

Phytoremediation and hyperaccumulator plants

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Abstract

Phytoremediation is a group of technologies that use plants to reduce, remove, degrade, or immobilize environmental toxins, primarily those of anthropogenic origin, with the aim of restoring area sites to a condition useable for private or public applications. Phytoremediation efforts have largely focused on the use of plants to accelerate degradation of organic contaminants, usually in concert with root rhizosphere microorganisms, or remove hazardous heavy metals from soils or water. Phytoremediation of contaminated sites is a relatively inexpensive and aesthetically pleasing to the public compared to alternate remediation strategies involving excavation/removal or chemical *in situ* stabilization/conversion. Many phytoremediation plans have multi-year timetables, but since most sites in need of remediation have been contaminated for more than ten years, as such a ten year remediation plan does not seem excessive. Seven aspects of phytoremediation are described in this chapter: phytoextraction, phytodegradation, rhizosphere degradation, rhizofiltration, phytostabilization, phytovolatilization, and phytorestoration. Combining technologies offer the greatest potential to efficiently phytoremediate contaminated sites. The major focus of this chapter is phytoextraction of arsenic, cadmium, chromium, copper, mercury, nickel, lead, selenium, and zinc.

1 Introduction to phytoremediation

Phytoremediation is a term applied to a group of technologies that use plants to reduce, remove, degrade, or immobilize environmental toxins, primarily those of anthropogenic origin, with the aim of restoring area sites to a condition useable for private or public applications. To date, phytoremediation efforts have focused on the use of plants to accelerate degradation of organic contaminants, usually in concert with root rhizosphere microorganisms, or remove hazardous heavy metals from soils or water. Phytoremediation of contaminated sites is appealing because it is relatively inexpensive and aesthetically pleasing to the public compared to alternate remediation strategies involving excavation/removal or chemical *in situ* stabilization/conversion. Seven aspects of phytoremediation are described in this chapter: phytoextraction, phytodegradation, rhizosphere degradation, rhizofiltration, phytostabilization, phytovolatilization, and phytorestoration. However, the major focus is on phytoextraction.

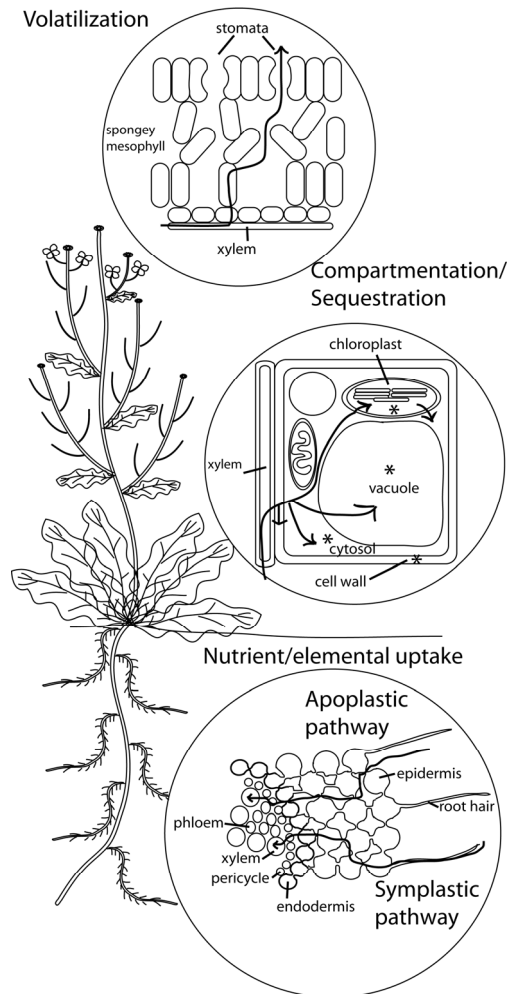


Fig. 1. Pathway of metal/nutrient uptake in plants. Soluble metals can enter into the root symplast by crossing the plasma membrane of the root endodermal cells or they can enter the root apoplast through the space between cells. If the metal is translocated to aerial tissues, then it must enter the xylem. To enter the xylem, solutes must cross the Casparian strip, a waxy coating which is impermeable to solutes, unless they pass through the cells of the endodermis probably through the action of a membrane pump or channel. Once loaded into the xylem, the flow of the xylem sap will transport the metal to the leaves, where it must be loaded into the cells of the leaf, again crossing a membrane. Once in the shoot or leaf tissues, metals can be stored in various cell types, depending on the species and the form of the metal, since it can be converted into less toxic forms (to the plant) through chemical conversion or complexation. The metal can be sequestered in several subcellular compartments (cell wall, cytosol, vacuole) or volatilized through the stomata.

1.1 Phytoextraction

Phytoextraction involves the removal of toxins, especially heavy metals and metalloids, by the roots of the plants with subsequent transport to aerial plant organs (Salt et al. 1998; Lombi et al. 2001a) (Fig. 1). Pollutants accumulated in stems and leaves are harvested with accumulating plants and removed from the site. Phytoextraction can be divided into two categories: continuous and induced (Salt et al. 1998). Continuous phytoextraction requires the use of plants that accumulate particularly high levels of the toxic contaminants throughout their lifetime (hyperaccumulators), while induced phytoextraction approaches enhance toxin accumulation at a single time point by addition of accelerants or chelators to the soil. In the case of heavy metals, chelators like EDTA assist in mobilization and subsequent accumulation of soil contaminants such as lead (Pb), cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), and zinc (Zn) in *Brassica juncea* (Indian mustard) and *Helianthus annuus* (sunflower) (Blaylock et al. 1997; Turgut et al. 2004). The ability of other metal chelators such as CDTA, DTPA, EGTA, EDDHA, and NTA to enhance metal accumulation has also been assessed in various plant species (Huang et al. 1997; Lombi et al. 2001b). However, there may be risks associated with using certain chelators considering the high water solubility of some chelator-toxin complexes which could result in movement of the complexes to deeper soil layers (Wu et al. 1999; Lombi et al. 2001b) and potential ground water and estuarine contamination.

1.2 Phytodegradation

In phytodegradation, organic pollutants are converted by internal or secreted enzymes into compounds with reduced toxicity (Schnoor 1997; Salt et al. 1998; Suresh and Ravishankar 2004). For instance, the major water and soil contaminant trichloroethylene (TCE) was found to be taken up by hybrid poplar trees, *Populus deltoides x nigra*, which break down the contaminant into its metabolic components (Newman et al. 1997). TCE and other chlorinated solvents can be degraded to form carbon dioxide, chloride ion and water (Schnoor et al. 1995). Poplars have also been shown to take up the ammunition wastes 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5 triazine (RDX), octahydro-1,3,5,7-tetranitro-1,3,5,7 tetrazocine (HMX) and partially transform them (Thompson et al. 1998; Yoon et al. 2002). Root exudates from *Datura innoxia* and *Lycopersicon peruvianum* containing peroxidase, laccase, and nitrilase have been shown to degrade soil pollutants (Schnoor et al. 1995; Lucero et al. 1999) and nitroreductase and laccase together can break down TNT, RDX, and HMX (Schnoor et al. 1995). The plants are then able to incorporate the broken ring structures into new plant material or organic soil components that are thought to be non-hazardous.

1.3 Phytovolatilization

Plants can also remove toxic substances, such as organics, from the soil through phytovolatilization. In this process, the soluble contaminants are taken up with water by the roots, transported to the leaves, and volatilized into the atmosphere through the stomata (Tollsten and Muller 1996; Newman et al. 1997; Davis 1998) (Fig. 1). The best example of this is the volatilization of mercury (Hg) by conversion to the elemental form in transgenic *Arabidopsis* and yellow poplars containing bacterial mercuric reductase (*merA*) (Rugh et al. 1996, 1998) (see Section 3.5). In a study where the movement of volatile organics was monitored by Fourier transform infrared spectrometry (FT-IR) in hybrid poplars (*Populus deltoides x nigra*), *Tamarix parviflora* (saltcedar), and *Medicago sativa* (alfalfa), chlorinated hydrocarbons were found to move readily through the plants, but less polar compounds like gasoline constituents did not (Davis et al. 1998). However, amounts of the contaminant transpired are in proportion to water flow and are relatively low, especially in the field. Rubin and Ramaswami (2001) found that poplar saplings can concentrate (100 ppb) and transpire methyl tertiary-butyl ether (MTBE), a compound added to gasoline which is commonly found as a groundwater pollutant. In a one week time period, they observed a 30% reduction in MTBE mass in hydroponic solution by saplings at both high (1600 ppb) and low (300 ppb) MTBE concentrations, which suggested that these plants could be successful in the phytoremediation of this toxin from groundwater (Rubin and Ramaswami 2001). Selenium (Se) is a special case of a metal that is taken up by plants and volatilized (see Section 3.8). Se can also be volatilized following conversion to dimethylselenide by microbes and algae (Neumann et al. 2003) (see Chapter 13).

1.4 Rhizosphere degradation

Like phytodegradation, rhizosphere degradation involves the enzymatic breakdown of organic pollutants, but through microbial enzymatic activity. These breakdown products are either volatilized or incorporated into the microorganisms and soil matrix of the rhizosphere. The types of plants growing in the contaminated area influence the amount, diversity, and activity of microbial populations (Jones et al. 2004; Kirk et al. 2005). Grasses with high root density, legumes that fix nitrogen, and alfalfa that fix nitrogen and have high evapotranspiration rates are associated with different microbial populations. These plants create a more aerobic environment in the soil that stimulates microbial activity that enhances oxidation of organic chemical residues (Anderson 1993; Schnoor et al. 1995; Narayanan 1998; Jones et al. 2004; Kirk et al. 2005). Secondary metabolites and other components of root exudates also stimulate microbial activity, a byproduct of which may be degradation of organic pollutants (Pieper et al. 2004).

