Review

Phytoremediation of soil contaminated with heavy metals: a technology for rehabilitation of the environment

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This paper reviews the chemical behaviour of heavy metals in soil, the effect of heavy metals on plants and humans, and describes phytoremediation, which is the use of green plants to remove soil contaminants. The physiological processes that support bio-accumulation of heavy metals by plants are also described.

Introduction

For environmental safety, the high concentration of heavy metals in the soil should be removed. Mining and manufacturing industries are main sources of heavy metals that pollute the soil, groundwater and air in South Africa. As one example, communities near Johannesburg in Gauteng, South Africa, lodged complaints in court about the toxicity of potable groundwater that was contaminated by heavy metals from industries (Ndaba 2002). Disposal of wastes from industries and management of contaminated soil and water from mines have become difficult in developed countries, because of new legislation that governments are forced to observe. For instance, the disposal of sewage sludge (now called biosolids) in oceans was banned in the 1970s, which encouraged the 'mushrooming' of numerous waste incinerators in many countries. Pollution from incinerating waste has been recognised in South Africa, and it has resulted in the signing of the 'Isipingo Declaration' by South Africa, Mozambique and Swaziland (Carnie 2002). This is a declaration of South Africa and the other two mentioned countries to ban waste incineration, because incineration of waste increases the atmospheric dioxins and cancer-associated heavy metals. The ban on dumping biosolids in the oceans has increased pressure for land application in the USA (Chaudri et al. 2001). Table 1 gives typical total concentrations of heavy metals in different fertilizer sources including biosolids, which can be an excellent source of plant nutrients and organic matter. But they also can harbour numerous organic and inorganic contaminants. The US Environmental Protection Agency (EPA) regulation limits for heavy-metal concentrations in biosolids and drinking water are presented in Table 2. Although land application of biosolids is not popular in South Africa, large quantities of animal waste (manure) are applied to agricultural fields every year. Livestock manure may contain high concentrations of heavy metals that originate from feed and medicines provided to the animals (Table 1). Normal and toxic concentrations of heavy metals in soil and plants are presented in Table 3.

According to Pierzynski et al. (1994), there are two reasons for concern over the increase of heavy metals (also referred to as 'trace elements') in the environment. [The term 'heavy metal' is usually restricted to those metals that have densities greater than 5.0 (Page 1974: 2).] First, humans and animals may ingest these toxic elements in contaminated food and fodder or inhale them as dust. A prevalence of chronic ailments, such as heart and kidney diseases, skin cancer and anaemia has been reported in people living for more than five years in areas polluted by heavy metals. Inhalation of arsenic (As) has been directly associated with lung cancer and skin cancer. Second, phytotoxic effects of elevated levels of heavy metals in soils cause poor vegetation establishment that makes the soils prone to erosion. This results in further dispersion of the pollutants to new areas, which threatens the health of greater numbers of people.

Plants grown in soil contaminated by metals accumulate higher concentrations of metals than plants grown in normal soil (Chang et al. 1992). Therefore, clean up of toxic metals from agricultural land is important, because agricultural products with high levels of toxic metals are barred from international markets (Chaudri et al. 2001). For instance, the European Union, Australia and New Zealand have a cadmium (Cd) regulation limit of 0.1mg Cd kg^{-1} fresh weight (Chaudri et al. 2001, Commission of the European

Table 1: Typical concentrations (mg kg^{-1}) of heavy metals in biosolids, farm manure, phosphate fertilizers and lime (adapted from Ross 1994b)

Metal	Biosolids	Farm manure	Phosphate fertilizers	Lime
Manganese (Mn)	60–3 900	30–969	40–2 000	40–1 200
Copper (Cu)	50–8 000	2–172	1–300	2–125
Zinc (Zn)	91–49 000	15–566	50–1 450	10–450
Nickel (Ni)	6–5 300	2.1–30	7–38	10–20
Cadmium (Cd)	1–3 410	0.1–0.8	50–190	0.04–0.1
Lead (Pb)	2–7 000	0.4–27	4–1 000	20–1 250
Mercury (Hg)	0.1–55	0.01–0.36	0.01–2	0.05

Table 2: The permitted limits of heavy metals in biosolids and drinking water

Metal	[a] Biosolids (mg kg^{-1})	[b] Drinking water (mg l^{-1})
Iron (Fe)	None	0.30
Manganese (Mn)	None	0.05
Copper (Cu)	4 300	1.30
Zinc (Zn)	7 500	5.00
Nickel (Ni)	420	None
Cadmium (Cd)	85	0.005
Lead (Pb)	840	0.015
Mercury (Hg)	57	0.002
Arsenic (As)	75	0.010

[a] US EPA (2002a); [b] US EPA (2002b)

Table 3: Normal and toxic total concentrations (µg g^{-1}) of heavy metals in the soil and plants (from Kirkham 1975, Alloway 1995, Fageria et al. 2002)

Metal element	Soil		Plants	
	Normal	Toxic	Normal	Toxic
Iron (Fe)	200	None	50	1 000
Copper (Cu)	2	60	5	20
Manganese (Mn)	7	1 000	30	300
Zinc (Zn)	1	70	20	100
Nickel (Ni)	0.4	100	0.1–5	10
Cadmium (Cd)	0.06	3	0.1–5	0.1
Lead (Pb)	10	100	0.1–12	30

Communities 2001). The 2004 World Congress on Environmental Health highlighted that environmental metal poisoning is becoming a major public health burden in many African countries due to rapid globalisation and industrialisation (Carnie 2004). The concentration of life-threatening heavy metals such as lead (Pb), As, mercury (Hg) and zinc (Zn) has been found to be increasing in water, soil and air in several African countries (Carnie 2004).

Here follows a literature review on pollution by heavy metals. It includes a method for their removal from soil called phytoremediation, which is the use of green plants to remove pollutants. The literature review will show that, despite the media reporting and public outcry with regard to environmental pollution by heavy metals, little work has been done in South Africa to explore the possibility of removing heavy metals from soil for the safety of the environment. The aim of this paper is to discuss the impact of heavy metals in the environment and the concept of phytoremediation.

Heavy Metal Impacts on Humans and Plants

The effects on plants and humans of eight heavy metals — Cd, copper (Cu), iron (Fe), Pb, manganese (Mn), Hg, nickel (Ni) and Zn — that are often of most concern in the environment are as follows:

Cadmium

The accumulation of Cd in water and soil has caused major environmental and human health problems (Salt et al. 1995a). Cadmium is usually less adsorbed by soil and

organic matter than several other heavy metals (e.g. Pb, Cu), which makes it more available to plants and more easily leached by groundwater (McBride 1994: 319, Basta and Sloan 1999, McLaughlin et al. 2000, Perronnet et al. 2000). Gonzalez et al. (1992) showed that the availability of Cd in biosolids-amended soil is controlled by phosphatic clay instead of organic matter. Other studies indicate that Cd is associated with Fe-oxides or an Fe-Mn oxide fraction in biosolids (Dudka and Chlopecka 1990, Bell et al. 1991).

