier, 1997; Van der Zaal *et al*., 1999.
3.	Nramp (Natural resistance associated macrophage proteins	Bacteria, yeast, insects, humans, plants, Algae	Bivalent metal ion transport	Belouchi *et al*., 1997; Williams *et al*., 2000
4.	YSI	Maize, *Arabidopsis*	Fe transporter	Curie *et al*., 2001
5.	ZIP	*Medicago* sp., *Truncatula* sp.	Cd, Zn, Mn, Fe, transporter	Lopez-Millan *et al*., 2004; Guerinot, 2000; Maiser *et al*., 2001
6.	Cation transporters	*Arabidopsis*	Manovalent and divalent cation transport	Schuurink *et al*., 1998; Arazi *et al*., 1999; White *et al*., 2002.

increase ion mobility in soil or promote biosurfactant – producing microbial populations. Plant roots and rhizosphere microorganisms have complex feedback mechanisms that permit them to adapt to changing soil conditions as they grow. In some plants growing in phosphorus deficient soil, the root exudates contain large amounts of citric acid, in an attempt to mobilize any phosphorous component present in the soil. Some rhizosphere microorganisms, also secrete plant hormones that increase root growth and thereby the secretion of root exudates.

Some bacterial strains also aid in pollutant detoxification *Xanthomonas maltophyla* (Blake *et al*., 1993), *Escherichia coli* and *Pseudomonas putida* (Lasat, 2002a) catalyze reduction and precipitation of highly mobile and environmentally less hazardous compounds.

Mycorrhiza, a well documented fungal symbiotic association with roots of majority of plants has also been reported in plants growing on heavy metal contaminated soil (Shetty *et al*., 1994; Chaudhry *et al*., 1998, 1999). Fungal symbiotic associations have the potential to enhance root absorption upto 47 – fold (Smith and Reed, 1997) and stimulate the acquisition of plant nutrients including metal ions (Khan *et al*., 2000). However, contradictory reports as to the effect of mycorrhizae on metal uptake are also available. An inhibition of Zn, Cu (Scheupp *et al*., 1987; Heggo *et al*., 1990) and Cd accumulation (Schutzendubel *et al*., 2002; Weissenhorn and Leyval, 1995; Joner and Leyval, 1997) in mycorrhizae plants has been reported. *Thlaspi praecox* colonized with arbuscular mycorrhizae fungi also showed reduced heavy metal (Cd and Pb) uptake (Vogel-Mikus *et al*., 2005). Inhibition of metal accumulation also suggests a role of mycorrhizae in protecting the plants from heavy metal toxicity, although the mechanism of protection is unclear. In addition to the effect on metal root uptake, mycorrhizae has also been reported to affect metal transport within plant. For example, mycorrhizae has been shown to alter the pattern of Zn translocation from root to shoot in the grass *Andropogon gerardii* and maize seedling (Khan *et al*., 2000). However, this effect appeared to be species specific since mycorrhizae did not affect Zn translocation pattern in *Festuca arrundinaceae* (Shetty *et al*., 1994). Mycorrhizal fungi can both enhance uptake of essential metals when metal levels are low and decrease plant metal uptake when metals are present at phytotoxic levels (Frey *et al*., 2000).

The mechanisms of three plant microbe interactions are still largely unclear; microbe mediated enhanced plant uptake may be due to a stimulatory effect on root growth, microbial production of metabolites that affect plant gene expression of transporter proteins or microbial effect on bioavailability of the element (DeSouza *et al*., 2000).

Role of metal chelators:

As mentioned earlier, the complex root secretions from plants contain natural chelating agents that affect pollutant solubility and uptake. Inside plant tissues such chelator compounds also play a role in tolerance, sequestration and transport (Ross, 1994). Phytosiderophores are chelators that facilitate uptake of Fe and perhaps other metals in grasses (Higuchi *et al*., 1999). Organic acids (*e.g.* citrate, malate, acetate) not only can facilitate uptake of metals with roots but also play a role in transport, sequestration, and tolerance of metals (Salt *et al*., 1995b; Von Wiren *et al*., 1999). As a tolerance and detoxification mechanism, chelated metals are effluxed from cytoplasm and sequestered in the vacuolar compartment, which excludes them from cellular sites where processes such as cell division and respiration occur, thus providing an effective protective mechanism (Chaney *et al*., 1997; Hall, 2002). Detoxification of Cd and Zn in *Thlaspi caerulescens* is achieved by vacuolar compartmentalization (Ma *et al*., 2005). Role of cysteine rich metallothioneins (MTs) (Hamer, 1986; Cobbett and

Table - 3: Some genetically engineered plants with the respective gene transferred, gene product, gene source and improved trait