1.5 Rhizofiltration

Rhizofiltration removes contaminants from water and aqueous waste streams, such as agricultural run off, industrial discharges, and nuclear material processing wastes (Salt et al. 1998; Suresh and Ravishankar 2004). Absorption and adsorption by plant roots play a key role in this technique, and consequently large root surface areas are usually required. In research associated with Epcot Center, closed systems with recirculating nutrients have exhibited the benefits of rhizofiltration and biofiltration using a variety of species (such as mosses and scented geraniums) (Negri and Hinchman 1996). Rhizofiltration was also shown to be useful in the San Francisco Bay study directed by Norman Terry (University of California, Berkeley) and supported by Chevron (Hansen et al. 1998). A wetland constructed next to the bay was shown to remove 89% of the Se from selenite-contaminated wastewater released from various oil refineries. The water flowing into the wetland was measured to have 20–30 $\mu\text{g L}^{-1}$ selenite, while the outflow from the wetland had less than 5 $\mu\text{g L}^{-1}$ selenite (Hansen et al. 1998). In a study of Se removal from agricultural subsoil drainage in the San Joaquin Valley (Gao et al. 2003), a flow-through wetland system was constructed with cells containing either a single species, or a combination of species [e.g. *Schoenoplectus robustus* (sturdy bulrush), *Juncus balticus* (baltic rush), *Spartina alterniflora* (smooth cordgrass), *Polypogon monspeliensis* (rabbit's foot grass), *Distichlis spicata* (saltgrass), *Typha latifolia* (cattail), *Schoenoplectus acutus* (Tule grass), and *Ruppia maritima* (widgeon grass)]. Four years after planting, comprehensive analysis showed that 59% of the Se remained in the wetland, mostly in the organic detrital layer and surface sediment, 35% in the outflow, 4% in seepage and 2% to volatilization. Wetland plant uptake of Se varies with species type, and parrot's feather (*Myriophyllum aquaticum*), iris-leaved rush (*Juncus xiphioides*), cattail, and sturdy bulrush were particularly noted for high Se uptake potential (Gao et al. 2003).

1.6 Phytostabilization

Erosion and leaching can mobilize soil contaminants resulting in aerial or waterborne pollution of additional sites. In phytostabilization, accumulation by plant roots or precipitation in the soil by root exudates immobilizes and reduces the availability of soil contaminants. Plants growing on polluted sites also stabilize the soil and can serve as a groundcover thereby reducing wind and water erosion and direct contact of the contaminants with animals. Significant phytostabilization projects have been employed in France and the Netherlands (Ernst et al. 1996; Bouwman et al. 2001; Marseille et al. 2000). A 2005-2010 superfund basic research program (Maier 2004) is developing a phytostabilization revegetation strategy to remediate mine tailings in arid and semi-arid ecosystems. The researchers will monitor the bioavailability of metals for the native metal- and drought-tolerant plant species used, and determine the permanence of expected toxicity reductions. Plants with high transpiration rates, such as grasses, sedges, forage

Table 1. Summary of elemental levels in background soil, metalliferous (or contaminated) soil, critical load in soil above which biodiversity and ecosystem function are adversely affected, and Commission des Communautés Européennes (EU) and Environmental Protection Agency (USA) permissible limits.

Element	Background soil levels (ppm) ^a	Metalliferous soil levels (ppm) ^c	Critical load in loam/silt (ppm)	CCE limits (ppm) ^h	EPA limits ^{k,i}
As	2.2 – 25	1 510	–	–	0.01 ppm
Cd	0.06 – 1.1	317	1.10	0.5 ⁱ	5 ppb
Cr	7 – 221	3 450 ^d	64.41	1.5 ⁱ	100 ppb
Cu	6 – 80	3 783	48.78	50 ^j	1.3 ppm
Hg	0.02 – 0.41	12 000 ^e	0.56	1.5	2 ppb
Ni	4 – 55	11 260	54.64	1 ⁱ	0.7 ppm
Pb	10 – 84	49 910	75.68	5 ⁱ	1.5 ppb
Se	0.01 – 0.09 ^b	>50 ^f	–	–	0.05 ppm
Zn	17 – 125	7 480	207.32	150 ^j	5 ppm

^a<http://www.sandia.gov>

^bLakin 1972

^cReeves and Baker 2000

^d<http://www.dtsc.ca.gov>

^e<http://www.deq.state.or.us>

^f<http://www.nwo.usace.army.mil>

^gBannick et al. 2002; Sand ~50% of loam/silt levels; clay ~1.5X loam/silt levels

^h<http://europa.eu.int>

ⁱH₂O

^jSoil

^k<http://www.epa.gov>

plants, and reeds are useful for phytostabilization by decreasing the amount of ground water migrating away from the site carrying contaminants (Suresh and Ravishankar 2004). Combining these plants with hardy, perennial, dense rooted or deep rooting trees (poplar, cottonwoods) can be an effective combination (Berti and Cuningham 2000).

1.7 Phytorestoration

Phytorestoration involves the complete remediation of contaminated soils to fully functioning soils (Bradshaw 1997). In particular, this subdivision of phytoremediation uses plants that are native to the particular area, in an attempt to return the land to its natural state. An examination of phytorestoration compared to the other forms of phytoremediation brings to light an important issue: what degree of decontamination do phytoremediation projects aim to achieve? There is a vast difference between removing just enough soil pollutants to reach legally defined levels of compliance, remediating soils to a level at which they can be used again, and completely restoring land from its contaminated state to an environmentally

uncontaminated state (Table 1). The objective of many phytoremediation projects is to restore the land to a legally acceptable level of contamination.

Lastly, a combination of phytoremediation approaches can be used for more effective environmental restoration. This may help to simultaneously remove different types of wastes from the same site. For example, a remediation system could include plants that hyperaccumulate toxic metals and plants that stimulate the activity of microbes that specialize in organic contaminant degradation.

2 Definitions of tolerant, indicator, and hyperaccumulator species

When categorizing plants that can grow in the presence of toxic elements, the terms “tolerant,” “indicator”, and “hyperaccumulator” are used. A tolerant species is one that can grow on soil with concentrations of a particular element that are toxic to most other plants. While both indicator species and hyperaccumulators are also tolerant, studies have shown the genetic distinction of the mechanisms involved (Assunção et al. 2001; Bert et al. 2003; Macnair et al. 1999). However, tolerant species are not necessarily indicators or hyperaccumulators, as tolerant non-accumulators can exclude metals from entering the root tissue. Examples of tolerant excluders include *Holcus lanatus* (Meharg and Macnair 1992a, 1992b), *Agrostis capillaris*, *Mimulus guttatus*, and *Silene vulgaris* (Pollard et al. 2002).

Indicator plants have been employed in biogeochemical prospecting. As early as 1865, F. Risse observed Zn accumulating plants, now known as *Thlaspi caerulescens*, growing near the German-Belgium border (Sachs 1865). This observation led others to associate the sites where these plants grew with soil containing elevated Zn. In the 1950s and 1960s, Helen Cannon and other members of the United States Geological Society cataloged indicator plants that were potentially important for “bioprospecting” for ore (Cannon 1960). Examples include mosses, which have been recognized as bioindicators of high metal concentrations in water (Gstoettner and Fisher 1995) and *Stanleya pinnata* (Prince’s Plume), which is a recognized Se indicator.

Hyperaccumulators take up particularly high amounts of a toxic substance, usually a metal or metalloid, in their shoots during normal growth and reproduction (Reeves 1992; Baker and Whiting 2002). Hyperaccumulation reported in senescing plants generally represents a breakdown of homeostatic mechanisms and is clearly not a function of normal growth processes, although such accumulations could be technologically useful. The metal/metalloid concentration that must be accumulated by the plant before it is designated a “hyperaccumulator” depends upon the particular metal or metalloid in question. In early hyperaccumulator studies, Brooks and coworkers (1977) defined nickel (Ni) hyperaccumulators as those accumulating greater than $1000 \mu\text{g Ni g}^{-1}$ dry weight in their leaves. Subsequently, Baker and Brooks (1989) defined threshold concentrations for other metals hyperaccumulated in plants as $100 \mu\text{g g}^{-1}$ dry weight for Cd, $1,000 \mu\text{g g}^{-1}$ dry weight for Ni, Cu, Co, Pb, and $10,000 \mu\text{g g}^{-1}$ dry weight for Zn and Mn. The defined levels of

these elements are typically at a concentration of one order of magnitude greater than those found in non-accumulator species (Salt and Kramer 2000). Hyperaccumulators are found in 45 different families, with the highest occurrence among the Brassicaceae (Reeves and Baker 2000). These plants are quite varied, from perennial shrubs and trees to small annual herbs.

While tolerance is necessary for accumulation, evidence suggests that tolerance and accumulation are independent traits. There is a strong positive correlation between glutathione (GSH), cysteine (Cys), O-acetyl-L-serine (OAS) levels, compounds associated with tolerance, and Ni accumulation in shoot tissues of *Thlaspi* species from serpentine soils (Freeman et al. 2004). *Thlaspi goesingense* has constitutively high levels of serine acetyltransferase (SAT) and glutathione reductase activity associated with resistance to Ni-induced oxidative stress in this plant (Freeman et al. 2004). Elevated levels of reduced GSH contribute to a decrease in Ni-induced lipid peroxidation in *T. goesingense* shoots and lower levels of Ni-induced reactive oxygen species in roots. Salicylic acid (SA) was observed to both regulate glutathione accumulation through post-translational activation of SAT, and to increase glutathione reductase activity in *A. thaliana* (Freeman et al. 2005). Changes in the levels of these compounds and enzymes appear to be associated with Ni tolerance required for hyperaccumulation (For additional details about Ni tolerance in plants, see Chapter 9; for additional details about heavy metal chelation, see Chapter 10).

Overexpression of *T. goesingense* mitochondrial SAT (TgSAT-m) in *A. thaliana* was found to confer increased Ni tolerance compared to controls (Freeman et al. 2004). These overexpressors also showed increased OAS, Cys and GSH biosynthesis (Freeman et al. 2004). However, *A. thaliana* SAT overexpressors and controls had the same shoot Ni content indicating that the SAT overexpressors neither hyperaccumulated nor excluded Ni, and chemical speciation showed that the Ni was not bound to thiols in these plants (Freeman et al. 2004). It is noteworthy that this demonstration of Ni tolerance without accumulation represents an example of the distinction between metal tolerance mechanisms and metal hyperaccumulation mechanisms and supports previous findings showing partial independent genetic control of hyperaccumulation and tolerance (Assunção et al. 2001; Bert et al. 2003; Macnair et al. 1999).