Cadmium is a toxic metal that can accumulate in the human body and has a half-life greater than 10 years. Elevated levels of Cd in the body can cause kidney damage in humans (Salt et al. 1997). Studies link renal dysfunction with a low level of Cd content in the diet (Salt et al. 1995b). Other diseases associated with Cd exposure are pulmonary emphysema and bone demineralisation (osteoporosis) (Bhattacharyya et al. 1988), because Cd replaces calcium (Ca) in bones.

Plants show a disturbed water balance when grown on Cd-laden soil (Poschenrieder et al. 1989). The metal is readily taken up by roots and translocated to aerial organs where it accumulates to high levels (Baryla et al. 2001). Cadmium affects stomatal function, water transport and cell wall elasticity (Bazzaz et al. 1974, Kirkham 1978, Baszynski et al. 1980). Poschenrieder et al. (1989) reported an increase in the stomatal resistance of plants that were treated with Cd, and similar results were reported by Kirkham (1978) and Baryla et al. (2001). The increase in stomatal resistance strongly correlated with increase of the abscisic acid (ABA) level in leaves (Poschenrieder et al. 1989). Inhibition of photosynthesis is another toxic effect of Cd, which is brought about by reduced stomatal

conductance in response to metal toxicity and sensitivity of photosystem II to high Cd concentration (Baryla *et al.* 2001). Cadmium may affect PS II on both the oxidising (donor) and reducing (acceptor) side (Haag-Kerwer *et al.* 1999). Rubisco activity in the Calvin cycle is inhibited by high Cd (Rivera-Becerril *et al.* 2002). The most clear symptom of Cd phytotoxicity is leaf chlorosis (Kirkham 1978, Baryla *et al.* 2001). Replacement of Fe by Cd in the centre of a precursor of the chlorophyll molecule was speculated as one of the causes of leaf chlorosis (Küpper *et al.* 1998).

High Cd concentration in the plant induces increased respiration and activities of the tricarboxylic acid cycle as well as other pathways of carbohydrate utilisation (Arisi *et al.* 2000). This increase in respiration was found to relate to the increased demand for ATP, which compensates for deficits in photophosphorylation (Ernst 1980).

Copper

Copper has been described by Alloway (1990) as an important pollutant of the air and agricultural soils. Intensive use of fungicides and herbicides, as well as sludge and manure application, has been identified as the main cause of agricultural soil contamination by Cu (Panou-Filotheou *et al.* 2001). Ingestion of elevated levels of Cu causes gastrointestinal distress, while long-term exposure to high Cu concentration causes liver and kidney damage (US EPA 2002a).

Panou-Filotheou *et al.* (2001) found that Cu toxicity resulted in reduction of stem height and root volume in the oregano plant (*Origanum vulgare*). Toxicity of Cu in roots is crucial, because roots provide entry into the plant of water and nutrients. Therefore, any remarkable reduction of root volume due to Cu toxicity also reduces water and nutrient uptake by the plant. Leaf chlorosis is another symptom of Cu phytotoxicity (Srivastava and Gupta 1996: 152). Leaf chlorosis due to Cu toxicity is strongly related to reduced volume and number of mesophyll cells (Panou-Filotheou *et al.* 2001) and displacement of Fe from physiologically active centres (Srivastava and Gupta 1996: 152).

Copper toxicity may cause damage to the plasma membrane of both plants and animals (Hall 2002, Demidchik *et al.* 2001), which results in the linking of the cytosolic electrolytes. Concentration of Cu above 3–5µmol l^{-1} increases non-specific plasma membrane permeability, inhibits Cl$^-$ channels, and suppresses plasma membrane H$^+$-ATPase (Demidchik *et al*. 1997). Non-specific conductance and H$^+$-ATPase inhibition are destructive to a cell because they are accompanied by plasma membrane depolarisation, disruption of ionic homeostasis and subsequent perturbation of enzymatic reactions (Demidchik *et al.* 2001, Hall 2002).

Iron

Although Fe is classified as an element with a low toxicity in plants (McBride 1994: 326), it is potentially noxious if taken up by plants in excess quantities. High levels of Fe in plants promote the formation of reactive oxygen species, which damage vital cellular constituents, especially membranes that are known to be susceptible due to lipid peroxidation (Schmidt 1999, Schützendübel and Polle 2002). Above-optimal levels of Fe may result in coalesced tissue, necrosis or bronzing, flaccidity and blackening of the roots (Laan *et al.* 1991). No serious human disease has been linked directly to an excessive concentration of Fe, which seems to be the reason that Fe has been given no regulation limit in biosolids by the US Environmental Protection Agency (Table 2).

Lead

Soil pollution by Pb occurs mostly through activities such as mining, smelting, land application of biosolids and the past use of antiknock gasoline additives such as tetramethyl and tetraethyl lead (Badawy *et al.* 2002).

People are usually exposed to Pb through drinking water, breathing Pb-laden dust and consuming food that accumulates high concentrations of Pb, because it has been grown on soil contaminated by Pb (Ogola *et al.* 2002). Lead impairs the nervous system and has effects on the foetus, infants and young children that results in a low intelligence quotient (United Nations 1998). Lead is classified as a possible human carcinogen because it can cause cancer. Low levels of exposure to Pb may cause ailments such as heart disease, abnormalities in children, testicular atrophy, anaemia and interstitial nephritis (United Nations 1998).

Lead toxicity can cause plasma membrane alteration in plants because Pb^{2+} is physiologically similar to Ca^{2+} (Srivastava and Gupta 1996: 221). Elevated Pb interferes with chlorophyll formation and the normal metabolism of Fe (Kacabova and Natr 1986). High concentration of Pb has been linked to poor seed germination, high stomatal resistance, inhibited CO$_2$ uptake and low photosynthetic rate (Poskuta *et al.* 1987).

Manganese

Manganese toxicity mostly occurs in waterlogged environments (McBride 1994: 334, Hopkins 1995). A symptom of Mn toxicity is the occurrence of dark brown spots on older leaves. These necrotic spots result from the local accumulation of oxidised Mn and phenolics (Horst 1988) and provide an index of the degree of Mn toxicity in plants (Horst and Fecht 1999, Wang *et al.* 2002). Elevated concentrations of Mn in the growing medium can also interfere with the absorption, translocation and utilisation of other elements such as Ca, magnesium (Mg), Fe and phosphorus (P) (Wang *et al.* 2002, Hopkins 1995). High concentrations of Mn in tissues can alter the activities of enzymes and hormones, which may render essential Mn-requiring processes non-functional or less active (Horst 1988). Effects of Mn toxicity on animals and humans are essentially not known.