S.No.	Transgenic plant	Transgene and its product	Gene source	Trait enhanced	References
1.	*S. cerevisiae*	Cup1 gene: MT's	*A. thaliana*	Zn & Cu tolerance	Robinson *et al.*, 1996
2.	*S. cerevisiae*	TaPCSI gene: PC's	Wheat	Cd tolerance	Vatamaniuk *et al.*, 1999
3.	*N. tobaccum and Liriodendron tulipifera*	merA: Hg(II) reductase	Gram-ve bacteria	Hg tolerance and volatilization	Heaton *et al.*, 1998
4.	*A. thaliana*	merA: Hg(II) reductase ; merB: organomercurial lyase	Gram-ve bacteria	Hg tolerance and volatilization	Rugh *et al.*, 1996
5.	*Brassica juncea*	APs gene: ATP-sulfurylase	*A. thaliana*	Se hyperaccumulation	Banuelos *et al.*, 2005
6.	*Lycopersicon esculentum*	ACC gene: 1-amino-cyclopropane 1-carboxilic acid deaminase	bacteria	Cd, Co, Cu, Mg, Ni, Pb, Zn tolerance	Gricheko *et al.*, 2000
7.	*A. thaliana*	PsMTA gene: MTs	Peas	Cu tolerance	Evans *et al.*, 1992
8.	*Brassica juncea*	gshI gene: g-glutamylcysteine	*E.coli*	Cd tolerance	Zhu *et al.*, 1999 a,b
9.	*N. tobaccum*	MT-1 gene: MT's	Mouse	Cd tolerance	Thomine *et al.*, 2000
10.	*N. tobaccum,* *Carcia papaya*	Citrate synthase gene	*Pseudomonas aeruginosa*	High levels of citrate	de la Fuente *et al.*, 1997
11.	*N. tobaccum*	FRE1, FRE2: Ferric reductass	*S. cerevisiae*	Ni hyperaccumulation	Samuelsen *et al.*, 1998

Goldsbrough, 2000) and thiol rich phytochelatins (PCs) as metal chelators is also well documented.

Metallothioneins are produced in animals and certain fungi in response to metal uptake (Hamer, 1986). In plants, MT proteins have been identified only in wheat (Lane *et al.*, 1987) and *Arabidopsis* (Murphy *et al.*, 1997). Although detection of plant MTs has been problematic, evidence suggests that they have high affinity for binding metal cations, such as Cd, Cu and Zn (Schmoger *et al.*, 2000; Cobbett and Goldsbrough, 2002). There are reports that MTs also play a role in heavy metal detoxification as overexpression of Fe or Cu chelating MTs and ferritin protects against metal induced oxidative injury (Fabisiak *et al.*, 1999). Phytochelatins (PCs) are also a family of metal-complexing peptides which are rapidly induced on overexposure to metals or metalloids in plants, animals and some yeasts (Schmoger *et al.*, 2000; Cobbett and Goldsbrough, 2002). PCs have been shown to be primarily involved in Cd and Cu tolerance (Rauser, 1995; Ow, 1996) but Schmoger *et al.* (2000) suggested that PCs may also be involved in arsenic detoxification as they possess high affinity to sulfhydryl groups. After chelation by PCs, the metal chelate complex is actively transported to the vacuole, where it is further complexed by sulfide (Cobbett and Goldsbrough, 2000; Lu *et al.*, 1997). However, more precise role of these different chelators in transport and detoxification of metals remains to be established.

Transgenic plants in phytoremediation: The plant species currently being developed for phytoremediation seem capable of effective bioaccumulation of targeted contaminant, but efficiency might be improved through the use of transgenic (genetically engineered) plants. Naturally occurring plant species that can be genetically engineered for improved phytoremediation include *Brassica juncea* for phytoremediation of heavy metals from soil (Dushenkov *et al.*, 1995), *Helianthus anus* (Dushenkov *et al.*, 1995) and *Chenopodium amaranticolor* (Eapen *et al.*, 2003) for rhizofiltration of uranium. In general, any dicotyledon plant species can be genetically engineered using the Agrobacterium vector system, while most monocotyledon plants can be transformed using particle gun or electroporation techniques. Some promising transgenics that show higher tolerance, accumulation, and /or degradation capacity for various pollutants have been developed. This was achieved either by overproducing metal chelating molecules such as citrate (de la Fuente *et al.*, 1997), PCs (Zhu *et al.*, 1999 a,b), MTs (Evans *et al.*, 1992; Hasegawa *et al.*, 1997) or ferritin (Goto *et al.*, 1999) or by overexpression of metal transporter proteins (Samuelsen *et al.*, 1998; Arazi *et al.*, 1999; Van der Zaal *et al.*, 1999; Curie *et al.*, 2001). Mercury volatilization and tolerance was also achieved in tobacco by introduction 3of a bacterial pathway (Rugh *et al.*, 1996; Bizily *et al.*, 2000; Kramer and Chardonnens, 2001 ; Pilon-Smits and Pilon, 2000). Table 3 lists some genetically engineered plants with the respective genes transferred, gene source and the improved trait of transgenics.

The increase in metal accumulation as the result of these genetic engineering approaches in typically two to threefold more metal per plant, which potentially enhances phytoremediation efficiency by the same factor. It is not yet clear how applicable these transgenics are for environmental clean up, since no field studies have been reported except one using transgenic Indian mustard plant that overexpresses enzymes involved in sulfate/ selenate reduction. (Pilon Smits *et al.*, 1999; Zhu *et al.*, 1999 a,b). Potential environmental impacts of transgenics such as competitiveness of transgenic to wild type, effect on birds, insects, *etc.*, that might feed on plant biomass containing high concentration of toxic metals and possibility of gene transfer to other plants by pollination require continuous monitoring. Genetic engineering of the chloroplast genome offers a novel way to obtain high expression without the risk of spreading the transgene via pollen (Ruiz *et al.*, 2003). In future, as more data on field trials and associated risk assessment would be available, transgenics will play an important role in commercial phytoremediation.