2.1 How do plants take up and transport metal?

The process of metal accumulation involves several steps, outlined in Fig. 1, one or more of which are enhanced in hyperaccumulators.

2.1.1 Solubilization of the metal from the soil matrix

Many metals are found in soil-insoluble forms. Plants use two methods to desorb metals from the soil matrix: acidification of the rhizosphere through the action of plasma membrane proton pumps and secretion of ligands capable of chelating the metal. Plants have evolved these processes to liberate essential metals from the

soil, but soils with high concentrations of toxic metals will release both essential and toxic metals to solution. To our knowledge, there are no reports of plants with the ability to solubilize Pb from the soil matrix, where most of soil Pb exists in an insoluble form (Blaylock and Huang 2000). Experiments demonstrating Pb hyperaccumulation have used $\text{Pb}(\text{NO}_3)_2$, a soluble form of Pb, though it must be questioned whether this is the most appropriate form of Pb for analysis. Aside from Pb, the solubilization mechanisms for hyperaccumulators are similar for metals discussed, and therefore will not be addressed independently for each metal. While no hyperaccumulators have evolved to handle high concentrations of toxic metals if they are present in solution, phytoremediator plants could be modified to solubilize contaminants that are bound to the soil.

2.1.2 Uptake into the root

Soluble metals can enter into the root symplast by crossing the plasma membrane of the root endodermal cells or they can enter the root apoplast through the space between cells (Fig. 1). While it is possible for solutes to travel up through the plant by apoplastic flow, the more efficient method of moving up the plant is through the vasculature of the plant, called the xylem. To enter the xylem, solutes must cross the Casparian strip, a waxy coating, which is impermeable to solutes, unless they pass through the cells of the endodermis (Fig. 1). Therefore, to enter the xylem, metals must cross a membrane, probably through the action of a membrane pump or channel. Most toxic metals are thought to cross these membranes through pumps and channels intended to transport essential elements. Excluder plants survive by enhancing specificity for the essential element or pumping the toxic metal back out of the plant (Hall 2002; Meharg and Macnair 1992a, 1992b).

2.1.3 Transport to the leaves

Once loaded into the xylem, the flow of the xylem sap will transport the metal to the leaves, where it must be loaded into the cells of the leaf, again crossing a membrane (Fig. 1). The cell types where the metals are deposited vary between hyperaccumulator species. For example, *T. caerulea* was found to have more Zn in its epidermis than in its mesophyll (Kupper et al. 1999), while *A. halleri* preferentially accumulates its Zn in its mesophyll cells instead of its epidermal cells (Kupper et al. 2000).

2.1.4 Detoxification/Chelation

At any point along the pathway, the metal could be converted to a less toxic form through chemical conversion or by complexation. Various oxidation states of toxic elements have very different uptake, transport, sequestration or toxicity characteristics in plants. Chelation of toxins by endogenous plant compounds can have similar effects on all of these properties as well. As many chelators use thiol groups as ligands, the sulfur (S) biosynthetic pathways have been shown to be

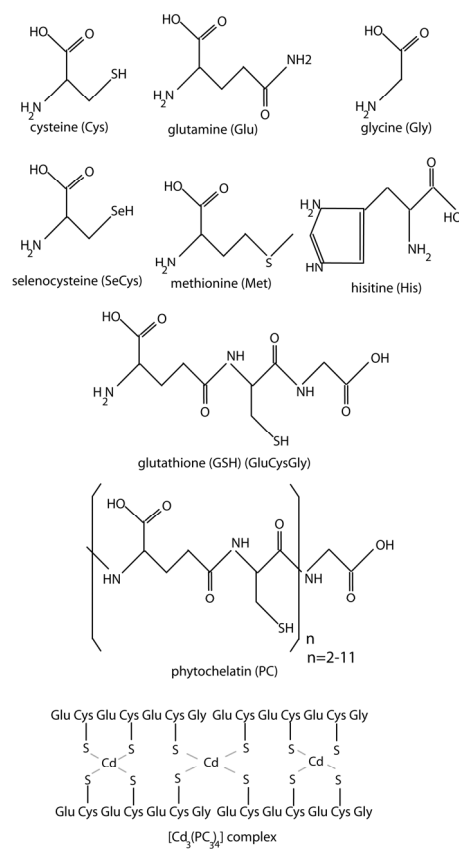


Fig. 2. Structures of some compounds that can bind metals: amino acids, glutathione, and phytochelatin.

Table 2. Summary of elements and the element-organic complexes that may be formed within plants.

Element	Analogue	Organic ligand
Arsenic	Phosphate	Phytochelatin, thiol, glutathione, ADP-As, ascorbic acid
Cadmium	Zn, Fe	Phytochelatin, glutathione, γ -glutamylcysteine, thiols
Chromium	Mn	Thiols
Copper	Cu	Citrate, metallothioneins, phytochelatin 2, phytochelatin 3
Mercury	Unknown ^a	Thiols
Nickel	Fe	Nicotianamine, histidine, thiols, citrate
Lead	Zn, Fe	Glutathione
Selenium	S	Cystiene, methionine, with and without methylation
Zinc	Zn	Phytochelatin, glutathione, γ -glutamylcysteine, thiols, citrate, malate

^aEnters cell through passive diffusion.

critical for hyperaccumulator function (Ng and Anderson 1979; Pickering et al. 2003; Van Huysen et al. 2004) and for possible phytoremediation strategies (Figs. 2 and 3; Table 2). Oxidative stress is one of the most common effects of heavy metal accumulation in plants, and the increased anti-oxidant capabilities of hyperaccumulators allow tolerance of higher concentrations of metals (Freeman et al. 2004).

2.1.5 Sequestration/Volatilization

The final step for the accumulation of most metals is the sequestration of the metal away from any cellular processes it might disrupt. Sequestration usually occurs in the plant vacuole, where the metal/metal-ligand must be transported across the vacuolar membrane. Metals may also remain in the cell wall instead of crossing the plasma membrane into the cell, as the negative charge sites on the cell walls may interact with polyvalent cations (Wang and Evangelou 1994). Selenium may also be volatilized through the stomata.

2.2 Strategies for phytoremediation using hyperaccumulators

The effectiveness of a phytoremediation plan is dependent on the selection of the appropriate plant or plants. Plants native to the target area should be considered since they are adapted to the local climate, insects, and diseases. Any plant used as a phytoremediator must be able to tolerate high concentrations of the toxic substance of interest, in addition to any other pollutants found at the particular site, as candidate sites for phytoremediation usually have multiple contaminants. In the United States, more than 80% of the metal contaminated Superfund, Department of Defense, and Department of Energy sites are also contaminated by organic pollutants (Ensley 2000).

Plants used for phytoextraction should develop a large amount of biomass quickly and be easy to cultivate and harvest, preferably multiple times per year (Newman 1997; Tong et al. 2004). For example, Mcgrath and Zhao (2003) have calculated that a plant with a bioconcentration factor of 40 can halve the concentration of metal in the top 20 cm of soil in 10 crops if it produces 5 tonnes ha⁻¹ crop⁻¹, but a plant with a bioconcentration factor of 20 must produce at least 10 tonnes ha⁻¹ crop⁻¹ to have the same effect. Since most metal hyperaccumulators are small plants with low biomass, efforts are being made to locate new hyperaccumulators, selectively breed for promising plant traits, and create transgenic phytoremediators. For example, hyperaccumulation of arsenic in the fern *Pteris vittata* was recently discovered (Ma et al. 2001). Plant breeding approaches used to increase crop plant biomass and improved nutrient compositions can be potentially used to increase hyperaccumulator biomass.

Numerous research groups have emphasized transgenic solutions to obtaining appropriate plants for phytoremediation. The general strategy underlying this approach is to introduce genes conferring the ability to tolerate and hyperaccumulate

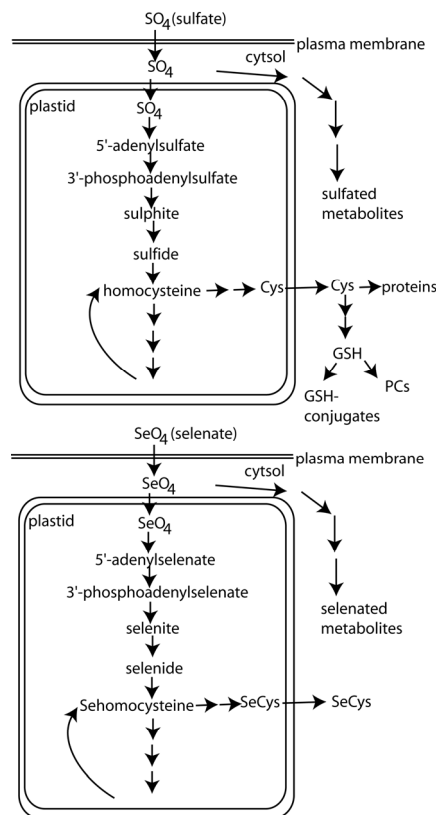


Fig. 3. Sulfur and selenium assimilation pathways in plants

toxic metals into larger plants capable of rapid development (reviewed in Salt et al. 1998; Clemens et al. 2002). Ideally, the genes could be introduced into plants which are accustomed to growing in the climate where they will be used. Plants could also be engineered to hyperaccumulate multiple different metals for sites with numerous contaminants. Existing hyperaccumulators could be engineered with increased biomass and metal storage capacity. Many studies have begun to elucidate the molecular mechanisms underlying metal hyperaccumulation and tolerance in plants (Ellis et al. 2004; Freeman et al. 2004; Pence et al. 2000). While it is possible that these discoveries mark the approaching widespread implementation of transgenic hyperaccumulators at phytoremediation sites, there is still much controversy surrounding the agricultural use of genetically modified organisms. Opposition to the deployment of transgenic plants in the field, especially in the promiscuous cruciferous species most closely related to the majority of hyperaccumulators, is potentially strong enough to prevent this type of phytoremediation technique from being widely used.

