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Heavy Metal Accumulation and Detoxification Mechanisms in Plants

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Abstract : Toxic metal contamination of soil, aqueous waste streams and groundwater causes major environmental and human health problems. The most commonly used methods for dealing with heavy metal pollution are still extremely costly. Phytoremediation is the use of plants to extract, sequester and/or detoxify pollutants and is a new and powerful technique for environmental clean-up. Plants are ideal agents for soil and water remediation because of their unique genetic, biochemical and physiological properties. Considerable advances have been made in recent decades in developing endemic or genetically engineered plants for the remediation of environmental clean-up. In the present review, current knowledge about metal accumulation and detoxification mechanism in plants is discussed. The importance and potential commercial applications of the phytoremediation are described.

Key Words: Plants, heavy metals, metallothionein, phytochelatins, phytoremediation

Bitkilerde Ağır Metal Toplanması ve Detoksifikasyon Mekanizmaları

Özet : Toprak, atık su ve akarsuların toksik metallerle kirliliği, çevre ve insan sağlığı açısından önemli bir sorun oluşturmaktadır. Ağır metal kirliliğini gidermek için günümüzde kullanılan metodlar fazlasıyla pahalıdır. Fitoremediasyon, kirleticileri özütlemek ve/ve ya detoksifiye etmek ve çevrenin temizlenmesi için bitkilerin kullanımını kapsayan oldukça güçlü bir tekniktir. Bitkiler, toprak ve su remediasyonu için, benzersiz genetik, biokimyasal ve fizyolojik özellikleri nedeniyle ideal araçlardır. Son yıllarda çevresel temizlemenin remediasyonu için endemik ya da genetik olarak elde edilmiş bitkilerin geliştirilmesinde farkedilir gelişmeler yaşanmıştır. Bu çalışmada, bitkilerdeki metal toplama ve detoksifikasyon mekanizması hakkındaki mevcut bilgiler tartışılmış ve fitoremediasyonun önemi ve potansiyel ticari uygulaması tanımlanmıştır.

Anahtar Sözcükler: Bitkiler, ağır metaller, metalotiyonin, fitokelatinler, fitoremediasyon

Introduction

Heavy metals are important environmental pollutants and many of them are toxic even at very low concentrations. Pollution of the biosphere with toxic metals has accelerated dramatically since the beginning of the industrial revolution (Nriogo, 1979). The primary sources of this pollution are the burning of fossil fuels, the mining and smelting of metalliferous ores, municipal wastes, fertilizers, pesticides, and sewage (Kabata-Pendias and Pendias, 1989).

Toxic metal contamination of soil, aqueous waste streams and groundwater poses a major environmental and human health problem, which is still in need of an

effective and affordable technological solution. In spite of the ever-growing number of toxic metal-contaminated sites, the most commonly used methods of dealing with heavy metal pollution are still either the extremely costly process of removal and burial or simply isolation of the contaminated sites. In addition to sites contaminated by human activity, natural mineral deposits containing particularly large quantities of heavy metals are present in many regions of the globe. These areas often support characteristic plant species that thrive in these metal-enriched environments. Some of these species can accumulate very high concentrations of toxic metals to levels which far exceed the soil levels (Baker and Brooks,

1989). In many ways, living plants can be compared to solar driven pumps which can extract and concentrate several elements from their environment. From soil and water, all plants have the ability to accumulate heavy metals which are essential for their growth and development. These metals include Mg, Fe, Mn, Zn, Cu, Mo and Ni (Langille and MacLean, 1976). Certain plants also have the ability to accumulate heavy metals which have no known biological function. These include Cd, Cr, Pb, Co, Ag, Se and Hg (Hanna and Grant, 1962; Baker and Brooks, 1989). However, excessive accumulation of these heavy metals can be toxic to most plants. The ability to both tolerate elevated levels of heavy metals and accumulate them in very high concentrations has evolved both independently and together in a number of different plant species (Ernst *et al.*, 1992).

In this review, we summarize current knowledge concerning metal accumulation and detoxification mechanisms in plants and the potential commercial application of this phenomenon in phytoremediation.

Plant Responses to Heavy Metals

Plants have developed three basic strategies for growing on contaminated and metalliferous soils (Baker and Walker, 1990).