Future requirements for phytoremediation:

Since last decade phytoremediation has gained acceptance as a technology and has been acknowledged as an important area of research. Phytoremediation's basic processes are still largely not clear and hence require further basic and applied research to optimize its field performance. Information collected from basic research at physiological, biochemical and genetic level in plants will be helpful in understanding the processes of passive adsorption, active uptake, translocation, accumulation and chelation mechanisms. Research aimed at better understanding of the interactive roles among plants roots and microbes will help scientists to utilize their integrative capacity for soil decontamination. Further, genetic evaluation of hyperaccumulators growing in metal contaminated soil and associated microbes would provide the researchers with a gene pool to be used in genetic manipulation of other non accumulators and production of transgenics.

However, new knowledge and plant material obtained from research is already being implemented for phytoremediation in the field. The first field tests with transgenics are showing promising results. As more results demonstrating the effectiveness of phytoremediation become available, its use may continue to grow, reducing clean up costs and enabling the clean up of more sites with the limited funds available (Rock, 2003). Currently a great deal of research is in progress in this direction and its impact will soon be felt in phytoremediation market.

An interesting development in phytoremediation could be the adoption of an integrated approach both for research and commercial purposes. Presently phytoremediation research is carried out by scientists with expertise in only certain fields *e.g.* plant molecular biology, plant biochemistry, plant physiology, ecology, plant biochemistry, plant physiology, ecology, toxicology or microbiology but phytoremediation being an integrated technology will be benefited more by a team of researchers with different backgrounds. Commercially to enhance public acceptance phytoremediation can be integrated with landscape architecture such as remediation of partially contaminated urban sites may be combined with an attractive design so that the area may be used as a park or some other recreational place by the public after the remediation process (Pilon-Smits, 2005).

It is obvious that phytoremediation is an effective technology for removing and detoxifying metals and metalloids such as Cd, Se and As from environment for recultivation and reclamation of polluted sites. Phytoremediatoin works best when supplemented by non biological remediation technologies for decontamination of most polluted sites. Because pollutant distribution and concentration are heterogeneous for sites, the most efficient and cost effective remediation solution may be a combination of different technologies such as excavation of the most contaminated spots followed by polishing the site with the use of plants. The identification of unique genes from natural hyperaccumulators and their subsequent transfer to fast growing species is another promising approach. To improve phytoremediation a number of agronomic enhancements are also possible ranging from traditional crop management techniques (use of pesticides, soil amendments, fertilizers *etc.*) to approaches more specific to phytoremediation such as improving metal solubility in soils through the use of chelators. Finally, advances in optimizing plants for phytoremediation will depend on gaining new knowledge about the fate and transport of metals/ metalloids in plants and innovative technologies to improve the acceptability of transgenic plants for phytoremediation.

References

Arazi, T., R. Sunkar, B. Kaplan and H. Fromm: A tobacco plasma membrane calmodulin binding transporter confers Ni^{2+} tolerance and Pb^{2+} hypersensitivity in transgenic plants. *Plant. J.,* **20**, 171-82 (1999).

Axelsen, K.B. and M.G. Palmgren: Inventory of the superfamily of P-type ion pumps in *Arabidopsis*. *Plant Physiol.,* **126**, 696-706 (2001).

Baker, A.J.M. and P.L. Walker: Ecophysiology of metal uptake by tolerant plants. *In*: Heavy metal tolerance in plants: Evolutionary aspects (*Ed*: A.J. Shaw). CRC Press, Boca Raton, F.L. pp. 155-177 (1990).

Baker, A.J.M., S.P. McGrath, R.D. Reeves and J.A.C. Smith: Metal hyperaccumulator plants: A review of the ecology and physiology of a biological resource for phytoremediation of metal-polluted soils. *In*: Phytoremediation of contaminated soil and water (*Eds*: N. Terry and G. Banuelos). Boca Raton, Lewis. pp. 85-108 (2000).

Banuelos, G., N. Terry, D.L. Leduc, E.A. Pilon Smits and B. Mackey: Field trial of transgenic Indian mustard plants shows enhanced phytoremediation of selenium contaminated sediment. *Environ. Sci. Technol.*, **39(6)**, 1771-1777 (2005).

Banuelos, G.S .: Factors influencing field phytoremediation of selenium laden soils. *In*: Phytoremediation of contaminated soil and water, (*Eds*: N. Terry, G. Banuelos). Boca Raton: Lewis. pp. 41-61 (2000).

Barber, S.A. and R.B. Lee.: The effect of microorganisms on the absorption of manganese by plants. *New Phytol.*, **73**, 97–106 (1974).

Baudouin, C., M. Charveron, R. Tarrouse and Y. Gall: Environmental pollutants and skin cancer. *Cell Biol. Toxicol.*, **18**, 341-348 (2002).

Beath, O.A., H.F. Eppsom and G.S. Gilbert: Selenium distribution in and seasonal variation of vegetation type occurring on seleniferous soils. *J. Am. Pharm. Assoc.*, **26**, 394-405 (2002).

Belouchi, A., T. Kwan and P. Gros: Cloning and characterization of the osnramp family from *Oryza sativa*, a new family of membrane proteins possibly implicated in the transport of metal ions. *Plant Mol. Biol.,* **33**, 1085-1092 (1997).