If it were possible to deploy genetically engineered phytoremediators, several issues must be considered before transgenes are introduced into the plants. Tissue-specific gene promoters that optimize transgene expression in tissues of interest must be tested for efficacy. The introduction of expression microarray techniques (Atgenex; Birnbaum et al. 2003) and activation tagged promoter lines (Alvarado et al. 2003) to profile gene expression in different tissues should accelerate this process. If transgenes from non-plant organisms were to be introduced, the proteins encoded by these transgenes must often be engineered to include signal sequences to correctly target the new proteins to the appropriate cellular compartment. Bizily et al. (2003) and Pilon et al. (2003) have recently shown that proper targeting of transgenically expressed proteins in phytoremediator plants can increase efficiency and remediation capacity.

3 Common elemental contaminants

Elements naturally occur in the earth's crust in a range of background levels that are generally below the critical load, i.e., the amount of the element above which there is a negative effect on biodiversity and ecosystem function (Table 1). However, the concentrations of elements in localized, naturally occurring metalliferous soils or in depositions from anthropogenic activity (e.g. mining, waste disposal, etc.) are considerably higher (Table 1). In the following section, plant mechanisms of tolerance and/or hyperaccumulation of common elemental contaminants are discussed.

3.1 Arsenic (As)

As is a naturally occurring metalloid, which has been used in pesticides and wood preservatives, leading to As contaminated sites (Meharg and Hartley-Whitaker 2002). For example, a Canberra, Australia suburb has As contamination from a pesticide spill (Ng et al. 1998), and localized soil contaminations resulting from use of As in pressure-treated lumber have been widely reported. In the alluvial planes of Bangladesh and West Bengal, India, As contamination of ground water from microbial degradation of peat has resulted in widespread well contamination and health risk. Irrigation has dispersed As contamination to surrounding soils, resulting in As poisoning of humans and other animals (McArthur et al. 2001). Similar contamination is seen in regions with As in subsoils worldwide.

Arsenite [AsO_2^- or As(III)] and arsenate [AsO_4^{3-} or As(V)] are the dominant inorganic arsenic moieties found in terrestrial plants. Both forms are phytotoxic, although via different mechanisms. Arsenate, the predominant form found in aerobic soils, is a phosphate analog. Formation of ADP-As complexes instead of ATP leads to cell death; arsenite can cause cell death by binding to and inhibiting enzymes with sulfhydryl groups. Arsenate is often designated as the more phytotoxic of the two arsenic species (Quaghebeur and Rengel 2003; Wang et al. 2002) but

the relative toxicities are species-specific (Wang et al. 2002a). As tolerant clones of the grass *H. lanatus* have a smaller proportion of their total As as arsenite compared to non-tolerant clones (Quaghebeur and Rengel 2003), while the As tolerant fern *Pteris vittata* (Chinese brake fern) almost exclusively accumulates arsenite in its fronds (Webb et al. 2003). While non-accumulators have a phytotoxic threshold at approximately 5-100 mg kg⁻¹ As dry weight, *H. lanatus* can accumulate up to 560 mg kg⁻¹ As (Porter 1975), and *P. vittata* can accumulate up to 27,000 mg kg⁻¹ As dry weight, with phytotoxic symptoms appearing around 10,000 mg kg⁻¹ As dry weight (Gumaelius et al. 2004). *Pteris cretica*, *Pteris longifolia*, and *Pteris umbrosa* are fern species that also hyperaccumulate As.

3.1.1 Uptake into the root

Pteris vittata accumulates As in contaminated and non-contaminated soils suggesting that hyperaccumulation is a constitutive trait (Wang et al. 2002). Arsenic accumulation is correlated with the phosphorus concentration of the media surrounding *P. vittata*. Wang et al. (2002) found that phosphate starvation resulted in a 2.5 fold increase in As net uptake, while the presence of phosphate in the media decreased arsenate influx. This increase in P/As uptake under starvation conditions is mediated by an increase in phosphate transporter gene expression and a consequent increase in the amount of protein (Liu et al. 1998; Muchhal and Raghobhama 1999; Poynton et al. 2004) (For an in depth description of metal-dependent transcriptional regulation, see Chapter 12). Arsenite does not compete with phosphate for uptake into *P. vittata* roots, suggesting that there is another mechanism for arsenite uptake (Wang et al. 2002).

3.1.2 Transport to the leaves

In *Pteris vittata*, arsenite is more efficiently translocated from roots to fronds than arsenate (Wang et al. 2002). In *B. juncea*, addition of the dithiol As chelator dimercaptosuccinate to nutrient solution increased arsenite transport from roots to shoots (Pickering et al. 2000), but it is not yet clear whether arsenite is complexed before xylem loading and transport in *P. vittata* (Wang et al. 2002). Consistent with arsenate competition for phosphate transport sites, high phosphorus levels were shown to result in significantly decreased As concentrations in *P. vittata* fronds (Wang et al. 2002).

3.1.3 Detoxification/Chelation

A detoxification pathway for arsenate (AsO₄⁻³) by conversion to arsenite (AsO₂⁻) upon its uptake into roots has been proposed (Meharg and Hartley-Whitaker 2002). Arsenate can be reduced to arsenite enzymatically by arsenate reductase as shown *in vitro* (Delnomdedieu et al. 1994) and non-enzymatically by glutathione (GSH) or ascorbic acid as in yeast (Mukhopadhyay et al. 2000) followed by the formation of an arsenite-thiol (AsO₂⁻-SH) complex. Phytochelatins (PCs) have also been proposed as As chelators in *H. lanatus* (Raab et al. 2004), and it has

been suggested that arsenite-PC complexes are stored in the vacuole (Meharg and Hartley-Whitaker 2002). It does not appear that arsenite-PC complexes are the dominant form of arsenite in *P. vittata* as neither PC nor total S are present in sufficient quantities in *P. vittata* for the expected As:thiol ratio which is found in populations of As tolerant non-accumulators (Zhao et al. 2002, 2003). Therefore, if PCs are important for *Pteris* hyperaccumulation, they may function as a cytoplasmic shuttle and not a storage complex (Raab et al. 2004). Furthermore, X-ray spectroscopy showed that, at most, 20% of the As in the fronds is coordinated to S suggesting that most of the As stored in the vacuole is aqueous, but uncomplexed with thiols (Lombi et al. 2002b).

3.1.4 Sequestration

Arsenic species are thought to be sequestered in extra- or sub-cellular compartments in *P. vittata* to prevent interaction between As species and cellular components. X-ray spectroscopy detected the majority of As intracellularly in the frond epidermal cells, probably in the vacuole (Lombi et al. 2002b). Arsenite-PC complexes in *H. lanatus* are most likely vacuolar (Meharg and Hartley-Whitaker 2002; Quaghebeur et al. 2003).

3.1.5 Phytoremediation of As

Various studies propose the use of *P. vittata* for phytoremediation of soil and water. Field experiments have indicated that *P. vittata* could remediate contaminated soil sites in 10 years or less (Salido et al. 2003) and can reduce arsenic levels in water to less than $10 \mu\text{g L}^{-1}$ (ppb) (Blaylock et al. 2001). In Albuquerque, New Mexico, a study was conducted where the ferns significantly decreased the level of arsenic in samples of the city's drinking water. While a study of the *Pteris* species could be beneficial in lending insight into hyperaccumulation mechanisms, using these plants for phytoremediation is not suggested. This is due to aforementioned evidence that the plants convert arsenate to arsenite. Although this conversion could make the arsenic less harmful for the plants, it is more harmful to animals and other organisms that might be exposed to the arsenite through plant contact. The ideal phytoremediator would accumulate arsenic at levels similar to *P. vittata*, but store it in a less toxic form. Arsenobetaine and arsenocholine, which are the major forms of arsenic found in fish (Lopez 2004), have low toxicity to humans and are readily excreted in urine. Transgenics with the ability to convert the inorganic forms of arsenic to these or similar compounds could be viable phytoremediators.

Dhankar et al. (2002) were able to greatly increase the arsenic tolerance and accumulation of *Arabidopsis* with only two genes. Constitutive overexpression of γ -glutamylcysteine synthetase (γ -ECS) from the glutathione biosynthesis pathway coupled with the leaf specific expression of arsenate reductase (*arsC*) from *E. coli* increased the fresh weight of arsenate challenged plants by ~5-fold and the shoot accumulation ~3-fold. While these significant improvements were not enough to make *Arabidopsis* into a viable phytoremediator, this shows promise for adding

arsenic tolerance and extraction capabilities to other hyperaccumulator species (Dhankher et al. 2002).

3.2 Cadmium (Cd)

Cd is a toxic metal and probable carcinogen associated with Zn mining and industrial operations where Cd has been used to prevent corrosion of machinery. Resulting air-borne Cd dust presents a significant health hazard. Ecotypes of *T. caerulescens* accumulate a wide range of Cd levels. The Ganges and Vivez ecotypes can accumulate up to 10,000 mg kg⁻¹ Cd dry weight and 12,500 mg kg⁻¹ Cd dry weight, respectively, without showing signs of toxicity; however, the Puy de Wolf and Prayon ecotypes only accumulate 2,300 mg kg⁻¹ Cd dry weight and 4,800 mg kg⁻¹ Cd dry weight, respectively (Lombi et al. 2000, 2001a, 2001b; Peer et al. 2003). Hyperaccumulation of Cd in *Arabidopsis halleri* has also been reported (Cosio et al. 2004; Kupper et al. 2000). However, reports of hyperaccumulation of Cd in *B. juncea* are questionable, although some Cd accumulation in this species is evident (Salt et al. 1997).

3.2.1 Uptake into the root

Cd uptake is likely mediated through transporters or channels for other divalent ions (Cosio et al. 2004). Several of the Zn and Fe transporting ZIP genes in plants have been shown to transport Cd, although with a wide range of affinities (Grotz et al. 1998; Pence et al. 2000; Ramesh et al. 2003; Vert et al. 2001). Excess divalent cations in the media, such as Zn, can reduce Cd uptake in many plant species, including *T. caerulescens* Prayon (Lombi et al. 2002a, 2001a). Significantly, divalent cations and Ca channel blockers had no effect on the Cd uptake of *T. caerulescens* Ganges, suggesting that this ecotype may have developed a novel Cd uptake system (Lombi et al. 2001a, 2002a).