Mercury

Mercury occurs in both organic and inorganic forms, but it is the organic form of Hg that is highly poisonous. The vapour from volatised Hg is also toxic to animals and humans (McBride 1994: 333). Coal combustion, metal refineries and

waste incineration are the main anthropogenic sources of Hg (US EPA 2002b, Shanley *et al.* 2002). Elevated concentrations of Hg in soil are strongly correlated with soil organic matter content (McBride 1994: 334). The toxicity of Hg is now taken seriously in the developed countries. For instance, the number of states in the USA that have issued Hg-related advisories on fish consumption increased from 27 states in 1993 to 43 states in 1999 (Shanley *et al.* 2002). However, Hg is still used for commercial applications such as making fluorescent bulbs, thermometers, electronic switches and other products (Shanley *et al.* 2002).

Methylated forms of Hg in the environment accumulate at the apices of food webs, which poses a health risk to children and pregnant women, especially those who eat fish (Shanley *et al.* 2002). Organic Hg enters the food chain mainly by its ingestion by fish and other aquatic organisms, which, when consumed by humans, is easily absorbed by the gastric and intestinal organs and then transported in the blood to the brain, liver, kidney and foetus (Ogola *et al*. 2002). Toxicity effects of Hg are confined primarily to the human central nervous systems (Shanley *et al.* 2002). Their effects are characterised by numbness and unsteadiness in the legs and hands, awkward movements, tiredness, ringing in the ears, narrowing of the field of vision, loss of hearing, sense of smell and taste, slurred speech, forgetfulness and kidney damage (Ogola *et al.* 2002, US EPA 2002a). Minamata is the Japanese name for the disease caused by eating Hg-contaminated fish or shellfish (Ogola *et al.* 2002). Specific effects of Hg toxicity in plants are essentially not known.

Nickel

Contamination of the environment by Ni is mostly from traffic or refinery emissions and industrial or municipal wastes (McBride 1994: 336, Barbafieri 2000). Nickel toxicity inhibits cell division in the meristem of the roots and limits the root expansion zone (Robertson 1985). It interferes with the translocation of Mn, Fe, Cu and Zn to the shoots (Anderson *et al.* 1973). This antagonistic effect causes symptoms typical of Mn and Fe deficiency in leaves (Anderson *et al.* 1973).

In animals and humans, Ni toxicity inhibits spermatogenesis, amylase enzymes, insulin formation and kidney function (Srivastava and Gupta 1996: 233). The most health-threatening form of Ni is nickel carbonyl ($Ni(Co)_4$) in cigarette smoke, which causes pulmonary fibrosis (respiration disorders) and renal disorders (Srivastava and Gupta 1996: 233).

Zinc

Primary sources of Zn pollution are industrial wastes and sewage sludge (McBride 1994: 329). Farm manures also have high concentrations of Zn (Mikkelsen 2000), which make them a promising amendment for Zn-deficient soils.

Zn toxicity affects plant growth by causing malformation of the nucleus and nucleolus of meristematic cells of the roots and also by disrupting cell division (Bobák 1985). Chlorophyll and root length are reduced with increased Zn concentrations in the growing media (Bekiaroglou and Karataglis 2002). Khurana and Chatterjee (2001) reported a reduction in biomass, seed number, seed weight and soluble proteins in sunflower (*Helianthus annuus*) plants grown in Zn-laden soil. Effects of Zn toxicity on humans and animals are unclear.

Bioavailability of Heavy Metals

Changes that control concentration and free metal activity of heavy metals in soil affect their bioavailability and uptake by plants (Spurgeon and Hopkin 1996). Soil properties that control the retention, transformation and mobility of metals include pH, redox potential, organic matter content and soil mineralogy (Calace *et al.* 2002). The effects of these different soil properties on plant metal uptake are detailed below.

pH

Soil pH is the major factor affecting metal availability for plant uptake (McBride 1994: 315, McLaughlin *et al.* 2000). Most heavy metals are soluble and mobile in acid soils. High pH increases the complexation of metals by functional groups of organic matter and oxides, which results in the reduction of metal concentration in the soil solution (Yoo and James 2002). According to Yoo and James (2002), pH controls the solubility of metals by influencing the extent of metal-complexation with organic C-based ligands. Lead (Pb^{2+}), for example, predominates in soil with a pH <6, and changes to the form $PbOH^+$ (solid phase) at pH levels between six and eleven (Pierzynski *et al.* 1994). The solid phases formed by heavy metals may also have pH-dependent solubilities that control their bioavailability (Pierzynski *et al.* 1994).

Redox potential

Soil redox potential is an important parameter that affects heavy metal transformation, solubility and uptake by plants (Carbonell-Barrachina *et al.* 1999). Metals with more than one oxidation state (e.g. Fe and Mn) are generally less soluble in their higher oxidation states (Ross 1994a). Reducing soil conditions in flooded areas promote high chemical reduction of Fe and Mn compounds, which results in increased solubility of Fe and Mn (McBride 1994: 317). The solubilised metals also can re-precipitate (Ross 1994a), limiting their movement to roots for absorption or uptake.

However, most heavy metals (e.g. Cu, Cd) are strongly immobilised by reducing conditions and are only available for plant uptake in oxidising environments (Yen *et al.* 1998, Pierzynski *et al.* 1994). For example, some studies show that Zn deficiency in rice grown in flooded paddy fields is a problem (Ross 1994b). The reduction of As^{5+} to As^{3+} increases the solubility and mobility of As in soils and sediments (Carbonell-Barrachina *et al.* 1999).

Organic matter, clay and oxide minerals

Bioavailability of metals decreases in soil with high amounts of organic matter, clay or oxides (McBride 1994: 121–164). Metals such as Cu and Pb form stable complexes with

organic matter. The quantities of organic matter, clay and oxides control metal speciation, movement and bioavailability, because the metal cations react with those components that have high specific areas and cation exchange capacity (Martinez and McBride 1999, Han *et al.* 2000). The complexation of metals by organic matter reduces the activities of metals in solution (Gardner 1999). Some heavy metals may be bound to humic substances in the inner-sphere complexes and become non-exchangeable (Xia *et al.* 1997, Yoo and James 2002).

Phytoremediation of Heavy Metals

Phytoremediation of soil contaminated by heavy metals is one of the emerging technologies that uses living plants either to extract these metals from the soil or render them harmless *in situ* (Lombi *et al.* 2001). Plant remediation provides a means of reducing environmental contamination (Salt *et al.* 1997). It has an advantage over other remedial options, because roots are present that can limit metal seepage in moist environments and dispersal by wind (Pierzynski *et al.* 1994). Phytoremediation allows a value-added, non-agricultural use of plants and will continue to expand in the future (Gleba *et al.* 1999).