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

Berti, W.R. and S.D. Cunningham: Phytostabilization of metals: Phytoremediation of toxic metals using plants to clean up the environment (*Eds*: I. Raskins and B.D. Ensley). Wiley, New York. pp. 71-88 (2000).

Bhatia, N.P., K.B. Walsh and A.J. Baker: Detection and quantification of ligands involved in nickel detoxification in a herbaceous Ni hyperaccumulator *Stackhousia tryonii* Bailey. *J. Exp. Bot.*, **56**, 1343-1349 (2005).

Bizily, S.P., C.L. Rugh and R.B. Meagher: Phytodetoxification of hazardous organomercurials by genetically engineered plants. *Nat. Biotechnol.,* **18**, 213-217 (2000).

Blake, R.C., D.M. Choate, S. Bardhan, N. Revis, L.L. Barton and T.G. Zocco: Chemical transformation of toxic metals by a *Pseudomonas* strain from a toxic waste site. *Environ. Toxicol. Chem.,* **12**, 1365-1376 (1993).

Blaylock, M.J. and J.W. Huang: Phytoextraction of metals. *In*: Phytoremediation of toxic metals using plants to clean up the environment (*Eds:* I. Raskin and B.D. Ensley). Wiley, New York. pp. 53-70 (2000).

Brooks, R.R.: Plants that hyperaccumulate heavy metals. CAB Intl., Wallingford (1998).

Brooks, R.R.: Copper and cobalt uptake by *Haumaniastrum* species. *Plant Soil,* **48**, 541-544 (1977).

Brown, S.L., C.L. Henry, R. Chaney, H. Compton and P.M. DeVolder: Using municipal biosolids in combination with other residuals to restore metal contaminated mining areas. *Plant Soil,* **249**, 203-215 (2003).

Bural, G.I., D.G. Dixon and B.R. Glick: Plant growth promoting bacteria that decrease heavy metal toxicity in plants. *Can. J. Microbiol.*, **46**, 237-245 (2000).

Burken, J.G., J.V. Shanks and P.L. Thompson: Phytoremediation and plant metabolism of explosives and nitroaromatic compounds. *In*: Biodegradation of nitroaromatic compounds and explosives (*Eds*: J.C. Spain *et al.*). Lewis, Washington, D.C. pp. 239-275 (2000).

Burken, J.G.: Uptake and metabolism of organic compounds: Green-liver model. *In*: Phytoremediation: Transformation and control of contaminants (*Eds*: S.C. McCutcheon and J.L. Schnoor). Wiley, New York. pp. 59-84 (2003).

Chandra Sekhar, K., C.T. Kamala, N.S. Chary, V. Balaram and G. Garcia: Potential of *Hemidesmus indicus* for phytoextraction of lead from industrially contaminated soils. *Chemosphere*, **58(4)**, 507-514 (2005).

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

Chaney, R.L., Y.M. Li , S.L. Brown , F.A. Homer and M. Malik : Improving metal hyperaccumulator wild plants to develop commercial phytoextraction systems: Approaches and progress. *In*: Phytoremediation of contaminated soil and water (*Eds*: N. Terry and G. Bañuelos). Boca Raton, Lewis. pp. 129-58 (2000).

Chaudhry, T.M., L. Hill, A.G. Khan and C. Keuk: Colonization of iron and zinc contaminated dumped filter cake waste by microbes, plants and associated mycorrhizae. *In:* Remediation and management of degraded land (*Eds*: M. H. Wong and A.J.M. Baker). CRC Press, Boca Raton, FL. pp. 275-283 (1999).

Chaudhry, T.M., W.J. Hayes, A.J. Khan and C.S. Khoo: Phytoremediation focusing on accumulator plants that remediate metal contaminated soils. *Aust. J. Ecotoxicol.,* **4**, 37-51 (1998).

Chen, H. and T. Cutright: EDTA and HEDTA effects on Cd, Cr, and Ni uptake by *Helianthus annuus*. *Chemosphere*, **45(1)**, 21-28 (2001).

Cobbett, C. and P. Goldsbrough: Phytochelatins and metallothioneins: Roles in heavy metal detoxification and homeostasis. *Ann. Rev. Plant Physiol. Plant Mol. Biol.,* **53**, 159-182 (2002).

Cobbett, C.S. and P.B. Goldsbrough: Mechanisms of metal resistance: Phytochelatins and metallothioneins. *In*: Phytoremediation of toxic metals using plants to clean up the environment (*Eds*: I. Raskin and B.D. Ensley). Wiley, New York. pp. 247-271 (2000).

Cosio, C., E. Martinoia and C. Keller: Hyperaccumulation of cadmium and zinc in *Thlaspi caerulescens* and *Arabidopsis halleri* at the leaf cellular level. *Plant Physiol.*, **134(2)**, 716-725 (2004).

Crowley, D.E., Y.C. Wang, C.P.P. Reid and P.J. Szansiszla: Mechanism of iron acquisition from siderophores by microorganisms and plants. *Plant Soil,* **130**, 179-198 (1991).