1. **Metal excluders:** These plants effectively prevent metal from entering their aerial parts over a broad range of metal concentrations in the soil; however, they can still contain large amounts of metals in their roots.
2. **Metal indicators:** These plants accumulate metals in their above-ground tissues and the metal levels in the tissues of these plants generally reflect metal levels in the soil.
3. **Accumulators:** These plant species (hyperaccumulators) can concentrate metals in their above-ground tissues to levels far exceeding those present in the soil or in the non-accumulating species growing nearby. It has been proposed that a plant containing more than 0.1% of Ni, Co, Cu, Cr or Pb or 1% of Zn in its leaves on a dry weight basis is called a hyperaccumulator, irrespective of the metal concentration in the soil (Baker and Walker, 1990). The information related to accumulator plants is most needed in four areas: first, the metal-accumulating ability of various species as a function of soil metal

concentrations, physical and chemical soil properties, physiological state of the plant, etc.; second, the specificity of metal uptake, transport and accumulation; third, the physiological, biochemical and molecular mechanisms of accumulation and hyperaccumulation; and fourth, the biological and evolutionary significance of metal accumulation.

Mechanisms of Metal Accumulation

Plants distribute metals internally in many different ways. They may localize selected metals mostly in roots and stems, or they may accumulate and store other metals in nontoxic form for latter distribution and use. A mechanism of tolerance or accumulation in some plants apparently involves binding potentially toxic metals at cell walls of roots and leaves, away from sensitive sites within the cell or storing them in a vacuolar compartment. A pressing environmental question about heavy metals concerns the amounts that plants can tolerate and accumulate without adverse effects. A suitable answer to this question will define the limits of plant growth relative to critical metal exposures. A further question relates more to metal form than to metal quantity. The metal form in plants appears to have a decisive role in metal transfer to other organisms. It is of great interest that plant species which have no exclusion mechanism in the roots absorb and translocate large concentrations of metals and accumulate them in their growing parts, especially in their leaves, without showing any toxicity symptoms, via a sort of internal resistance or accumulation mechanism. Many types of heavy metal resistance and tolerance mechanisms have been suggested, especially for Cu, Zn, Ni and Cr, in plants growing on metalliferous soils (Turner, 1970; Turner and Marshall, 1971; Antonovics *et al.*, 1971). Fe, Mn and Cu (Turner and Marshall, 1971; Memon *et al.*, 1979), Ni and Co (Memon *et al.*, 1980a), Cd and Zn (Memon *et al.*, 1980b), Pb (Brooks, 1983), and Se (Banuelos and Meek, 1990) accumulator plants have been reported.

Memon and co-workers, while working with 62 plant species in 39 genera and 27 families from the natural forest of Central Japan, reported several multi-accumulator plant species concentrating several hundred-fold levels of Mn, Cu, Zn, Cd, Co and Ni in their leaves (Memon *et al.* 1979; Memon *et al.* 1980a; Memon *et al.* 1980b) compared to non-accumulator plants. Very high accumulations of these metals were found in leaves of

Acanthopanax sciadophylloides Franch. & Sav. (Mn: 4600 ppm), *Ilex crenata* Thunb. (Mn: 1155 ppm, Zn: 730 ppm) *Clethra barbinervis* Siebold. & Zucc. (Mn: 1374 ppm, Co: 25 ppm) and *Sasa borealis* Makino. & Siebata (Ni: 16 ppm). The concentration ratios of the elements (content in leaves/content in A horizon soil) were as follows: *A. sciadophylloides* (Mn: 767), *Ilex crenata* (Mn: 191, Zn: 177), *Clethra barbinervis* (Mn: 227, Co: 125) and *Sasa*

borealis (Ni: 30). These values were many times higher than those of low metal content plant species. Mn in *Acanthopanax sciadophylloides* was 180 times higher, Zn in *I. crenata* was 90 times higher, Co in *C. barbinervis* was 50 times higher and Ni in *Sasa borealis* was 8 times higher than in low metal content plant species. Characteristic accumulation patterns of Mn are shown in Table 1.

Table 1. Manganese content in the leaves of accumulator plants.