3.2.2 Transport to the leaves

Piñeros and Kochian (2003) demonstrated that *T. caerulescens* and *T. arvense* mesophyll cells exhibit different plasma membrane ion transport properties, but the differences cannot be directly linked to the differences in Zn and Cd accumulation. Analysis of Cd/Zn transport capacity in leaf mesophyll protoplasts demonstrated that the constitutive transport capacity and affinity for these metals were indistinguishable in *T. caerulescens* Ganges, *A. halleri*, and *T. caerulescens* Prayon; however, Cd accumulation increased in Ganges protoplasts but decreased in *A. halleri* protoplasts in conjunction with Cd pre-exposure (Cosio et al. 2004). Therefore, there may be multiple Cd transport systems in leaves. This suggests that in addition to its novel Cd root uptake pathway, Ganges has developed mechanisms in leaves to facilitate hyperaccumulation.

3.2.3 Detoxification/Chelation

Upon Cd exposure, *Nicotiana tabaccum* hairy roots had 5 times more reactive oxygen species (ROS) than *T. caerulescens* hairy roots (Boominathan and Doran 2003a). As GSH has been shown to act as an antioxidant in other species, it was hypothesized that increased GSH synthesis might account for increased tolerance in *T. caerulescens*. However, exposure to the GSH synthesis inhibitor buthionine sulfoximine (BSO) did not significantly affect ROS levels in *T. caerulescens* compared to controls, suggesting that GSH was not required for Cd tolerance in *T. caerulescens* (Boominathan and Doran 2003). *Thlaspi caerulescens* has constitutively high levels of antioxidant enzyme activity like catalase, 300-fold higher than *N. tabaccum* (Boominathan and Doran 2003), therefore, this may contribute to Cd tolerance. While Cd treatment does not induce phytochelatin (PC) synthesis in non-tolerant plants like *A. thaliana*, most metal tolerant plants do not accumulate phytochelatin-metal complexes in response to metal toxicity (Cobbett and Goldsbrough 2002). Although *T. caerulescens* and *T. arvense* had increased PCs following Cd treatment, total PCs were lower in the hyperaccumulator *T. caerulescens*, and PC levels did not correlate with increased tolerance in this plant (Ebbs et al. 2002) (For a detailed discussion of phytochelatin, see Chapter 10).

3.2.4 Sequestration

Few studies have addressed the sequestration of Cd. Vázquez et al. (1992) found Cd in the apoplast and vacuoles of *T. caerulescens*, and most Cd in *T. caerulescens* hairy roots appears to be localized in the cell walls (Boominathan and Doran 2003). More recently, Cosio et al. (2005) demonstrated that ~35% Cd taken up accumulates in the cell wall/apoplast in *T. caerulescens* leaves.

3.2.5 Phytoremediation of Cd

Pilot studies of Cd and Zn phytoremediation have been attempted with contaminated soils collected from a Zn smelter site in Palmerton, PA (Brown et al. 1994), a Zn smelter in France, sewer sludge contaminated agricultural soil from the UK (Lombi et al. 2001b), and a site contaminated by mine tailings in Silver Bow Creek, MT (Ebbs et al. 1997). In each case, *T. caerulescens* removed Cd and Zn from the soils, but at rates that would require more than 15 years to remove most of the metals, and only from a narrow soil horizon. In the UK, agricultural soil, Cu toxicity limited the growth of *T. caerulescens*, demonstrating the need for phytoremediator plants that can tolerate toxic concentrations of multiple pollutants (Lombi et al. 2001b). The remediation potential of *T. caerulescens* is also limited by its small stature and biomass. In one study, even though *T. caerulescens* accumulated the highest concentrations of Cd and Zn in its leaves, *B. juncea* removed more Zn and equivalent amounts Cd due to its larger size (Ebbs et al. 1997). Transgenic approaches to either make *T. caerulescens* grow larger or to make *B. juncea* accumulate more Cd and Zn could make Cd phytoextraction feasible.

Brassica juncea plants genetically modified with bacterial genes to overproduce γ -glutamylcysteine synthetase (ECS) or glutathione synthetase (GS) were found to accumulate 1.5 times more Cd and Zn compared to wild type *B. juncea* growing on metal-contaminated soil from a USEPA Superfund site near Leadville, CO (Bennett et al. 2003). Both ECS and GS overexpressors were able to remove up to 25% of the soil Cd, and Bennet et al. (2003) predict that such transgenic plants should be 1.5 to 3 fold more efficient in phytoextraction than wild type plants.

Zhu et al. (1999) overexpressed the *E. coli* gene *gshI* (with a chloroplast targeting sequence) in *B. juncea*, which resulted in five times the ECS activity in the transgenic plants compared to wild type. Transgenic seedlings also had increased Cd tolerance, PCs, γ -glutamylcysteine (γ -GluCys), GSH, total non-protein thiols, and Cd accumulation (40-90%). These results indicate that ECS is important in Cd accumulation and tolerance and that overexpressing ECS could potentially be an effective phytoremediation strategy (Zhu et al. 1999).

Lee et al. (2003) overexpressed an *Arabidopsis* PC synthase (*AtPCS1*) in *Arabidopsis* resulting in 1.3 to 2.1-fold increase PCs; however, the transgenic lines were hypersensitive to Cd stress as measured by root growth and this hypersensitivity could be alleviated by the addition of glutathione. This suggests that the regulation of glutathione levels and perhaps the entire S assimilation pathway is important for Cd tolerance and accumulation. In a contrasting study, PC-deficient *Arabidopsis* (*cad1-3*) were transformed with wheat *TaPCS1* cDNA (Gong et al. 2003). While the Cd sensitivity of the transgenic *cad1-3* plants was complemented by *TaPCS1* expression, these *TaPCS1* expressing plants accumulated less Cd than the *cad1-3* mutants but increased Cd transport to leaves (Gong et al. 2003). Finally, Song et al. (2003) report that expression of the yeast vacuolar glutathione-Cd transporter YCF1 in *Arabidopsis* increased biomass and Cd uptake was ~2-fold greater than wild type. In addition, preliminary data suggest that expressing YCF1 in poplar also increases biomass (Song et al. 2003). Thus far, transgenic plants do not have sufficiently high levels of accumulation needed for significant Cd phytoremediation; however, a combined approach could result in an effective Cd phytoremediation technology.

3.3 Chromium (Cr)

Cr(III) is an essential nutrient for animals and is present in many oxidation states in the environment [Cr(II) to Cr(VI)], including the most common forms Cr(0), Cr(III), and Cr(VI). Cr(VI) and the steel component Cr(0) are usually byproducts of industrial processes. Cr(VI) is considered to be 1,000 times more toxic than Cr(III), and the World Health Agency and EPA has determined that Cr(VI) is a carcinogen. Cr(VI) contamination in the soil and groundwater has been detected in various parts of southern California and in Tennessee (EPA 2000; EPA 2004), where wells were closed because Cr(VI) levels exceeded EPA limits (Table 1). Areas in and around Glasgow, Scotland are also heavily contaminated by Cr used

for ore processing in the 19th century which subsequently leached into the ground water supply and accumulated at toxic levels (Farmer et al. 1999).

3.3.1 Phytoremediation of Cr

Although Cr(IV) is oxidized to the relatively non-hazardous Cr(III) in soil (ATSDR, 2001), Cr(IV), and Cr(VI) in groundwater and estuaries can pose health hazards and disrupt ecosystems. *Betula* and *Salix* trees are able to take up Cr, and therefore, would be useful for phytoremediation of Cr(IV) contaminated groundwater (Pulford et al. 2001) while Cr(VI) in estuaries can be absorbed by coconut husks and bagasse (Krishnani et al. 2004; Parimala et al. 2004). A recent report of *Salsola kali* (tumbleweed) accumulating Cr(VI) may prove to be useful for phytoremediation of Cr(VI) in the soil (Gardea-Torresdey et al. 2005).

3.4 Copper (Cu)

Cu is an essential element and enzyme co-factor for oxidases (cytochrome c oxidase, superoxide dismutase) and tyrosinases; however, animals and plants can accumulate toxic levels of Cu. Cu contamination for soil and groundwater usually results from mine sites like Blackbird Creek, ID (Mebane 1997) or munitions research or disposal sites like the Picatinny Arsenal, in northern New Jersey (EPA 1989). The Deer Lodge Valley and Anaconda areas of the Beaverell pedon in Montana, a region affected by long-term Cu smelting, has soil polluted with high levels of Cu and lower concentrations of As, Zn, and Pb (Burt et al. 2000, 2003). Cu contamination is also problematic in European soils exposed to historical mining and smelting activity. During the 19th century, the Devon Great Consols Mine in the Tamar Valley of Devon, UK, was the world's largest producer of Cu. As a result, Cu in the form of chalcopyrite has permeated the soil in this region (Lombi et al. 2004). In France, due to the continuous treatment of vine downy mildew with Bordeaux mix since the end of the 19th century, extensive deposits of Cu have accumulated in many vineyard soils (Brun et al. 2001). In Kayseri, Anatolia, high Cu concentrations were found in Zn-producing industrial sites (Aksoy et al. 2000), while a study in Denizli found that high levels of Cu characterize urban roadsides associated with road traffic (Celik et al. 2005). Cyprus (Kupros) has an extensive history of Cu mining extending back over 2,000 years. Cu mining wastes from this prolonged activity have had a significant impact on the environment and biota of this island (Pyatt 2001). Greece is also afflicted by Cu contamination: lake ecosystems, such as Lake Pamvotis in north-western Greece, exhibit high concentrations of this metal (Papagiannis et al. 2004), and Cu poisoning from high Cu content in local food has occurred in areas such as Veria county (Zan-topoulos et al. 1999).