Curie, C., Z. Panaviene, C. Loulergue, S.L. Dellaporta, J.F. Briat and E.L. Walker: Maize yellow stripe 1 encodes a membrane protein directly involved in Fe(III) uptake. *Nature,* **409**,346-349 (2001).

de la Fuente, J.M. , V. Ramírez-Rodriguez, J.L. Cabrera-Ponce and L. Herrera-Estrella: Aluminum tolerance in transgenic plants by alteration of citrate synthesis. *Science,* **276**, 1566-1568 (1997).

de la Rosa G., J.R. Peralta Videa, M. Montes, J.G. Parsons, I. Cano-Aguilera and J.L. Gardea-Torresdey: Cadmium uptake and translocation in tumbleweed (*Salsola kali*), a potential Cd-hyperaccumulator desert plant species: ICP/OES and XAS studies. *Chemosphere*, **55(9)**, 1159-1168 (2004).

DeSouza, M.P., E.A.H. Pilon-Smiths and N. Terry: The physiology and biochemistry of selenium volatilization by plants. *In*: Phytoremediation of toxic metals: Using plants to clean up the environment (*Eds*: B.D. Ensley and I. Raskin). Wiley, New York. pp. 171-190 (2000).

Dushenkov, S. and Y. Kapulnik: Phytofiltration of metals. *In*: Phytoremediation of toxic metals using plants to clean up the environment (*Eds*. I. Raskin and B.D. Ensley). Wiley, New York. pp. 89-106 (2000).

Dushenkov, S.: Trends in phytoremediation of radionuclides. *Plant Soil,* **249**, 167-75 (2003).

Dushenkov, V., P.B.A.N. Kumar, H. Motto and I. Ruskin: Rhizofiltration: The use of plants to remove heavy metals from aqueous streams. *Environ. Sci. Technol.*, **29,** 1239-1245 (1995).

Eapen, S., K.N. Suseelan, S. Tivarekar, S.A. Kotwal and R. Mitra: Potential for rhizofiltration of uranium using hairy root cultures of *Brassica juncea* and *Chenopodium amaranticolor*. *Environ. Res*., **91(2)**, 127-133 (2003).

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

Fabisiak, J.P., L.L. Pearce, G.G. Borisenko, Y.Y. Tyhuriana, V.A. Tyurin, J. Razzack, J.S. Lazo, B.R. Pitt and V.E. Kagan: Bifunctional anti pro-oxidant potential of metallothionein: Redox signaling of copper binding and release. *Antioxidant Redox. Signal*, **1**, 349-364 (1999).

Frey, B. , K. Zierold and I. Brunner: Extracellular complexation of Cd in the Hartig net and cytosolic Zn sequestration in the fungal mantle of *Picea abies–Hebeloma crustuliniforme* ectomycorrhizas. *Plant Cell Environ.,* **23**, 1257-1265 (2000).

Goto, F., T. Yoshihara , N. Shigemoto, S. Toki and F. Takaiwa: Iron fortification of rice seed by the soybean ferritin gene. *Nature Biotechnol.,* **17**, 282-286 (1999).

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

Guerinot, M.L.: The ZIP family of metal transporters. *Biochim. Biophys. Acta*., **1465**, 190-198 (2000).

Hall, J.L.: Cellular mechanisms for heavy metal detoxification and tolerance. *J. Exp. Biol.,* **53**, 1-11(2002).

Hamer, D.H.: Metallothioneins. *Ann. Rev. Biochem*., **55**, 913-951 (1986).

Hansen, D. , P.J. Duda, A. Zayed and N. Terry: Selenium removal by constructed wetlands: Role of biological volatilization. *Environ. Sci. Technol.,* **32**, 591-597 (1998).

Hasegawa, I. , E. Terada, M. Sunairi, H. Wakita and F. Shinmachi: Genetic improvement of heavy metal tolerance in plants by transfer of the yeast metallothionein gene (CUP1). *Plant Soil,* **196**, 277-281 (1997).

Hawkesford, M.J.: Transporter gene families in plants: the sulphate transporter gene family–redundancy or specialization? *Physiol. Plant,* **117**, 155-163 (2003).

Heaton, A.C.P., C.L. Rugh, N.J. Wang and R.B. Meagher: Phytoremediation of mercury and methylmercury polluted soils using genetically engineered plants. *J. Soil Contam.,* **7**, 497-507 (1998).

Heggo, A., J.S. Angle and R.L. Chaney: Effects of vesicular arbuscular mycorrhizal fungi on heavy metal uptake by soybean. *Soil Biol. Biochem*., **22**, 856-869 (1990).

Higuchi, K., K. Suzuki, H. Nakanishi, H. Yamaguchi, N.K. Nishizawa and S. Mori: Cloning of nicotianamine synthase genes, novel genes involved in the biosynthesis of phytosiderophores. *Plant Physiol*., **119**, 471-479 (1999).

Horne, A.J.: Phytoremediation by constructed wetlands. *In*: Phytoremediation of contaminated soil and water (*Eds*: N. Terry and G. Bañuelos). Boca Raton, Lewis. pp. 13-40 (2000).

Jaffre, T., R.R. Brooks, J. Lee and R.D. Reeves: *Sebertia acuminata*: A nickel-accumulating plant from new Caledonia. *Science*, **193**, 579-580 (1976).

Jiang, L.Y. and X.E. Yang: Chelators effect on soil Cu extractability and uptake by *Elsholtzia splendens*. *J. Zhejiang Univ. Sci.,* **5(4)**, 450-456 (2004).

Joner, E.J. and C. Leyval: Uptake of ^{109}Cd by roots and hyphae of a *Glomus mosseae*/*Tripholium subterraneum* mycorrhiza from soil amended with high and low concentration of cadmium. *New Phytol.*, **135**, 353-360 (1997).