Plant name	Plant D.W.**	Soil Available	Concentration ratio***	Reference
Mn content, ppm				
<i>Acanthopanax sciadophylloides</i> Franc. & Sav.	4632	6± 5.7	767	Memon <i>et al.</i> (1979)
<i>Pieris japonica</i> D.Don ex G.Don	3286	6± 5.7	544	Memon <i>et al.</i> (1979)
<i>Ilex crenata</i> Thunb.	2022	6± 5.7	335	Memon <i>et al.</i> (1979)
<i>Rhododendron Semibarbatum</i> Maxim.	1919	6±5.7	318	Memon <i>et al.</i> (1979)
<i>Acer sieboldianum</i> Miq.	1687	6± 5.7	279	Memon <i>et al.</i> (1979)
<i>Acer rufinerve</i> Siebold & Zucc.	1627	6± 5.7	269	Memon <i>et al.</i> (1979)
<i>Acer micranthum</i> Siebold & Zucc.	1558	6± 5.7	258	Memon <i>et al.</i> (1979)
<i>Lindera Erythrocarpa</i> Makino.	1416	6± 5.7	234	Memon <i>et al.</i> (1979)
<i>Clethra barbinevris</i> Siebold & Zucc.	1374	6± 5.7	227	Memon <i>et al.</i> (1979)
<i>Macadamia neurophylla</i> (Gauill.) Virost	40733	100 ~ 200	200 ~ 400	Jaffere (1979)
<i>M. augustifolia</i> Virost	11109	100~ 200	55 ~ 110	Kelley <i>et al.</i> (1975)
<i>Betula verrucosa</i> Ehrh.	1500*	35 ~ 70	20 ~ 40	Lounma (1956)
<i>Sorbus aucuparia</i> L.	1300*	35 ~ 70	20 ~ 40	
<i>Clethra barbinervis</i> Siebold & Zucc.	800*	70 ~ 150	5 ~ 10	Yamagata <i>et al.</i> (1960)
<i>Castanea crenata</i> Siebold & Zucc.	1100*	45 ~ 90	7 ~ 15	
<i>Quercus</i> L. spp.	800*	90 ~ 180	5 ~ 10	
<i>Black gum</i>	900*	3 ~ 7	130 ~ 300	Connor and Shacklette (1975)
<i>Sumac</i>	1400*	3 ~ 7	200 ~ 470	
<i>Sweet gum</i>	1300*	8 ~16	80 ~ 160	

*Calculated by dividing the concentration in ash by 10 (estimating that ash will be 10% of dry matter)

Dry weight, * Calculated by dividing Mn concentration in dry matter by available Mn in soil

To determine the metal distribution pattern at the sub-cellular level, electron probe X-ray microanalysis was performed with the fresh leaves, and petiole specimens which were unfixed and frozen in liquid nitrogen to minimize artifacts. Figures 1 and 2 show the distribution of Mn in the petioles of *A. sciadophylloides* and in the leaves of the tea plant (*Thea sinensis* L.), respectively. Most of the Mn was accumulated in the cell walls of epidermis, collenchyma, bundle sheath cells and in a vacuolar compartment (Figs. 1 & 2), away from metabolically active compartments, e.g., cytosol,

mitochondria and chloroplast (Memon, 1980; Memon, 1981). Cell fractionation analysis with *A. sciadophylloides* leaves confirmed the results of X-ray microprobe analysis and showed that most of the Mn was present in cell walls and in supernatant (Table 2) (Memon and Yatazawa, 1984). Gel chromatography analysis of supernatant with Sephadex G-10 showed that a very large amount of Mn in this fraction was present in the region, indicating a molecular weight of approximately 145 (Fig. 3). High performance liquid chromatography and high voltage paper electrophoresis analysis showed that Mn was