3.4.1 Phytoremediation of Cu

At superoptimal levels, Cu is highly toxic to plants and Cu ligands in plants are citrate, PC₂, PC₃, and metallothioneins (Murphy et al. 1999; Rauser 1999). Correspondingly, most Cu-tolerant plants are excluders, and no confirmed Cu accumulators have been identified. It was originally thought that *Elsholtzia splendens* was a Cu hyperaccumulator, but after further investigation it was concluded to be a tolerant excluder like *Elsholtzia argyi* (Jiang et al. 2004b), *Silene vulgaris* (Song et al. 2004) and *Mimulus guttatus* (Harper et al. 1998). While 37 taxa of Cu hyperaccumulators have been reported, mainly from the Shaban Copper Arc of Congo, more research is needed to determine if the high Cu levels are due to hyperaccumulation or depositions of Cu dust on leaves (Song et al. 2004). *Salix nigra* was shown to accumulate more Cd and Cu than other *Salix* species, and field studies should determine the feasibility of this species for phytoremediation (Kuzovlina et al. 2004). In addition, soil amendments, like phosphate, increase Cu uptake, and therefore, may further phytoremediation efforts (Wu et al. 2004) (For a detailed discussion of Cu in plants, see Chapter 9). For aqueous Cu contamination, *Eichhornia crassipes*, the water hyacinth, is estimated to absorb 21.62 kg Cu ha⁻¹, and could potentially be used for phytoremediation of low level Cu contamination in waste water (Liao and Chang 2004).

3.5 Mercury (Hg)

Mercury is toxic to humans, and depending on the form it takes can cause severe neurological disorders (Carty and Malone 1979). Mercury exposure to humans is mostly through ingestion of fish, as Hg is biomagnified through the aquatic food chain, and amalgam dental fillings. Over the past century, several thousand tons of Hg have been released to the environment by human activity (Andren and Nriagu 1979). Many developing countries use elemental Hg-Au amalgamation mining practices, which results in significant Hg contamination of surrounding soil and water. Examples include artisanal Au mines in Suriname and the Amazon basin (Gray et al. 2002). Mercury in soil can be converted to cinnabar (HgS) as a result of sulfate reduction after the deposition and burial of mercury-contaminated soils. However, mercury release from solid forms, such as cinnabar, can also create environmental hazards. Studies of contaminated soil from industrial mercury dumping at the headwaters of East Fork Poplar Creek in Oak Ridge, TN, and in a Florida Everglades study indicate that organic matter could increase mercury mobilization from cinnabar and affect mercury bioavailability (Barnett et al. 1997; Ravichandran et al. 1998). These are candidate areas for phytoremediation.

The most toxic forms of mercury are organomercurials like methyl-Hg and phenyl mercuric acetate, followed by ionic Hg(II), with elemental Hg(0) as the least toxic form. Organomercurials and ionic Hg are toxic to plants, and to date Hg hyperaccumulating plants have not been identified. However, a Hg hyperaccumulating mushroom *Amanita muscaria* has been found that accumulates 96-1900 ng g⁻¹ dry wt in the caps and 61-920 ng g⁻¹ dry wt in the stalks depending on the col-

lection site (Falandysz et al. 2003). A recent study investigating Hg accumulation among *Salix* spp. found that the majority of the Hg is accumulated and retained in the cell wall of the roots and only 0.45-0.65% was translocated to the shoots (Wang and Greger 2004).

3.5.1 Phytoremediation of Hg

Instead of using plants to phytoextract mercury, several studies have focused on converting the organomercurials to Hg(0), which is volatile and is released into the atmosphere. The Meagher group at the University of Georgia has accomplished this transformation by expressing the bacterial genes *merB*, which encodes organomercury lyase (Bizily et al. 1999), and *merA*, encoding mercuric reductase in plants (Rugh et al. 1996). MerB severs the mercury-carbon bond and MerA reduces ionic mercury to elemental mercury. Transgenic poplar and cottonwood trees expressing *merA* and/or *merB* could be used as phytoremediators which do not require harvesting or replanting each season (Rugh et al. 1998; Che et al. 2004). In an elegant demonstration of the importance of proper subcellular targeting, Bizily et al. (2003) created ER and cell wall targeted versions of MerB. This appears to have targeted the MerB activity to the secretory pathway, which is thought to be the main location of hydrophobic organomercurials within the cells. Even though the plants produced tenfold or less targeted MerB than the untargeted MerB, Bizily et al. (2003) were able to identify lines that converted equivalent amounts of elemental Hg(0). Ruiz et al. (2003) were able to express *merA* and *merB* in chloroplasts which allows for high levels of protein production as well as other possible advantages. While these approaches show great promise from a scientific and technical perspective, there is a great deal of public resistance to a technology which volatilizes mercury, even if it is in a form that is 200 times less toxic than the form present in soil and water.

3.6 Nickel (Ni)

Ni is an essential element that can be toxic and possibly carcinogenic in high concentrations (ATSDR 2003). Ni toxicity in humans usually results from repeated occupational exposure resulting in dermatitis, asthma or headaches (Davies 1986; Akeeson and Skerfing 1985), but Ni contamination of soils is primarily restricted to regions surrounding Ni smelting operations such as Sudbury, Ontario, and Harare, Zimbabwe (Johnson and Hale 2004; Lupankwa et al. 2004). As, Cd, and Pb are often present in Ni mining and smelting wastes, and soil and water heavy metal concentrations from Ni mining operations can exceed governmental safety limits (Lupankwa et al. 2004). Serpentine and ultramafic soils are naturally occurring regions of high Ni concentrations characterized by unique Ni-tolerant flora. The majority of Ni hyperaccumulators have been collected from these soils.

Alyssum lesbiacum and *Thlaspi goesingense* are both Ni hyperaccumulating plants in the Brassicaceae family. In the genus *Alyssum* alone, 48 different species have been discovered containing between 1,000 $\mu\text{g g}^{-1}$ and 30,000 $\mu\text{g g}^{-1}$ Ni in leaf

dry biomass (Baker and Brooks 1989; Kerkeb and Kramer 2003). *Thlaspi goesingense* has been reported to accumulate 9,490 mg Ni g⁻¹ dry weight. (Freeman et al. 2004; Kramer et al. 1997; Reeves and Brooks 1983) (For a detailed description of Ni in plants, see Chapter 9). Ni phytoextraction using hyperaccumulators has been patented (Chaney et al. 1999).

3.6.1 Uptake into the root

Little is known about Ni uptake into roots. Evidence that histidine (His) chelates Ni suggests that His might assist root uptake of Ni. *Alyssum lesbiacum* has constitutively high free His levels, and when *Salmonella typhimurium* ATP phosphoribosyl transferase enzyme (StHisG) was expressed in *A. thaliana*, the His increased twofold and biomass increased 14-40-fold when grown on Ni (Wycisk et al. 2004). But, a comparison of the uptake mechanisms of *A. lesbiacum* and *B. juncea*, a non-accumulator, indicated that Ni and His are taken up independently, as His uptake inhibitors had no effect on the Ni uptake, and Ni was taken up as a free cation (Kerkeb and Kramer 2003). Furthermore, Salt et al. (1999) found that, while root exudation of histidine and citrate may help reduce Ni uptake for the nonaccumulator *T. arvense*, these exudates did not appear to be involved in the hyperaccumulation of Ni by *T. goesingense*.

3.6.2 Transport to the leaves

Ni and His loading into the xylem appear to be correlated (Kerkeb and Kramer 2003), and nicotianamine-Ni complexes have been shown to be transported from the roots to the shoots and across plant membranes in a manner similar to nicotianamine-Fe complexes (Becher et al. 2004; DiDonato et al. 2004; Koike et al. 2004; Vacchina et al. 2003; Weber et al. 2004).

3.6.3 Detoxification/Chelation

Nicotianamine is thought to be involved in Ni detoxification in *T. caerulescens* (Vacchina et al. 2003). Nicotianamine synthase (NAS) is constitutively expressed at high levels in both *T. caerulescens* and *A. halleri* which strongly suggests a role for nicotianamine in Ni/Zn hyperaccumulation (Becher et al. 2004; Vacchina et al. 2003; Weber et al. 2004). Kramer and coworkers found that much of the intracellular Ni of *T. goesingense* associated with citrate, and it is likely that citrate-Ni association is vacuolar (Kramer et al. 2000). His (or a His-like ligand) could facilitate shuttling Ni across the cytoplasm in *T. goesingense* (Kramer et al. 2000). Ni has a higher affinity for both nitrogen and oxygen ligands than S ligands, and the observed absence of Ni-S ligands indicates a lack of PC binding (Freeman et al. 2004; Kramer et al. 2000).

3.6.4 Sequestration

Ni is sequestered in multiple locations in *T. goesingense*. When cell walls from *T. arvense* and *T. goesingense* were incubated in levels of Ni normally toxic to *T. arvense* plants, more Ni was found bound to *T. goesingense* cell walls compared to *T. arvense*, suggesting cell wall modifications in *T. goesingense* might be conducive to enhanced Ni binding (Kramer et al. 2000). Reduced cell wall binding in *T. arvense* might alternatively be explained by pH changes resulting from exposure to a toxic concentration of Ni. Cell fractionation analyses also indicate that the majority of intracellular Ni is localized in the vacuole, and *T. goesingense* accumulates twice as much as *T. arvense* even though there was no observed difference in the vacuole sizes of the two species (Kramer et al. 2000; Salt and Persans 2000). Overexpression of *T. goesingense* metal-tolerance proteins (MTPs), members of the cation diffusion facilitator (CDF) family, conferred resistance to Ni, Cd, Co, and Zn in yeast (Persans et al. 2001). *TgMTP1* (*T. goesingense* MTP1) was expressed in yeast, and *in vivo* and *in vitro* staining with hemagglutinin (HA)-tagged TgMTP::1HA showed that TgMTP1 is localized to both the vacuole and the plasma membrane (Kim et al. 2004). Furthermore, TgMTP1 is constitutively expressed at high levels in *T. goesingense* (Persans et al. 2001). TgMTP1 may enhance vacuolar sequestration and act as a metal efflux pump at the plasma membrane (Kim et al. 2004). The CDF homologs *ZTPI* in *T. caerulescens* and *AhMTP1* in *A. halleri* are also constitutively expressed at elevated levels (Assunção et al. 2001; Becher et al. 2004).