Khan, A.G., C. Keuk, T.M. Chaudhry, C.S. Khoo and W.J. Hayes: Role of plants, mycorrhizae and phytochelators in heavy metal contaminated land remediation. *Chemosphere*, **41**, 197-207 (2000).

Knasmuller, S., E. Gottmann, H. Steinkellner, A. Fomin, C. Pickl, A. Paschke, R. God and M. Kundi: Detection of genotoxic effects of heavy metal contaminated soils with plant bioassays. *Mutat. Res.,* **420**, 37-48 (1998).

Kramer, U. and A.N. Chardonnens: The use of transgenic plants in the bioremediation of soils contaminated with trace elements. *Appl. Microbiol. Biotechnol.,* **55**, 661-672 (2001).

Kramer, U., I.J. Pickering, R.C. Prince, I. Raskin and D.E. Salt.: Subcellular localization and speciation of nickel in hyperaccumulator and non accumulator *Thlaspi* species. *Plant Physiol.*, **122**, 1343-1353. (2000)

Lane, B., R. Kajioka and T. Kennedy: The wheat germ E$_c$ protein is a zinc containing metallothionein. *Biochem. Cell Biol.*, **65**, 1001-1005 (1987).

Lasat, M.M.: Phytoextraction of toxic metals. *J. Environ. Quality*, **31**, 109-120 (2002b).

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

Le Duc, D.L., A.S. Tarun, M. Montes-Bayon, J. Meija and M.F. Malit: Overexpression of selenocysteine methyltransferase in *Arabidopsis* and Indian mustard increases selenium tolerance and accumulation. *Plant Physiol.,* **135**, 377-383 (2004).

Li, T.Q., X.E. Yang, X.F. Jin, Z.L. He, P.J. Stoffella and Q.H. Hu: Root responses and metal accumulation in two contrasting ecotypes of *Sedum alfredii* Hance under lead and zinc toxic stress. *J. Environ. Sci. Hlth. Toxcol. Hazard Subst. Environ. Eng.*, **40(5)**, 1081-1096 (2005).

Li, Y.M , R. Chaney, E. Brewer, R. Roseberg and S.J. Angle: Development of a technology for commercial phytoextraction of nickel: Economic and technical considerations. *Plant Soil,* **249**, 107-115 (2003).

Lin, Z.Q. , R.S. Schemenauer, V. Cervinka, A. Zayed, A. Lee and N. Terry : Selenium volatilization from a soil plant system for the remediation of contaminated water and soil in the San Joaquin Valley. *J. Environ. Qual.*, **29**, 1048-1056 (2000).

Lopez-Millan, A.F., D.R. Ellis and M.A. Grusak: Identification and characterization of several new members of the ZIP family of metal ion transporters in *Medicago truncatula*. *Plant Mol Biol.*, **54(4)**, 583-596 (2004).

Lu, Y.P. , Z.S. Li and P.A. Rea: AtMRP1 gene of *Arabidopsis* encodes a glutathione S-conjugate pump: isolation and functional definition of a plant ATP-binding cassette transporter gene. *Proc. Natl. Acad. Sci. USA,* **94**, 8243-8248 (1997).

Lytle, C.M., F.W. Lytle, N. Yang, H. Qian, D. Hansen, A. Zayed and N. Terry: Reduction of Cr(VI) to Cr(III) by wetland plants: Potential for *in situ* heavy metal detoxification. *Environ. Sci. Technol.,* **32**, 3087-3093 (1998).

Ma, L.Q. , K.M. Komar and C. Tu: A fern that accumulates arsenic. *Nature,* **409**, 579 (2001).

Ma, X., A.R. Richter, S. Albers and J.G. Burken: Phytoremediation of MTBE with hybrid poplar trees. *Int. Phytoremediation*, **6(2)**, 157-167 (2005).

Marschner, H.: Mineral nutrition of higher plants. Academic Press, San Diego. pp. 889 (1995).

Maiser, P.S. Thomine, J.I. Schroeder, J.M. Ward, K. Hirschi, H, Sze, I.N. Talke, A. Amtmann, F.J.M. Maathuis, D. Sanders, J.F. Harper, J. Tchieu, M. Gribskov, M.W. Persans, D.E. Salt, S.A. Kim and M.L. Guerinot: Phylogenetic relationships within cation transporter families of *Arabidopsis*. *Plant Physiol.*, **126,** 1646-1667 (2001).

Meagher, R.B., C.L. Rugh, M.K. Kandasamy, G. Gragson and N.J. Wang: Engineered phytoremediation of mercury pollution in soil and water using bacterial genes. *In:* Phytoremediation of contaminated soil and water (*Eds*: N. Terry and G. Banuelos). Boca Raton, Lewis. pp. 201-221 (2000).

Murphy, A., J. Zhou, P.B. Goldsbrough and L. Taiz: Purification and immunological identification of metallothioneins 1 and 2 from *Arabidopsis thaliana*. *Plant Physiol.*, **113**, 1293-1301 (1997).

Negri, M.C. and R.R. Hinchman: The use of plants for the treatment of radionuclides. *In*: Phytoremediation of toxic metals using plants to clean up the environment (*Eds*: I. Raskin and B.D. Ensley). Wiley, New York. pp. 107-132 (2000).