Croplands polluted by heavy metals need to be rehabilitated, because stricter laws limiting concentrations of toxic metals in food crops will limit their availability for crop cultivation (Grčman *et al.* 2001). The rehabilitation of metal-contaminated sites is necessary to restore sites, keep them continually productive and limit human exposure to toxic elements.

Methods other than phytoremediation to clean up metal-contaminated sites have been applied. They include complete excavation of contaminated material, which is followed by treatment or *in situ* encapsulation (Pierzynski *et al.* 1994). A conventional remediation method involves soil excavation, transport to a decontaminating site, soil cleansing using chemical or physical treatments, and then return of the clean soil to its original site (Lasat *et al.* 2001). Conventional methods disturb the soil's physical properties and landscape, while *in situ* phytoremediation maintains them (Perronnet *et al.* 2000). Phytoremediation technology is cheaper to implement and has greater environmental benefits compared to conventional engineering methods such as excavation. The market for phytoremediation in the USA was estimated to be between $1 million and $2 million in 1997, and it is projected to reach $70–100 million by the year 2005 (Glass 2000). Phytoremediation of heavy metals can be divided into three types: phytoextraction, phytostabilisation and phytovolatilisation.

Phytoextraction

In phytoextraction, soil metal pollutants absorbed by plants accumulate in the shoots (Salt *et al.* 1998), which are harvested and incinerated. The ashes are then disposed of in secured sites to prevent further pollution (Blaylock *et al.* 1997, Robinson 2001). The ratio of metal concentrations in the soil and the plant is used to determine the effectiveness of the plant species in metal phytoextraction (Barman *et al.*

2000). A ratio greater than 1.0 indicates higher accumulation of metals in plant parts than in the soil. Plant species that have a ratio >1.0 are considered efficient for phytoremediation (Barman *et al.* 2000). The final amount of metal pollutant extracted is determined by the total biomass harvested, number of harvests carried out and the metal concentration in harvested portions of the plant (Cunningham and Ow 1996). Phytoextraction may be in the form of continuous (natural) phytoextraction, which involves natural hyper-accumulator plants or as induced phytoextraction, which involves adding soil amendments, especially synthetic chelating agents, to increase metal bioavailability and uptake (Salt *et al.* 1998). Chelates will be discussed in the next section.

Mejáre and Bülow (2001) divided metal-hyper-accumulating plants into three groups according to the metal that they tend to accrue, namely: Cu/cobalt(Co), Zn/Cd/Pb and Ni. Hyper-accumulators are usually small, weedy plants. The most studied metal hyper-accumulators to date include *Brassica juncea*, *Brassica oleracea*, *Berkeya coddii*, *Allysum bertolonii* and *Thlaspi caerulescens*, some of which can accumulate more than 1% of a specific metal in their shoot dry weight. Baker and Brooks (1989) defined metal hyper-accumulators as plants that can accumulate greater than $100\mu g\ g^{-1}$ (0.01%) of Cd; $1\ 000\mu g\ g^{-1}$ (0.1%) of Co, Cu, chromium (Cr), Pb and Ni; or more than $10\ 000\mu g\ g^{-1}$ (1%) of Mn or Zn in their tissues. There exist natural hyper-accumulators for specific metals. For instance, *B. juncea* is a hyper-accumulator for Cd, *T. caerulescens* of Zn and Cd, and *B. coddii*, *A. bertonii* and *Thlaspi goesingense* of Ni (Lasat *et al.* 2000). The limitation associated with this kind of phytoextraction is that hyper-accumulator plant species are rare and often grow slowly, producing small amounts of harvestable biomass (Ebbs *et al.* 1997, Salt *et al.* 1998). Some metals such as Pb are mostly immobile in soil, which reduces their bioavailability and thus their uptake by the plant (Lombi *et al.* 2001). Consequently, hyper-accumulators of Pb are uncommon.

One important feature that is found only in metal hyper-accumulators is that they allocate a smaller concentration of a heavy metal to the roots compared with leaves and stems (Baker *et al.* 1994). This is attributed to the efficient translocation of such metals from the roots to the shoots (Küpper *et al.* 2000) and is considered an advantageous strategy in plant heavy-metal tolerance, because the primary target of heavy-metal toxicity is the root system (Godbold *et al.* 1984).

Studies have shown that leaves are the main sinks for metal accumulation in hyper-accumulators (Psaras and Manetas 2001). Within the leaf, heavy metals are allocated predominantly to the epidermal cells and trichomes (Psaras and Manetas 2001, Salt *et al.* 1995a). The heavy metal allocation to trichomes may be a strategy for detoxification, because trichomes are part of the external tissue of the leaf (Salt *et al.* 1995a). However, plant species differ in the types of heavy metals that they sequester in their leaf trichomes. For example, Pb accumulates in the trichomes of *Nicotiana tabacum*, Mn in *Heliathus annuus*, Cd in *Brassica juncea* and Ni in *Alyssum lesbiacum* (Martell 1974, Blamey *et al.* 1986, Salt *et al.* 1995a, Kramer *et al.* 1997). Accumulation of

potentially toxic metals in leaves is thought to be a plant's defensive strategy against herbivores.

One strategy plants may adopt to increase heavy metal translocation from the roots to the shoot via the xylem stream is to increase their transpiration rate (Gleba *et al.* 1999). Accumulation of metals is thought to be driven primarily by mass flow caused by transpiration (Salt *et al.* 1995b). In fact, in a study in which plants were treated with ABA, it was found that a large reduction in Cd concentrations in leaves was strongly correlated with increased stomatal resistance (Salt *et al.* 1995b). However, metal translocation may be reduced by a high cation exchange capacity on the xylem cell walls (Salt *et al.* 1998) and may explain why a neutralisation of heavy metal cation charge by chelating agents enhances the translocation of metals to the shoots. Some studies suggest that the binding of heavy metals in cell walls may provide a means of detoxifying or sequestering them (Vögeli-Lange and Wagner 1996, Hart *et al.* 1998). In some plants, this is achieved by depositing some metals in the form of carbonates on the cell wall (Cunningham *et al.* 1995).

Several studies have shown that wheat grains accumulate high quantities of Cd in heavy-metal-polluted sites, but it still is unclear whether seeds of other taxa accumulate high concentrations of heavy metals. Brooks (1998) reported that heavy metal concentrations in seeds were negligible compared with those in other plant parts. However, Psaras and Manetas (2001) have reported high accumulation of Ni in seeds of *Thlaspi pindicum*. In non-hyper-accumulator plants heavy metals such as Cd are allocated more or less equally to various plant parts. In hyper-accumulator plants metals preferentially allocated to above-ground parts are stored in cellular vacuoles where they are matched with low-weight molecular compounds or adsorbed onto cell walls (Salt *et al.* 1999).