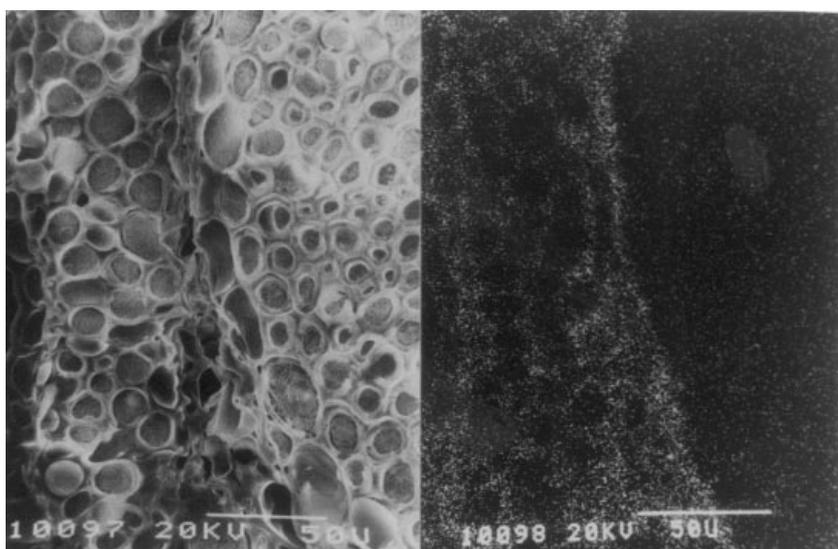


Figure 1. a. Secondary electron image (SEI). b. Mn X-ray image of the petiole of *Acanthopanax sciadophylloides* Franch & Sav.

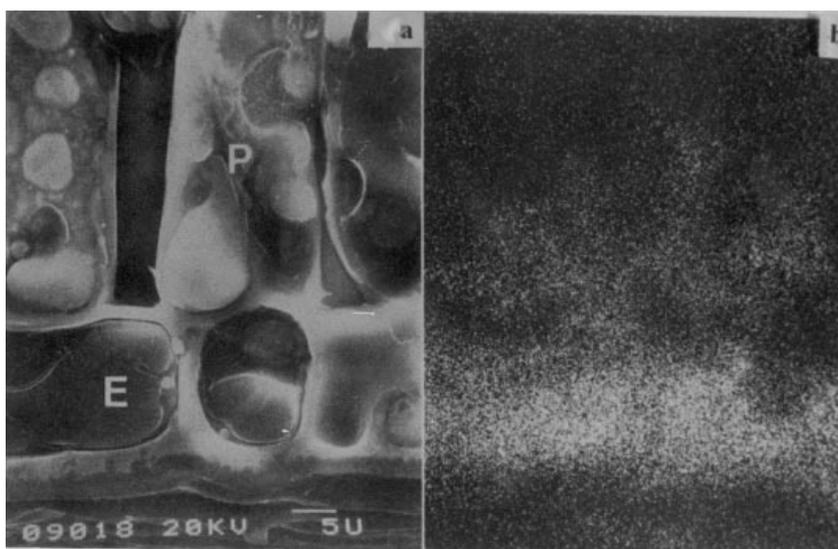


Figure 2. a. Highly magnified secondary electron image (SEI). b. Mn X-ray image of the epidermis (E) and parenchyma (P) cells of *Thea sinensis* L.

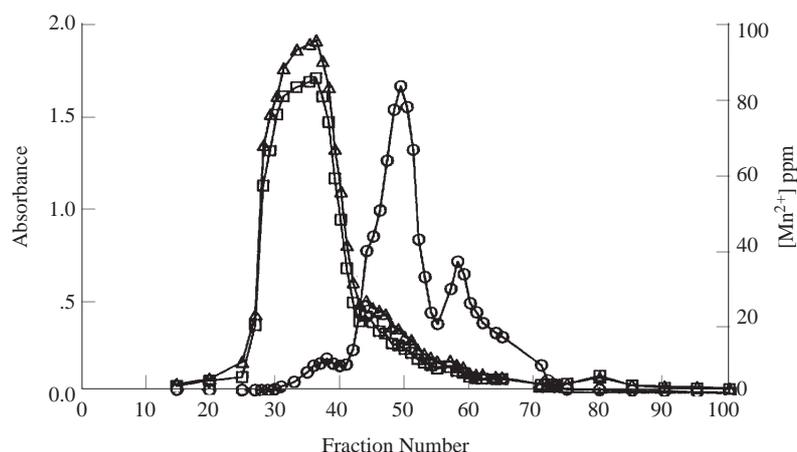


Figure 3. Separation of Mn complexes in the supernatant of *Acanthopanax sciadophylloides* by gel chromatography. Gel filtration of supernatant taken at 105000 x g (plant material was ground with Tris-HCl buffer, pH 7.5 + 0.5 M sucrose) on sephadex G-10. Column 1.6 x 75; eluent Tris-HCl buffer solution (pH 7.5) containing 1 M KCl; flow rate 20 ml h⁻¹; fraction size 2 ml. Absorbance at 260 nm (Δ), at 280 nm (\square), Mn (\circ).