Nies, D.H.: Microbial heavy-metal resistance. *Appl. Microbiol. Biotechnol.,* **51**, 730-750 (1999).

Nzengung, V.A. and S.C. McCutcheon: Phytoremediation of perchlorate. *In:* Phytoremediation: Transformation and control of contaminants (*Eds*: S.C. McCutcheon and J.L. Schnoor). Wiley, New York. pp. 863-885 (2003).

Ow, D.W.: Heavy metal tolerance genes: Prospective tools for bioremediation. *Res. Conserv. Recycling,* **18**, 135-149 (1996).

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

Piechalak, A., B. Tomaszewska, D. Baralkiewicz and A. Malecka: Accumulation and detoxification of lead ions in legumes. *Phytochemistry*, **60 (2)**, 153-162 (2002).

Pilon-Smits, E.A.: Phytoremediation. *Ann. Rev. Plant Biology.,* **56**, 15-39 (2005).

Pilon-Smits, E.A.H. and M. Pilon: Breeding mercury-breathing plants for environmental cleanup. *Trends Plant Sci.*, **5**, 235–236 (2000).

Pilon-Smits, E.A.H., M.P. de Souza, G. Hong , A. Amini and R.C. Bravo : Selenium volatilization and accumulation by twenty aquatic plant species. *J. Environ. Qual.,* **28**, 1011-1017 (1999).

Raskin, I., P.B.A.N. Kumar, S. Dushenkov and D.E. Salt: Bioconcentration of heavy metals by plants. *Curr. Opin. Biotechnol.,* **5**, 285-290 (1994).

Raskin, I., R.D. Smith and D.E. Salt: Phytoremediation of metals: Using plants to remove pollutants from the environment. *Curr. Opin. Biotechnol.,* **8**, 221-126 (1997).

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

Reeves, R.D. and A.J.M. Baker: Phytoremediation of toxic metals. Wiley, New York. pp. 193-229 (2000).

Reeves, R.D. and R.R. Brooks: European species of *Thlaspi* L. (Cruciferae) as indicators of nickel and zinc. *J. Geochem. Explor.*, **18**, 275-283 (1983).

Robinson, N.J., J.R. Wilson and J.S. Turner: Expression of the type 2 metallotionein-like gene *MT2* from *Arabidopsis thaliana* in Zn^{2+}-metallothionein deficient *Synechococcus* PCC 7942: Putative role for MT2 in Zn^{2+}-metabolism. *Plant Mol. Biol.*, **30**, 1169-1179 (1996).

Rock, S.A .: Field evaluations of phytotechnologies. *In*: Phytoremediation: Transformation and control of contaminants (*Eds*: S.C. McCutcheon and J.L. Schnoor). Wiley, New York. pp. 905-924 (2003).

Ross, S.M. : Toxic metals in soil-plant systems. Chichester: Wiley, England (1994).

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

Ruiz, O.N., H.S. Hussein, N. Terry and H. Daniell: Phytoremediation of organomercurial compounds via chloroplast genetic engineering. *Plant Physiol.,* **132**, 1344-1352 (2003).

Salt, D.E. and V. Kramer: Mechanism of metal hyperaccumulation in plants. *In*: Phytoremediation of toxic metals: Using plants to clean up the environment (*Eds*: I. Raskin and B.D. Ensley). Wiley, New York. pp 231-246 (2000).

Salt, D.E., M. Blaylock, P.B.A.N. Kumar, V. Dushenkov, B.D. Ensley, I. Chet, and I. Raskin: Phytoremediation: A novel strategy for the removal of toxic metals from the environment using plants. *Biotechnol.*, **13**, 468-475 (1995a).

Salt, D.E., R.C. Prince, I.J. Pickering and I. Raskin: Mechanisms of cadmium mobility and accumulation in Indian mustard. *Plant Physiol.* **109**, 1427-1433 (1995b).

Salt, D.E., R.D. Smith and I. Raskin: Phytoremediation. *Annu. Rev. Plant Physiol. Plant Mol. Biol.,* **49**, 643-668 (1998).

Samuelsen, A.I., R.C. Martin, D.W.S. Mok and C.M. Machteld: Expression of the yeast FRE genes in transgenic tobacco. *Plant Physiol.* **118**, 51-58 (1998).

Scheupp, H., B. Dehn and H. Sticher: Interaktionen zwischen VA-mycorrhizen und schwermetallbelastungen. *Agnew Botanik*, **61**, 85-96 (1987).

Schmoger, M.E., M. Oven and E. Grill: Detoxification of arsenic by phytochelatins in plants. *Plant Physiol.*, **122**, 793-801 (2000).

Schutzendubel, A. and A. Polle: Plant responses to abiotic stresses: Heavy metal induced oxidative stress and protection by mycorrhization. *J. Exp. Botany*, **53(372)**, 1351-1365 (2002).

Schuurink, R.C., S.F. Shartzer, A. Fath and R.L. Jones: Characterization of a calmodulin-binding transporter from the plasma membrane of barley aleurone. *Proc. Natl. Acad. Sci. USA,* **95,** 1944-1949 (1998).

Shetty, K.G., B.A.D. Hetrick, D.A.H. Figge and A.P. Schwab: Effects of mycorrhizae and other soil microbes on revegetation of heavy metal contaminated mine spoil. *Environ. Pollut*., **86**, 181-188 (1994).