One way to improve phytoextraction is through the transfer of the genes that regulate hyper-accumulation. Insertion of them into rapidly-growing plants with high biomass is seen as an alternative for improving phytoextraction (Lasat *et al.* 2000). Genetic engineering has allowed the transfer of a bacterial gene for the transcription of mercuric reductase into *Arabidopsis thaliana*, mutants that tolerate and volatilise Hg (Rugh *et al.* 1996). Such engineering programmes may reduce the cost of phytoremediation (Salt *et al.* 1998).

Phytostabilisation

Phytostabilisation is the process by which plants immobilise metal contaminants in the soil. This is achieved through their absorption and accumulation by roots, adsorption onto roots, precipitation within the root zone and physical stabilisation in the soil (US EPA 2000). This type of phytoremediation decreases the bioavailability and mobility of metal contaminants and their percolation and erosion, which thereby prevents air and groundwater contamination (Miller 1996). This technology has been used in the treatment of contaminated soils, sediments and sludge, and was tested at Kansas State University and the University of Iowa in an effort to remediate mine-tailing sites with high levels of Cd, Pb and Zn (US EPA 2000). Successful phytostabilisation of Pb, Zn, Cd and As in soils has been achieved using hybrid poplars (Schnoor 1997).

Phytovolatilisation

Some plants take up heavy metals such as Hg, selenium (Se) and As and transpire them into the atmosphere or volatilise them into modified, harmless forms. Phytovolatilisation minimises the entry of Se into the food chain, because most of the Se may be volatilised below ground in the roots (Zayed and Terry 1994). The Se accumulator *Astragalus racemosus* volatilises Se as dimethyl diselenide (Evans *et al.* 1968, Parker *et al.* 1991). Root-symbiotic bacteria assist the plants in volatilising Se and As in the root zone (Salt *et al.* 1998).

As noted, the introduction of a modified bacterial mercuric ion reductase into transgenic *Arabidopsis thaliana* has increased the conversion of Hg^{2+} into Hg^0 making the transgenic Arabidopsis plant effective in Hg volatilisation (Rugh *et al.* 1996). Factors that increase the transpiration rate would probably increase the effectiveness of this technology. The problem with phytovolatilisation is that contaminants or hazardous metabolites can accumulate in the vegetation and be translocated into edible products such as fruit (Newman *et al.* 1997, US EPA 2000).

Use of Synthetic Chelating Agents for Phytoextraction

Unavailable forms of heavy metals are likely to be excluded by the plant-uptake process unless some chemical modification of the soil environment occurs to increase their bioavailability (Barbafieri 2000). When a chelating agent is added to soil, the formation of metal-chelate complexes in the soil solution decreases free metal activity, and this results in the desorption or dissolution of the soil-bound metals to compensate for the shift in equilibrium (Dushenkov *et al.* 1997). The dissolution of metals continues until either the chelate is saturated with metals, the supply of the metal from the solid phases is exhausted or the solid phase is no longer soluble. Chelate-assisted phytoextraction involves the release of bound metals into soil solution accompanied by transport of metals to the harvestable shoot (Salt *et al.* 1998).

There are two advantages associated with chemically-enhanced phytoextraction. First, it is applicable even in
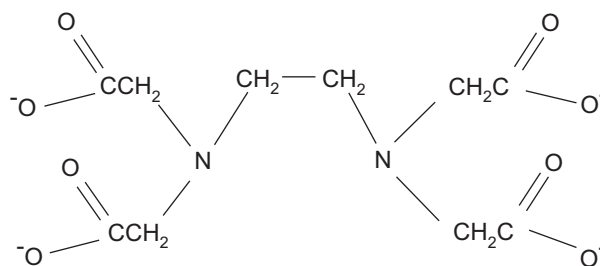

Figure 1: The chemical structure of ethylenediamine-tetraacetic acid (EDTA) that is used in phytoremediation (adapted from Sinex 2004)

situations where metals are less mobile and available for plant uptake. Second, it is a relevant technology if no natural hyper-accumulator for the metal is known (McGrath *et al.* 2002). The chemical amendments mostly used for phytoextraction are ethylenediamine-tetraacetic acid (EDTA), diethylenetriamine-pentaacetic acid (DTPA), ethylenebis-(oxyethylenenitrolo)-tetraacetic acid (EGTA), ethylenediaminedi(o-hydroxyphenylacetic) acid (EDDHA), N-(2-hydroxyethyl)ethylenediamine-tetraacetic acid (HEDTA) and citric acid. Of these, EDTA has been most frequently used as an amendment for phytoextraction, because it has a strong affinity for different heavy metals (Norvell 1991). Its chemical structure is presented in Figure 1. The effectiveness of different ethylene-based chelating agents in increasing heavy-metal solubility in soil solution, plant uptake and shoot accumulation has been reported in the sequence EDTA > HEDTA > DTPA > EGTA > EDDHA (Huang *et al.* 1997, Blaylock *et al.* 1997).

EDTA has been used to increase the accumulation of metals in shoots of plants such as Indian mustard (*Brassica juncea)* (Blaylock *et al.* 1997, Huang *et al.* 1997, Wu *et al.* 1999), Chinese cabbage (*Brassica rapa*) (Grčman *et al.* 2001) and sunflower (*Heliathus annuus*) (Kirkham 2000, Liphadzi *et al.* 2003). Haag-Kerwer *et al.* (1999) showed that about 80% of the total soil metal is solubilised and becomes available for phytoremediation. Furthermore, application of EDTA to Pb-contaminated fields planted with corn (*Zea mays*) resulted in a 140-fold increase in Pb concentration in the xylem sap, and a net increase in Pb translocation from roots to shoots via the transpiration stream (Huang *et al.* 1997, Salt *et al.* 1998).

Synthetic chelating agents such as EDTA also allow plants not classified as hyper-accumulators to be usable for phytoremediation purposes, because EDTA induces them to take up more heavy metals than they normally can accumulate. The enhancement of metal uptake and translocation by EDTA has been attributed to an alteration of membrane permeability caused by the removal of Zn and Ca in the plasma membrane (McGrath *et al.* 2002), which leads to an efflux of K^+ from the cytosol to the apoplast (Vazquez *et al.* 1999). Another way in which EDTA promotes heavy metal accumulation is that it prevents cell wall binding and deposition of heavy metals, thereby enhancing metal translocation to the plant shoots (Blaylock *et al.* 1997).