Table 2. Distribution of manganese among differential centrifugation fractions of the leaves of *Acanthopanax sciadophylloides*.

Fractions	Concentration of Mn* $\mu\text{g/g}$ (F. W.)
A (Cell Wall fraction)	22
B (Chloroplast fraction)	5
C (Mitochondrial fraction)	37
D (Ribosomal fraction)	7
E Supernatant	259
Total	330
Total in plant leaves	526
Difference	196

* Please see ref. Memon et al. (1984).

chelated with oxalic acid in a vacuolar compartment (Memon and Yatazawa, 1984). The following mechanism of Mn detoxification was suggested from these experiments: Mn^{2+} is taken up at the plasma membrane and binds with malate in the cytoplasm and this Mn-malate complex is transported through the tonoplast membrane to the vacuole where Mn dissociates from malate and complexes with oxalate. Here malate functions as a "transport vehicle" through the cytoplasm

and oxalate as the "terminal acceptor" in the vacuole (Memon and Yatazawa, 1984). Several other mechanisms may contribute to heavy metal tolerance, depending on the type of metal and plant species, among them:

1) Induction of Metal Chelating Proteins – Phytochelatins and Metallothioneins

Induction of metal chelating proteins related to phytochelatins (γ -glutamylcysteinyl isopeptides) (Zenk, 1996; Clemens *et al.* 1999; Cobbet, 2000), and/or metallothioneins (Robinson *et al.* 1993; Robinson *et al.* 1997; Rauser, 1999), which by modifying the cell metabolism increases the level of cell tolerance to excess metal ions.

Phytochelatins

Phytochelatins form a family of peptides that consists of repetitions of the γ -Glu-Cys dipeptide followed by a terminal Gly, the basic structure being $(\gamma\text{-Glu-Cys})_n\text{-Gly}[\text{PC}]_n$, where n is generally in the range of two to five. Phytochelatins are synthesized enzymatically from glutathione (GSH) in response to many metals (Rauser, 1990). They are structurally related to glutathione (GSH) and are presumed to be the products of a biosynthetic pathway $\text{Gly}+\text{Cys} \xrightarrow{\text{GCS}} \gamma\text{-Glu-Cys} + \text{Glu} \xrightarrow{\text{GS}} \text{GSH} \xrightarrow{\text{PSC}} \text{PC} \xrightarrow{\text{PC-Cd}} \text{PC-Cd} \xrightarrow{\text{HMTI}} \text{Vacuole}$; where GCS= γ -glutamylcysteine synthetase, GS=glutathione synthetase, PCS=phytochelatin synthetase, HMTI=heavy metal tolerance 1, ABC type vacuolar membrane transporter of PC-Cd complexes). A number of other structural variants of PCs, such as $(\gamma\text{-Glu-Cys})_n\text{-}\beta\text{-Ala}$, $(\gamma\text{-Glu-Cys})_n\text{-Ser}$ and