Smith, S.E. and D.J. Reed: Mycorrhizal symbiosis. Academic Press, London. p. 589 (1997).

Taiz, L. and E. Zeiger: *Plant Physiology*. Sinauer, Sunderland, MA. pp. 690 (2002).

Terry, N., A.D. Zayed, M.P. de Souza and A.S. Tarun: Selenium in higher plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol*., **51**, 401-432 (2000).

Terry, N., C. Carlson, T.K. Raab and A.M. Zayed: Rates of selenium volatilization among crop species. *J. Environ. Qual*., **21**, 341-344 (1992).

Thomine, S., R. Wang, J.M. Ward, N.M. Crawford and J.I. Schroeder: Cadmium and iron transport by members of a plant metal transporter family in *Arabidopsis* with homology to Nramp genes. *Proc. Natl. Acad. Sci. USA*, **97**, 4991-4996 (2000).

Tu, C. and L.Q. Ma: Effects of arsenic on concentration and distribution of nutrients in the trends of the darsenic hyperaculator *Pteris vittala* L. *Environ. Pollut.,* **135(2),** 333-340 (2005).

Turgut, C., M. Katie Pepe and T.J. Cutright: The effect of EDTA and citric acid on phytoremediation of Cd, Cr and Ni from soil using Helianthus annuus. *Environ Pollut*., **131(1)**, 147-154 (2004).

Vallini, G., S. Di Gregorio and S. Lampis: Rhizosphere induced selenium precipitation for possible applications in phytoremediation of se polluted effluents. *Z. Naturforsch* (C), **60(3-4)**, 349-356 (2005).

Van der Zaal, B.J. , L.W. Neuteboom , J.E. Pinas, A.N. Chardonnens and H. Schat: Overexpression of a novel *Arabidopsis* gene related to putative zinc-transporter genes from animals can lead to enhanced zinc resistance and accumulation. *Plant Physiol.,* **119**, 1047-1055 (1999).

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

Vassil, A.D., Y. Kapulmik, I. Raskin and D.E. Salt: The role of EDTA in lead transport and accumulation by Indian mustard. *Plant Physiol*., **117**, 447-453 (1998).

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

Vogel-Mikus, K., D. Drobne and M. Regvar: Zn, Cd and Pb accumulation and arbuscular mycorrhizal colonisation of pennycress *Thlaspi praecox Wulf*. (Brassicaceae) from the vicinity of a lead mine and smelter in Slovenia. *Environ. Pollut*., **133(2)**, 233-242 (2005).

Volkering, F., A.M. Breure and W.H. Rulkens : Microbiological aspects of surfactant use for biological soil remediation. *Biodegradation,* **8**, 401-417 (1998).

Von Wiren, N., S. Klair, S.Bansal, J.F. Briat, H. Khodr and T. Shiori: Nicotianamine chelates both Fe III and Fe II. Implications for metal transport in plants. *Plant Physiol*., **119**, 1107-1114 (1999).

Weissenhorn, I. and C. Leyval: Root colonization of maize by a Cd-sensitive and a Cd-tolerant *Glomus mossae* and cadmium uptake in sand culture. *Plant Soil,* **175**, 223-238 (1995).

White, P.J., H.C. Bowen, V. Demidchik, C. Nichols and J.M. Davies: Genes for calcium-permeable channels in the plasma membrane of plant root cells. *Biochimicaet Biophysica Acta*, **1564**, 299-309 (2002).

Williams, L.E., J.K. Pittman and J.L. Hall: Emerging mechanisms for heavy metal transport in plants. *Biochim. Biophys Acta*, **1465(1),** 104-126 (2000).

Winter Sydnor, M.E. and E.F. Redente: Reclamation of high elevation, acidic mine waste with organic amendments and topsoil. *J. Environ. Qual.,* **31**, 1528-1537 (2002).

Yanqun, Z., L. Yuan, C. Jianjun, C. Haiyan, Q. Li and C. Schvartz: Hyperaccumulation of Pb, Zn and Cd in herbaceous grown on lead-zinc mining area in Yunnan, China. *Environ. Int.,* **31(5)**, 755-762 (2005).

Zayed, A., E. Pilon Smits, M. deSouza, Z.Q. Lin and N. Terry: Remediation of selenium polluted soils and waters by phytovolatilization. *In*: Phytoremediation of contaminated soil and water (*Eds*: N. Terry and G. Bañuelos). Boca Raton, Lewis. pp. 61-83 (2000).

Zhang, W, Y. Cai, K.R. Downum and L.Q. Ma: Arsenic complexes in the arsenic hyperaccumulator *Pteris vittata*, Chinese brake fern. *J. Chromatogr. A.*, **1043(2)**, 249-254 (2004).

Zhu, Y , E.A.H. Pilon-Smits, L. Jouanin and N Terry: Overexpression of glutathione synthetase in *Brassica juncea* enhances cadmium tolerance and accumulation. *Plant Physiol.,* **119**, 73-79 (1999a).

Zhu, Y., E.A.H. Pilon Smits, A. Tarun, S.U. Weber, L. Jouanin and N. Terry: Cadmium tolerance and accumulation in Indian mustard is enhanced by overexpressing glutamylcysteine synthetase. *Plant Physiol.,* **121**, 1169-1177 (1999b).