Phytotoxicity at locations highly contaminated by metals may occur even before the application of the synthetic chelate, and this reduces the chance of success with phytoextraction (Sun *et al.* 2001). Application of EDTA after flowering should allow perennial plants to develop a larger biomass in the subsequent growing season (Salt *et al.* 1998, Sun *et al.* 2001) before they suffer from the phytotoxic effects of EDTA and EDTA-metal complexes. The harmful effects of EDTA at high concentrations have been attributed to its behaviour as a detergent (Sillanpaeae and Oikari 1996, Dirilge 1998, Shahandeh and Hossner 2000).

The main environmental concern about the application of EDTA to soils for phytoextraction and soil amendment purposes is that the heavy metals solubilised or complexed by EDTA, if not taken up by roots, may be leached down the soil profile and contaminate groundwater (Grčman *et al.*

2001, McGrath *et al.* 2002). The biological stability of EDTA may allow metal-EDTA complexes to remain in the soil over a whole growing season (Hong *et al.* 1999, Nortemann 1999, Satroutdinov *et al.* 2000), a disadvantage in high rainfall areas with shallow ground-water tables.

Mechanisms for Metal Acquisition by Plants

Plant roots can produce exudates that may solubilise and/or chelate metals for uptake. They also may be involved in metal translocation and detoxification (Hall 2002). Numerous studies have shown that phytosiderophores produced by plants such as durum wheat (*Triticum durum*) and barley (*Hordeum vulgare*) chelate metals, particularly Fe, which facilitates their uptake (Tagaki *et al.* 1984, Zhang *et al.* 1991, Römheld 1991, Hopkins *et al.* 1998, Clemens 2001). When a metal is chelated by phytosiderophores, the phytosiderophore-metal complex is transported across the cell membrane via specialised transporters (Von Wiren *et al.* 1995, 1996). Grain crops such as durum wheat are known to accumulate high concentrations of Cd in the grains when grown on Cd-polluted sites. This tendency by wheat and other monocots to accumulate Cd is associated with their high phytosiderophore production (Römheld 1991). Uptake and shoot accumulation of Cu, Zn and Mn also increases with phytosiderophore production in Fe-deficient soil (Shenker *et al.* 2001).

Dicotyledonous plants improve metal bioavailability in soil by extrusion of protons (H^+) into the rhizosphere (Lasat 2002). Most heavy metals are soluble at acidic pH. Moreover, an acidic environment induces the reduction of ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}), which is readily taken up by plants (Lasat 2002).

Various transporters for different metals have been identified on the plasma membrane and tonoplast in plants (Figure 2). The main transporters of Zn across the plasma membrane include a zinc transporter (ZNT1), two zinc-regulated transporters 1 and 2 (ZRT1–2) and four zinc inducible proteins (ZIP1–4) (Clemens 2001). The zinc inducible proteins 1, 2 and 3 are confined to the roots, while ZIP4 is found in both the shoots and the roots (Clemens 2001).

Studies on *Arabidopsis* indicate that Fe uptake by the roots from the soil is mediated by an iron-regulated transporter (IRT1) (Clemens 2001). Korshunova *et al.* (1999) found that IRT1 also transports Mn, Zn and Cd (Clemens 2001). Deficiency in Fe can induce a high uptake of other metal ions because, when soil Fe is limited, there is an expression of Fe-transporter proteins that facilitates conveyance of Fe and other metals (Cohen *et al.* 1998). Another family of transport proteins involved in uptake and transport of Fe is a natural resistant associated macrophage protein (Nramp) (Vidal *et al.* 1993). There also exists a Cu transporter protein (COPT1), which is involved exclusively in Cu translocation within the plant and is not present in the roots (Clemens 2001).

Cadmium has no specific transporter and is thought to be conveyed by transporters of other essential elements such as Nramp (Guerinot 2000, Thomine *et al.* 2000). Conveyance of Ca^{2+} also may facilitate transport of Cd across the plasma membrane in wheat (Clemens *et al.*
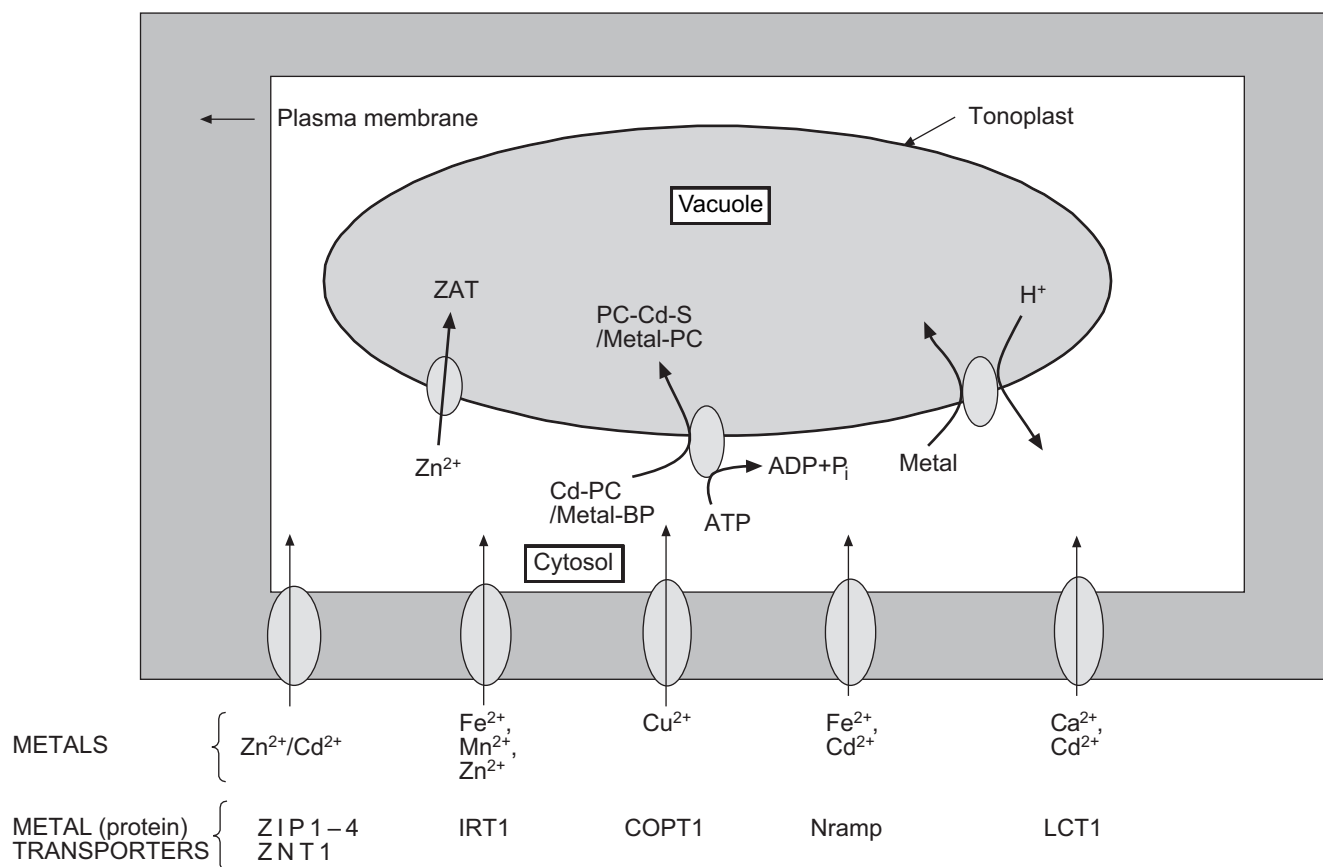
Figure 2: Identified metal transporters (or transport proteins) in the plasma membrane and tonoplast in plants, where PC = phytochelatins, BP = binding proteins, P_i = inorganic phosphate, S = sulfhydryl group, ZIP = zinc inducible protein, ZNT = zinc transporter, IRT = iron-regulated transporter, COPT = copper transporter, Nramp = natural resistant associated macrophage proteins and LCT = lead-calcium transporter (adapted from Clemens 2001, Rauser 1995a, 1995b)