3.6.5 Phytoremediation of Ni

Different species of *Alyssum* Ni hyperaccumulators have been evaluated for phytoremediation of mine sites (McGrath and Zhao 2003), and *Alyssum* hybrids have been bred with suitable traits for phytomining Ni on serpentine soils in Oregon and Washington (Chaney et al. 1999; Li et al. 2003b), and phytomining technology has been commercialized (Li et al. 2003a). Other metal hyperaccumulators are being investigated for their uses in the phytomining of Ni, thallium, and gold from soils (Anderson et al. 1999; Boominathan et al. 2004).

The overexpression of genes from different hyperaccumulators, like the MTPs, could elevate shoot Ni accumulation. In addition, overexpression of multiple MTP genes in conjunction with SAT metal tolerance genes could enhance both metal accumulation and tolerance, thereby improving Ni phytomining and phytoremediation technologies.

3.7 Lead (Pb)

Pb is an extremely toxic heavy metal which is a serious threat to the health of children and wildlife (EPA 2005a). The main sources of Pb poisoning include lead paint and old gasoline spills (PbBrCl , $2\text{PbBrCl}\cdot\text{NH}_4\text{Cl}$) resulting in dust and soil contamination of food and water (Xintaras 1992). Other areas with high Pb con-

centrations include Pb mines and smelters (PbSO_4 , $\text{PbO}\cdot\text{PbSO}_4$, and PbS), such as the Leadington mine in Leadington, MO (Tom and Miles 1935), shooting ranges, and disposal sites for old batteries. A shooting range in Cortland, NY was estimated to have accumulated 500 tons of Pb after 30 years of use. The New York Department of Environmental Conservation found that the Pb concentrations at this site posed a health threat to people and wildlife, and the site received clean-up order from the EPA (Cape Cod Times 1997). College Grove, TN, has been identified as an area of concern due to Pb contamination from old battery cases on railroad property, with Pb levels ranging from 2,700 to 5,500 ppm (Chavez 1999).

Elemental Pb is insoluble and the most water soluble forms of Pb compounds are lead acetate (2 mg ml^{-1}), lead chloride (0.009 mg ml^{-1}), and lead nitrate (5 mg ml^{-1}) (Xintaras 1992). Atmospheric Pb mostly exists as PbSO_4 and PbCO_3 . Although many plants may have a strategy of Pb exclusion as *Thlaspi praecox*, which hyperaccumulates Cd and Zn but excludes Pb (Vogel-Mikus et al. 2005), several plant species can hyperaccumulate soluble Pb in the soil. It has been reported that *Sesbania drummondii*, a leguminous shrub, and several *Brassica* species can accumulate significant amounts of Pb in their roots (Blaylock et al. 1997; Sahi et al. 2002; Wong et al. 2001), and *Piptathertan miliacetall*, a grass, accumulates Pb directly correlating to soil concentrations without symptoms of toxicity for 3 weeks (Garcia et al. 2004). Sahi et al. (2002) have noted that *S. drummondii* can tolerate Pb levels up to 1500 mg L^{-1} and accumulate $\sim 40 \text{ g kg}^{-1}$ shoot dry weight. *Brassica juncea* shows reduced growth at 645 ug g^{-1} Pb in the soil substrate, but can accumulate 34.5 g kg^{-1} shoot dry weight, although significant shoot accumulation is not observed until Pb reaches saturation levels in the roots. Most of the shoot accumulation was found in stems and not leaves suggesting that Pb is relatively insoluble (Kumar et al. 1995). Microanalysis spectra data through *S. drummondii* root sections show a decreasing gradient of Pb contents from the epidermis to the root central axis, and electron microscopy of *S. drummondii* roots revealed Pb deposition in the cell membrane and cell wall (Sahi et al. 2002).

3.7.1 Phytoremediation of Pb

The biggest challenge to effective phytoremediation of Pb is its extremely low solubility, as only $\sim 0.1\%$ of soil Pb is available for extraction (Huang et al. 1997). Efforts at phytoremediation of Pb have concentrated on using soil amendments like EDTA to increase the available Pb uptake (Blaylock et al. 1997; Huang et al. 1997; Wu et al. 1999). Addition of chelators does increase the solubility and uptake, but the amount Pb transferred to shoots is still low in comparison to the amount of Pb in the soil, and increases the likelihood that the mobilized Pb-EDTA will leach out of the soil and contaminate groundwater (Wu et al. 1999). The prospects for phytoremediation of Pb will depend on the development of novel systems for solubilizing Pb and for transporting it to the leaves. Lastly, the expression of the glutathione-Cd vacuolar transporter YCF-1 in *Arabidopsis* has been found to increase the tolerance and slightly increases the accumulation of Pb (Song et al. 2003).

3.8 Selenium (Se)

Se is an essential element for animals, but so far has not been demonstrated essential for plants; mammalian Se-glutathione peroxidase protects against oxidative stress (Michiels et al. 1994), and Se also has anti-cancer/cancer preventative activities when present in compounds like methylselenocysteine (MeSeCys) (Ellis et al. 2004). Livestock in the southeastern United States with low-Se soils exhibit nutrition-related deformities, while livestock grazing on high-Se soils in Western states exhibit toxicity symptoms (Cosgrove 2001). Se naturally leaches from the soil, but Se becomes concentrated where leachates from highly irrigated soils or waste releases from petroleum refining accumulate to toxic levels in shallow groundwater regions or wetlands. Kesterson National Wildlife Refuge in California is a notable example of toxic Se accumulation that resulted in deformities in water fowl and other wildlife. In 1971 a manmade reservoir was built to catch agricultural run-off as part of a plan to conduct the run-off to the Pacific Ocean, but the conduit was never completed, and the Kesterson Reservoir became a dead-end holding area instead of a flow-through-regulating reservoir. In 1978, the reservoir began to receive drainage water, and by 1982, the ecological disaster was evident: the salt and pesticide accumulations were expected, but the Se accumulations were not, and Se remediation came to the forefront of public and scientific concern (US DOI 2004). Other Se remediation sites are the Stillwater Wildlife Management Area in Nevada, the Salton Sea irrigation area in California, the Middle Green River Basin Area in Utah, the Kendrick Reclamation Project in Wyoming, and the Gunnison/Grand Valley area in Colorado.

Se hyperaccumulators such as *Astragalus bisulcatus*, the two-grooved milk-vetch, have been shown to accumulate Se up to 0.65% (w/w) (Pickering et al. 2003), and *B. juncea* accumulated 50 mg kg⁻¹ dry mass in the field (Banuelos et al. 1997). *A. bisulcatus* accumulates high concentrations of Se-methylseleno-Cysteine (Se-MeSeCys) in young leaves, while mature leaves have predominately selenate and 40 to 60-fold less Se-MeSeCys. Seleno-Cys methyl transferase (SMT1), which catalyzes Se-MeSeCys from seleno-Cys (SeCys) and S-methyl-transferase, is present in leaves of all ages. This suggests that the synthesis of Se-MeSeCys in older leaves must be blocked at an earlier metabolic step and that mature leaves cannot reduce selenate (SeO₄⁻²) to selenite (SeO₃⁻²) (Pickering et al. 2003).

3.8.1 Uptake into the root

Shibagaki et al. (2002) have demonstrated that selenate is taken up through the sulfate transporter *Sultr1;2* (Fig. 3). *Arabidopsis* mutants in the sulfate transporter gene *Sultr1;2* were resistant to selenate, and *Sultr1;2* expression is localized in the root tip, root cortex, and lateral roots (Shibagaki et al. 2002).

3.8.2 Transport to the leaves

Biochemical forms of Se isolated from plants suggests that Se metabolism is similar to the S metabolic pathway and that Se analogs of S assimilated into S path-

ways (Leustek 2002), and selenate (SeO_4^{2-}), like sulfate (SO_4^{2-}), is transported to the chloroplasts after uptake into the roots (Ellis et al. 2003; Leustek 2002) (Fig. 3).

3.8.3 Detoxification/Chelation

Selenate (SeO_4^{2-}) and sulfate (SO_4^{2-}) metabolism in plants parallel selenate and sulfate accumulations in mature *A. bisulcatus* leaves and non-accumulators (Ellis et al. 2003; Pickering et al. 2003) (Fig. 3). While there is no direct evidence, selenate reduction in *A. bisulcatus* most likely occurs via the ATP sulfurylase/APS reductase pathway, as a Se-specific selenate reductase has not been identified.

Se non-accumulating species accumulate seleno-Methionine (SeMet) and Selenomethionine (Se-MeSeMet) (McCluskey et al. 1986; Virupaksha and Shrift 1965) while MeSeCys accumulates *A. bisulcatus*. If SeMet or Se-MeSeMet is incorporated into proteins, the seleno-protein is non-functional resulting in cellular toxicity. In contrast, *A. bisulcatus* forms MeSeCys from the methylation of SeCys by SMT (Wang et al. 1999). MeSeCys and proteins incorporating it are not toxic to the plant, and therefore, accumulate to high concentrations. Pickering et al. (2003) have suggested that mature leaves of *A. bisulcatus* could export Se-MeSeCys to younger tissues, and Se-MeSeCys is likely incorporated in seeds.

MeSeCys is an intermediate in the formation of dimethyl diselenide, a volatile form of Se. Dimethyl diselenide is the primary volatile of *A. bisulcatus*, the distinctive malodorous, signature smell of the plants (Pickering et al. 2003). Identification of all of the enzymes involved in the metabolic pathway of Se-MeSeCys in *A. bisulcatus* will clarify this pathway, and further elucidate the mechanisms whereby this plant establishes its hyperaccumulation capabilities.

3.8.4 Phytoremediation of Se

When ATP sulfurylase, an enzyme that reduces selenate to selenite, was overexpressed in *B. juncea*, Se accumulation in shoots was twofold greater and greater biomass than the Se hyperaccumulator *Stanleya pinnata* (Van Huysen et al. 2004) indicating that *B. juncea* ATP sulfurylase overexpressors have the potential to successfully phytoremediate Se contaminated sites. As *B. juncea* plants overexpressing ATP sulfurylase already have a bioconcentration factor of ~10, any improvement in accumulation or volatilization could make these plants suitable for efficient phytoremediation.