(γ -Glu-Cys) $_n$ -Glu, have been identified in some plant species (Rausser, 1999). Phytochelatins (PCs) are rapidly induced *in vivo* by a wide range of heavy metal ions, and the enzyme, which synthesizes PCs from GSH, is a γ -Glu-Cys dipeptididyl transpeptidase (EC 2.3.2.15). The other common name of this enzyme is PC synthase (Grill *et al.*, 1989). Mutants of *Arabidopsis thaliana* (L.) Heynh. that lack Phytochelatase-synthase (PC-synthase) are unable to synthesize PCs and are hypersensitive to Cd and Hg. The *cad 1* mutant of *Arabidopsis* is cadmium-sensitive and its GSH level is similar to that of wild type but is deficient in PC and lacks PC synthase activity *in vitro*. It is predicted that *CAD1* is the structural gene for PC synthase (Howden *et al.*, 1995). The *Arabidopsis CAD 1* gene (referred to as *AtPCS1*) (Ha *et al.*, 1999; Vatamanuik *et al.*, 1999) and a similar gene in wheat (*TaPCS1*) (Clemens *et al.*, 1999) have been shown to confer resistance to Cd when expressed in yeast *Saccharomyces cerevisiae* Hansen. However, these mutants have essentially wild type levels of tolerance to Cu and Zn (Howden *et al.*, 1995). PC-synthase has an isoelectric point near pH 4.8 and has optimum temperature of 35°C and pH 7.9. The molecular weight of the enzyme is around 95 kDa and seems to be composed of four subunits. The occurrence of PC-synthase in different higher plants has been confirmed (Clemens *et al.*, 1999). The vacuole is the ultimate storage site for those heavy metal ions that happened to enter the cytosol of a given plant cell. These ions will activate PC-synthase, which synthesizes at the expense of GSH, and PC molecules of varying chain lengths thus chelate the metal. The metal-PC complex is subsequently actively transported from the cytosol to the vacuole (Salt and Rausser, 1995). Heavy metal ions such as Cd²⁺ enter the plant cell by transporters for essential cations such as Fe²⁺ (Thomine *et al.*, 2000). *AtNramps* genes in *Arabidopsis* encode the metal transporter, which transports both the metal nutrient iron and the toxic metal cadmium.

PC synthase activity is the major determinant of the rate of the PC synthesis and is immediately activated by the presence of metal ions. It uses GSH present in the cytosol in mM concentration. The metal binding peptides are synthesized and chelate and inactivate every toxic metal ion entering the cytosol before they can inactivate the enzymes of essential metabolic routes. Aside from detoxification, PC plays a role in homeostasis of heavy metals in plants, and this is the mechanism that regulates

the metal ion availability in plant cells (Thomine *et al.*, 2000).

Metallothioneins

Metallothioneins (MT) are the other low molecular weight proteins which bind heavy metals and are found throughout the animal and plant kingdoms. These proteins also play an important role in detoxification by sequestering metals in plant cells. Plants have been found to contain a number of genes encoding MT-like proteins having sequence similarity to animal MT proteins. They are subdivided into two types, MT1 and MT2, on the basis of arrangements of cysteine residues. Until 1997, the only plant protein that could unequivocally be designated as a metallothionein was the wheat Ec (early cysteine-labeled) protein (Lane *et al.*, 1987). Murphy *et al.* (1997) identified and purified protein products of MT1 and MT2 genes from *Arabidopsis*, and therefore the list of plant MTs was enlarged with those proteins. *Arabidopsis* contains a large family of genes encoding MTs (Zhou and Goldsbrough, 1994). A total of 8 MT genes have been identified to date including one pseudogene. Two *Arabidopsis* MTs (MT4a and MT4b) are homologous to the wheat Ec MT and are expressed during seed development. The other 5 genes are expressed in vegetative tissues, exhibiting a variety of patterns of expression and responsiveness to environmental conditions. MT1 mRNA is expressed predominantly in roots, whereas mRNAs for both MT2 and MT3 are more abundant in shoots.

Treatment of plants with Cu can induce MT mRNAs, notably in tissues where the normal level of expression is low. It has been demonstrated recently that proteins encoded by *Arabidopsis* MT genes follow a similar pattern of tissue expression and metal induction (Murphy *et al.*, 1997). MT promoter GUS fusions are used to study the expression of individual genes. The MT2b promoter drives GUS expression primarily in vascular tissues and is not affected by Cu. In contrast, GUS activity in seedlings with the MT2a: GUS constructs increases by as much as 10-fold in response to Cu treatment. Antisense RNA and targeted gene disruption are used to produce plants with reduced expression of specific MTs in order to understand the function of MTs. Transgenic plants with reduced expression of either MT1 or MT2 are somewhat more sensitive to root growth inhibition by Cu, but are not affected by Cd, indicating that MTs are involved in some aspects of Cu tolerance.