1998). Inhibition of Cd uptake by Zn in soybean (*Glycine max*) suggests that Cd uptake is mediated by a Zn transport system (Cataldo *et al.* 1983). This suggestion is supported by findings of Dowdy and Larson (1975), Cunningham *et al.* (1975), and Haghiri (1974), who observed an antagonistic relationship between Zn and Cd in which high Zn levels reduced Cd uptake. The transport protein for Pb and Ca^{2+} is lead-calcium transporter (LCT1) (Clemens *et al.* 1998).

Transportation of heavy metals into the cell vacuole for compartmentation may occur by a metal/H^+ antiport in which metal conveyance into the vacuole is accompanied by a simultaneous movement of H^+ out of the vacuole. Alternatively, it may involve the activities of ATP-dependent transporters located at the tonoplast (Salt and Wagner 1993, Salt and Rauser 1995, Rea *et al.* 1998). Compartmentation of heavy metals into vacuoles is an effective mechanism that plants use to reduce toxicity of heavy metals in the cytosol (Vögeli-Lange and Wagner 1990, Apse and Blumwald 2002). Several studies show that compartmentation of Ni, Zn and Cd or Cd-PC occurs in the vacuole of plants tolerant of metal-polluted soils (Davies *et al.* 1991, Ernst *et al.* 1992, Brune *et al.* 1994, De 2000).

Another way in which heavy-metal uptake occurs in plants is through the destruction of the integrity of the plasma membrane (Vazquez *et al.* 1999). Toxicity of heavy metals

increases the permeability of the plasma membrane, a mechanism linked to the displacement of Ca^{2+} from the plasma membrane (Vazquez *et al.* 1999). When the plasma membrane is damaged, K^+, which is normally present in the cytosol at high concentrations, flows out of the cytosol to the apoplast. High K^+ in the apoplast cause the development of an electrochemical gradient between the cytosol and the apoplast (Vazquez *et al.* 1999). For charge balancing, the heavy metals already surrounding the cell then enter the cell. This mechanism of metal uptake also was reported by Zhu *et al.* (2000), who found that K^+ deprivation in wheat enhanced caesium (Cs) uptake by roots.

Translocation of heavy metals from the roots to the shoot is thought to occur via the xylem, driven by the transpiration force established in the leaves (Salt *et al.* 1995a, Hart *et al.* 1998). Translocation of these metals from the roots to shoots may also be enhanced by metal-binding ligands produced by plants (Vögeli-Lange and Wagner 1990).

Detoxification Strategies in Plants

Plants have several mechanisms at the cellular and subcellular levels that are involved in the sequestration or detoxification of toxic heavy metals (Hall 2002). Antioxidation and chelation are the most studied

mechanisms. Chelation is a means of avoiding the build-up of toxic metals at or near sensitive organelles in the cell and thus preventing their damage (Hall 2002). In heavy metal-tolerant plants, toxic metals are bound by chelators and chaperones (Clemens 2001). Chelators such as metal-binding peptides (including metallothioneins and phytochelatins), organic acids and amino acids are involved in metal detoxification by buffering cytosolic metal concentrations. Chaperones deliver the metal ions to the vacuole for vacuolisation and binding to metal requiring proteins (Clemens 2001). Known mechanisms that plants use to detoxify toxic metals are as follows.

Antioxidants

Heavy-metal toxicity enhances the production of reactive oxygen species, which are deleterious to sensitive organelles. Some plants tolerate these effects by reducing the level of reactive oxygen species (ROS) in their tissues. Tolerant plants keep the ROS level down through activities of the antioxidative defence systems (Schützendübel and Polle 2002), which include the metabolites ascobate, glutathione and tocopherol, as well as enzymatic scavengers of activated oxygen such as superoxide dismutases (SOD), peroxidases and catalases (CAT), ascobate peroxidases (APX), glutathione S-transferases (GST) and glutathione peroxidases (GPX) (Noctor and Foyer 1998, Asada 1999, Apse and Blumwald 2002, Kawano *et al.* 2002). Some Cd- tolerant plants overcome the metal's toxicity by increasing their production of glutathione (Schützendübel and Polle 2002). In general, toxicity from transition heavy metals (e.g. Fe and Cu) may cause an increase in production of reactive oxygen species (ROS), which are superoxide radicals ($O_2^{.}$), hydroxyl radicals ($HO^{.}$) and hydrogen peroxide (H_2O_2) (Dietz *et al*. 1999, Schützendübel and Polle 2002). ROS are produced when metabolism that occurs in chloroplasts and mitochondria is inhibited during stress (Apse and Blumwald 2002). Transition metals produce ROS by auto-oxidation, because they are redox-active metals (Schützendübel and Polle 2002). Although ROS may be important for a plant's defence system against pathogens, they also are potentially destructive to the cell because they cause oxidation of proteins and membrane lipids or cause DNA injury (Apse and Blumwald 2002, Schützendübel and Polle 2002).

High levels of Cd and Hg (non-redox-reactive heavy metals) in the plant tissue cause oxidative stress, which results in lipid peroxidation, H_2O_2 accumulation and an oxidative burst (Schützendübel and Polle 2002). Elevated Cd in plants causes a transient depletion of glutathione (GSH) and an inhibition of anti-oxidative enzymes, especially glutathione reductase (Schützendübel and Polle 2002). One damaging mechanism of non-redox-reactive heavy metals in plants is that they bind strongly to oxygen, nitrogen and sulphur atoms (Nieboer and Richardson 1980), which results in blocking of essential functional groups in biomolecules (Schützendübel and Polle 2002). For instance, these heavy metals can inactivate enzymes by binding to cysteine residues or sulfhydryl groups of enzymes or structural proteins (Van Assche and Clijsters 1990, Vögeli-

Lange and Wagner 1990). The displacement of Mg by heavy metals such as Ni and Zn in enzymes inactivates or inhibits activities of the enzymes (Van Assche and Clijsters 1986).