Other studies have focused on overexpressing enzymes in Se metabolism. Overexpression of the mammalian selenocysteine lyase, which converts SeCys to elemental Se and alanine, in *Arabidopsis* slightly increases the amount of Se accumulated and slightly reduces the amount of Se incorporated into proteins (Pilon et al. 2003). Interestingly, cytosolic versions of selenocysteine lyase increased tolerance to Se while chloroplastic versions decreased tolerance, illustrating the importance of proper sub-cellular localization of novel proteins in transgenic plants. Two biochemical pathways can convert SeCys to a volatile compound, either to dimethyl selenide or dimethyl diselenide. Cystathionine- γ -synthase catalyzes Se-

Cys to dimethyl selenide, and overexpression of cystathionine- γ -synthase in *B. juncea* increased Se tolerance and enhanced Se volatilization (Van Huysen et al. 2004). Overexpression of SMT from *A. bisulcatus* in *Arabidopsis* and *B. juncea* increased Se tolerance, accumulation of MeSeCys and volatilization of Se (Ellis et al. 2004; LeDuc et al. 2004). These transgenic plants were more tolerant to selenite than to selenate, indicating that the reduction of selenate to selenite is limiting. Overexpression of ATP sulfurylase with selenocysteine lyase, cystathionine- γ -synthase or SMT could therefore have synergistic effects.

3.9 Zinc (Zn)

Zn is an essential microelement, but is toxic to animals and plants at high concentrations (Cobbett and Goldsbrough 2002; Gupta and Gupta 1998). The first Zn hyperaccumulator identified was *T. caerulescens*. This plant was reported to accumulate between 25,000 and 30,000 $\mu\text{g g}^{-1}$ total Zn before exhibiting symptoms of toxicity, although *T. caerulescens* can accumulate a maximum dry weight of 40,000 $\mu\text{g g}^{-1}$ Zn in its shoots (Pence et al. 2000). *Arabidopsis halleri* has also been found to increase in its shoot Zn concentration from 300 $\mu\text{g g}^{-1}$ dry wt at 1 μM Zn to 32 000 $\mu\text{g g}^{-1}$ at 1000 μM Zn without phytotoxicity (Zhao et al. 2000). *Arabidopsis lyrata* ssp. *Friedensville* accumulates high leaf concentrations of Zn in the field (Cannon 1960), but exhibits variable accumulation in the axenic culture.

3.9.1 Uptake into the root

The ZIP family of proteins (ZRT/IRT-like proteins) transport Zn into the plants (Grotz et al. 1998; Ramesh et al. 2003). ZNT1 from *T. caerulescens* mediates low affinity Zn uptake as expected for a plant that grows on high concentrations of Zn (Pence et al. 2000). ZNT1 expression is higher in the hyperaccumulator *T. caerulescens* than in the non-accumulator *T. arvense*, possibly leading to a higher density of Zn transporters in the root-cell plasma membrane (Pence et al. 2000). This difference in transporter concentration could account for the observation that the hyperaccumulator and the nonaccumulator have the same affinity for Zn, but the hyperaccumulator has a higher rate of uptake (Lasat et al. 1996).

3.9.2 Transport to the leaves

Despite lower rates of uptake, the roots of *T. arvense* were found to accumulate substantially more Zn than in *T. caerulescens* (Lasat et al. 1996). This difference is likely due to better transport to the leaves in the hyperaccumulator. *T. caerulescens* had five times more xylem sap Zn (Lasat et al. 1998) and ten times more Zn was translocated to the shoots in *T. caerulescens* than in *T. arvense* (Lasat et al. 1996). The leaf cells of the hyperaccumulator are able to accumulate more Zn when leaf sections are subjected to high Zn (1mM) conditions (Lasat et al. 1998).

The molecular mechanisms of this increased uptake are unknown. Additional studies of leaf Zn and Cd uptake are included in Section 3.2.

3.9.3 Detoxification/Chelation

Mechanisms of Zn detoxification, chelation, and sequestration are species-specific. Zn was mostly found coordinated to malate in *A. halleri* leaves (Sarret et al. 2002). Although malate is the most common organic acid in *T. caerulescens* shoots (Tolra et al. 1996) no Zn-malate complexes were detected with X-ray absorption spectroscopy (Salt et al. 1999). Instead, the predominant form of Zn in the roots was Zn-histidine with the remaining 30% bound to the cell wall. In the xylem sap, most of the Zn exists as the free hydrated Zn²⁺ cation with ~20% as Zn-citrate, while in the leaves, all four forms are found with citrate being the most common (For detailed description of heavy metal chelation, see Chapter 10).

3.9.4 Sequestration

Both *T. caerulescens* and *T. arvense* store similar amounts of Zn in their root apoplasts, indicating that cell wall compartmentation is not a tolerance mechanism. The higher concentration of Zn in the root vacuoles of the non-accumulator noted above suggests that root vacuole accumulation is a tolerance mechanism for non accumulators which lack a mechanism to transport to the leaves.

Leaf vacuoles are the primary site of Zn sequestration in *T. caerulescens* (Kupper et al. 1999). X-ray microanalysis of shoot tissue indicated that Zn is sequestered in the vacuoles of epidermal and sub-epidermal leaf cells in *T. caerulescens* (Frey et al. 2000; Vazquez et al. 1992), but in the mesophyll cells of *A. halleri* (Kupper et al. 2000).

3.9.5 Phytoremediation of Zn

See Section 3.2 (Cd) for discussion of Zn and Cd phytoremediators.

4 Future outlook

Phytoremediation is a technology with great potential. Phytoextraction using a combination of high-biomass with hyperaccumulator mechanisms will successfully remove heavy metal contaminants from the environment. The underlying mechanisms of hyperaccumulation can be applied to many different technologies. For example, trace metal deficiency could be reduced or eliminated in humans and animals if essential mineral nutrient levels were elevated in food crops (Gstoettner and Fisher 1995). Phytomining with hyperaccumulating plants selected to uptake high levels of valuable metals, such as gold and Ni, would eliminate the need for traditional mining technologies which have heavy metal contamination as a by-product.

Among the limitations of phytoremediation strategies has been that *in situ* remediation often takes many years to accomplish compared to traditional decontamination approaches to substantially restore a polluted area (Suresh and Ravishankar 2004; Schnoor et al. 1995, 1997). However, phytoremediation costs at least ten times less than traditional methods of excavation and removal (Schnoor 1997), and if an additional economic incentive were present (not only an environmental benefit) such as phytomining or forestry, then phytoremediation would be viewed as economically viable (Robinson et al. 2003).

Another limitation to consider is the availability of the contaminants in question to the plants. Schnoor et al. (1995) note that areas where contamination is less than 5 m in depth are the best suited sites for phytoremediation. Furthermore, the solubility of the contaminant in the soil determines whether phytoextraction is possible: in the case of metals, only metals found as free metal ions, soil soluble metal complexes, or metals adsorbed to inorganic soil constituents at ion exchange sites are readily available for uptake by the plants (Lasat et al. 2000). Metals that are bound to soil organic matter, precipitated (oxides, hydroxides, carbonates), or embedded in the structure of silicate minerals are not available to the plants. It has been suggested that phytoremediation is best suited for removing moderately hydrophobic pollutants such as BTEX compounds (benzene, toluene, ethylbenzene, and xylenes), chlorinated solvents, or nitrotoluene ammunition wastes; or excess nutrients (nitrate, ammonium, and phosphate) (Schnoor et al. 1995). The concentrations of soil pollutants should also be considered, as at some contaminated sites such as the Iron Mountain superfund site in Redding, CA, high levels of contaminants are toxic to plants and prevents successful phytoremediation (Schroder et al. 2002).

While many examples in both literature and patents propose the potential of different plants to remove soil heavy metals, McGrath and Zhao (2003) explain how the bioconcentration factor of many of these plants is not conducive to actual phytoremediation. The bioconcentration factor is the ratio of the plants shoot metal concentration to the soil metal concentration, which can be interpreted as the ability of a plant to take up the metal and transport it to its shoots. While most plants have a bioconcentration factor for heavy metals and metalloids of less than one (including many reported putative phytoremediators), a much greater value is required for phytoremediation. McGrath and Zhao note that even if one assumes a high biomass production of twenty tonnes per hectare per crop, a bioconcentration factor of greater than ten is required to reduce soil metals by half in less than ten crops. Ten tonnes per hectare per crop is possible for many agricultural crops, and the bioconcentration factor would need to be twenty or greater to reduce soil metals by half in less than ten crops. Many of these sites have been contaminated for more than ten years, as such a ten year remediation plan does not seem excessive. In general, the benefits and disadvantages of phytoremediation must be assessed for a particular project to determine whether this kind of remediation is the most appropriate for the task. Combining technologies described above (1) offer the greatest potential to efficiently phytoremediate contaminated sites.

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List of abbreviations

EDTA, CDTA, DTPA, EGTA, EDDHA and NTA, metal chelators;
TCE: trichloroethylene
TNT: 2,4,6-trinitrotoluene
RDX: hexahydro-1,3,5-trinitro-1,3,5 triazine
HMX: octahydro-1,3,5,7-tetranitro-1,3,5,7 tetrazocine
MTBE: methyl tertiary-butyl ether
BTEX compounds: benzene, toluene, ethylbenzene, and xylenes; organic pollutants;
ROS: reactive oxygen species

BSO: buthionine sulfoximine
SA: salicylic acid
MTPs: metal-tolerance proteins
CDF: cation diffusion facilitator
SAT: serine acetyltransferase
 γ -ECS: γ -glutamylcysteine synthetase
ECS: γ -glutamylcysteine synthetase
GS: glutathione synthetase
 γ -GluCys: γ -glutamylcysteine
NAS: nicotianamine synthase
SMT1: seleno-Cys methyl transferase
PC: phytochelatin
Cys: cysteine
His: histidine
Met: methionine
GSH: glutathione
OAS: O-acetyl-L-serine
SeCys: seleno-Cys
SeMet: seleno-Methionine
MeSeCys: methylselenocysteine
Se-MeSeCys: Se-methylseleno-Cysteine
Se-MeSeMet: Se-methylseleno-Met

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