2) Induction of Heat Shock Proteins

Inductions of heat shock proteins both by several transition metals (Zn, Cu, Cd, Hg) and by the sulfhydryl reagent arsenite have recently been reported. These induced heat shock proteins protect membranes and proteins in a similar way as under heat stress (Neumann, *et al.*, 1994). The induction of mRNA for heat shock proteins or the synthesis of heat shock proteins under heavy metal stress has been observed in different plants or plant cell cultures (Wollgiehn and Neumann, 1995). However, the putative role of heat shock proteins in heavy metal tolerance is largely unknown.

Phytoremediation

Phytoremediation defines the use of plants to extract, sequester, and/or detoxify various kinds of environmental pollutant (Salt *et al.*, 1998). It is a newly evolving field of biotechnology that uses plants to clean-up polluted soil, water, and air (Salt *et al.*, 1998). Plants can be genetically modified by genetic engineering methods and can be used to remove a wide variety of environmental contaminants. This field has generated great excitement because it may offer a reasonable cost effective means to restore the hundreds of thousands of square miles of land and water that have been polluted by human activities (Salt *et al.*, 1995; Cunnigham *et al.*, 1996; Salt *et al.*, 1998).

There are two types of phytoremediation process. One is elemental and the other one organic. Elemental pollutants include toxic heavy metals and radionucleotides, such as arsenic, cadmium, caesium, chromium, lead, mercury, strontium, technetium, tritium, and uranium (Dushenkof *et al.*, 1997; Salt *et al.*, 1998; Salt and Kramer, 1999). Organic pollutants that are potentially important targets for phytoremediation include polychlorinated biphenyls (PCBs) such as dioxin, polycyclic aromatic hydrocarbons (PAHs) such as benzoapyrene, nitroaromatics such as trinitrotoluene (TNT), and linear halogenated hydrocarbons such as trichloroethylene (TCE). Many of these compounds are not only toxic and teratogenic, but are also carcinogenic (Cunnigham *et al.*, 1996). The goal for the organic compound is to completely mineralize them into relatively non-toxic compounds such as carbon dioxide, nitrate, chlorine and ammonia.

Applying several approaches can increase the efficiency of phytoremediation. First, plant species or

varieties can be screened and those with a superior potential for remediation for certain pollutants can be selected. Second, several agronomical practices can be developed to optimize the remediation process (e.g., pH adjustment, addition of chelators). Finally, biotechnological methods can be applied to enhance a plant's capacity for super phytoremediation.

Phytoremediation by Use of Metal-Accumulating plants

As a result of their association with specific ore deposits, many metallophyte plants are used in prospecting for mineral deposits (Brooks, 1983). Only recently the value of metal accumulating terrestrial plants for environmental remediation has been fully realized. Phytoremediation of heavy metals is an emerging technology and four subsets of this technology are being developed (Salt *et al.*, 1995; Pilon-Smiths and Pilon, 2000).

- 1) Phytoextraction, in which metal-accumulating plants are used to transport and concentrate metals from soil into the harvestable parts of roots and above-ground shoots (Brown *et al.*, 1994; Kumar *et al.*, 1995).
- 2) Rhizofiltration, in which plant roots absorb, precipitate and concentrate toxic metals from polluted effluents (Smith and Bradshaw, 1979; Dushenkov *et al.*, 1995).
- 3) Phytostabilization, in which heavy metal tolerant plants are used to reduce the mobility of heavy metals, thereby reducing the risk of further environmental degradation by leaching into the ground water or by airborne spread (Smith and Bradshaw, 1979; Kumar *et al.*, 1995).
- 4) Plant assisted bioremediation, in which plant roots in conjunction with their rhizospheric microorganisms are used to remediate soils contaminated with organics (Walton and Anderson, 1992; Anderson *et al.*, 1993).

The use of metal-accumulating plants for removal of metals from contaminated soils and waters has a number of advantages such as lower cost, generation of a recyclable metal-rich plant residue, applicability to a range of toxic metals and radionuclides, minimal environmental disturbance, elimination of secondary air or water-borne wastes, and public acceptance.