Polyphenolics

Polyphenolics, which include tannins and lignin precursors (Strack *et al.* 1989), are potential antioxidants and have the ability to chelate heavy metals such as Fe (Rice-Evans *et al.* 1996). Lummerzheim *et al.* (1998) found that the response to Pb toxicity by *Arabidopsis thaliana* was accompanied by the accumulation of polyphenolics. Polymerisation of polyphenolics by peroxidases, which increases after heavy metal uptake and detoxification, is responsible for the binding of heavy metals in waterlily (Nymphaea) epidermal glands (Lavid *et al.* 2001). Plants that are rich in tannins such as tea plants are tolerant of elevated levels of Mn, because tannins reduce Mn toxicity by chelating Mn (Aoba 1986).

Metal-binding peptides

Metal-binding peptides provide another mechanism for metal tolerance by chelating heavy metals. These reduce the intracellular concentration of free toxic heavy metals or render them unavailable for interaction with metabolically-active cellular compartments (Vögeli-Lange and Wagner 1990). Cadmium-binding proteins (CdBPs), which are induced by the presence of Cd, have a high affinity for this metal and are involved in its detoxification (Vögeli-Lange and Wagner 1990). Studies indicate a positive correlation between the occurrence of CdBPs and tolerance to Cd (Stefens *et al*. 1986, Grill *et al.* 1987, Reese and Wagner 1987, Scheller *et al.* 1987).

Biosynthesis of metal-binding proteins in the cytoplasm occurs when the plant is exposed to elevated levels of the toxic metal. For instance, CdBPs form metal-binding peptide complexes in the cytosol and are then translocated into the vacuole (see Figure 2) (Vögeli-Lange and Wagner 1990). In the high acidic environment in the vacuole, the metal-binding peptide complexes are dissociated from the metal-binding peptides (Reese and Wagner 1987) and form complexes with organic acids or amino acids present in the vacuole (Krotz *et al.* 1989). Also, the metal-binding peptides may serve as a shuttle for transferring metals from the cytosol into the vacuole (Vögeli-Lange and Wagner 1990). Two types of cysteine-rich peptides that bind heavy metals in the cell are phytochelatins and metallothioneins (Hall 2002).

Metallothioneins are cysteine-rich proteins with low molecular weights that bind metal ions in metal-thiolate clusters (Hamer 1986, Rauser 1995a, 1995b). Metallothioneins are involved in the detoxification of metals, buffering of cytosolic metals, scavenging of metals during leaf senescence or metal secretion by the leaf trichomes (Garcia-Hernandez *et al.* 1998, Rauser 1995b).

Phytochelatins (PCs) are small peptides with a general structural formula of γ-(Glu-Cys)$_n$-Gly (n = 2–11) that bind metal ions (Kondo *et al.* 1984, Jackson *et al*. 1987, Hall 2002). These cysteine-rich polypeptides are synthesised from glutathione (GSH) by γ-glutamylcysteine synthetase and phytochelatin synthase (Zenk 1996, Cobbett *et al.* 1998,

Ha *et al.* 1999). Plants produce phytochelatins from reduced GSH in response to heavy-metal toxicity (Hall 2002). Phytochelatin biosynthesis is induced by different levels of a variety of heavy metals, the most effective being Ag, Cd, As, Cu, Pb and Hg (Cobbett 2000, Mejáre and Bülow 2001).

The sequestration of heavy metals like Cd by phytochelatins (Schützendübel and Polle 2002) involves the formation of low molecular weight Cd-thiolate (Cd-S) complexes (Strasdeit *et al.* 1991) in the cytosol which are transported to the tonoplast (Figure 2). They then are taken up by active transport systems (e.g. metal/H^+ antiporter) and deposited in the vacuole, where the phytochelatin-metal forms stable high molecular weight Cd-PC complexes by reacting with sulphides (Tommasini *et al.* 1998, Rea 1999, Clemens 2001, Hall 2002). The incorporation of sulphides into the high molecular weight complexes of Cd-PCs (PC-Cd-S) not only increases stability of the complexes, but also increases the amount of Cd per molecule (Cobbett 2000).

Organic acids

The production of organic acids by plants assists in the detoxification of heavy metals in the rhizosphere and cytosol, thereby enhancing plant tolerance of these metals (Shahandeh and Hossner 2000). The involvement of organic acids in aluminum detoxification has been clearly documented (Cobbett 2000). Citrate (Ni-citrate) is also thought to be a Ni detoxification agent and involved in the transportation of toxic Ni from roots to leaves (Brooks 1998). Organic acids are also involved in xylem transport and metal storage in the shoots (Salt *et al.* 1999). Citric acid is considered to be the main ligand or chelator for Cd when Cd is present at low concentrations (Wagner 1993) and is also involved in plant Zn and Ni tolerance (Godbold *et al.* 1984, Sagner *et al.* 1998). Citrate has a high affinity for heavy metals, and the metal-citrate complexes formed seem stable in vacuoles with an acid pH of 5.5 (McGrath *et al.* 2002). Another chelator for Zn in Zn-tolerant plants is malate (Mathys 1977).

Amino acids

Histidine influences metal (especially Ni) accumulation, tolerance and transport to shoots in both hyper-accumulator plant species and other taxa (Shahandeh and Hossner 2000). The amount of histidine present in xylem sap strongly correlates with the Ni concentration (Kramer *et al.* 1996) with an increase in Ni concentration in the xylem sap inducing a concomitant increase in histidine (Clemens 2001). Also, Salt *et al.* (1999) reported a positive association between Zn and histidine in roots.

Nicotianamine is a non-proteinaceous amino acid synthesised from three molecules of s-adenosyl methionine (Clemens 2001). Nicotianamine is a chelator of Fe and several other divalent metal ions in the plant (Von Wiren *et al.* 1999). Although nicotianamine is a precursor of a phytosiderophore, it is not exuded by roots (Clemens 2001).

Recommendations

Decision makers should support remediation of the environment polluted by by-products of mines and industries. They should consider phytoremediation, which has become a multimillion dollar industry in developed countries. Besides being cheap, it is an on-site operation. The construction of drainage systems below contaminated soil layers to capture the heavy metal-laden leachates for recycling could defray remediation costs. Furthermore, phytoremediation may provide a means of retrieving essential elements (e.g. Fe, Zn) in food crops to cure or prevent diseases that are caused by lack of these nutrients.

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