In the phytoextraction process, several sequential crops of laboratory-improved hyperaccumulating plants may be used to reduce soil concentrations of heavy metals to environmentally acceptable levels. Preliminary trials with Ni and Zn hyperaccumulator plants from *Brassicaceae* family were successful in partially removing heavy metals from soils contaminated by long-term application of heavy metal containing sludge (Brown *et al.*, 1994; Brown *et al.*, 1995). Dried, ashed or composted plant residues, highly enriched in heavy metals, may be isolated as hazardous waste or recycled as bio-metal ore. Although the most heavily contaminated soils do not support plant growth, sites with light to moderate toxic metal contamination might be suitable for growing hyperaccumulating plants for toxic metal clean-up. Plants that accumulate toxic metals can be grown and harvested economically, leaving the soil or water with a greatly reduced level of toxic metal contamination (Lasat *et al.*, 2000; Lombi *et al.*, 2000).

Recently, there has been growing interest in the use of metal-accumulating roots and rhizomes of aquatic or semiaquatic vascular plants for the removal of heavy metals from contaminated aqueous streams. For example, water hyacinth (*Eichhornia crassipes* (C.F.P.Mart) Solms) (Kay *et al.*, 1984), pennywort (*Hydrocotyle umbellata* L.) (Dierberg, *et al.*, 1987), duckweed (*Lemna minor* L.) and water velvet (*Azolla pinnata* R.Br.) (Jain *et al.*, 1989) take up Pb, Cu, Cd, Fe and Hg from contaminated solutions. In a related development, cell suspension cultures of *Datura innoxia* Miller were found to remove a wide variety of metal ions from solutions (Jackson *et al.*, 1990; Jackson *et al.*, 1993). Most of the removed metals were tightly chelated by unidentified components of cell walls in a process that did not require metabolic activity. The observation that hydroponically grown roots of terrestrial plants are extremely effective in removing Cu, Zn, Cd, Cr, Ni and Pb from water has laid the foundation for the development of rhizofiltration in several laboratories in the USA and Europe. For example, 1.1 g dry weight of either sunflower (*Helianthus annuus* L.) or Indian mustard (*Brassica juncea* Czern.) roots, immersed in 400 ml of water containing 300 $\mu\text{g ml}^{-1}$ of Pb, brought the Pb concentration to below 1 $\mu\text{g ml}^{-1}$ in 8 hours (Dushenkov *et al.*, 1995). Disappearance of Pb from the solution was accompanied by a dramatically increased concentration of Pb in the root tissue, over 10% on a dry weight basis. These reports indicate that, at least in some instances,

rhizofiltration may provide an attractive alternative to current methods of chemical and microbial precipitation of heavy metals.

Phytoremediation of Mercury

Mercury is among the most hazardous of the heavy metals and its pollution is regarded as one of the most serious environmental problems (Rugh *et al.*, 1998; Bizly *et al.*, 1999; Bizly *et al.*, in press). Elemental mercury and mercury ions (Hg^{2+}) are released into the environment as a result of gold mining, industry, burning fossil fuels and medical waste. Once in the environment, these forms of mercury are converted by sulphate reducing bacteria to the extremely toxic compound methylmercury, which bioaccumulates in the food chain. Organomercurials are 1-2 orders of magnitude more toxic in some eukaryotes and are more likely to biomagnify across trophic levels than ionic mercury [Hg (II)] (Rugh *et al.*, 1996). The biophysical behaviour of organic mercury is thought to be due to its hydrophobicity and efficient membrane permeability.

Mercury remediation by conventional methods is very expensive, and thus many areas polluted by mercury are presently left unreclaimed. Generally, plants cannot detoxify methylmercury, and accumulation in plant tissues can be toxic to wildlife. Plant tolerance to mercury is quite low and therefore phytoremediation can be limited by plant tolerance. Meager and colleagues set out a new approach to introduce bacterial genes that converts methylmercury to volatile elemental mercury in plants (Bizly *et al.*, 1999; Bizly *et al.*, in press). This pathway involves the sequential action of two enzymes in which first organomercurial lyase (encoded by the *MerB* gene) converts methylmercury to Hg^{2+} . The second enzyme, mercuric reductase (encoded by the *MerA* gene), reduces Hg^{2+} to elemental mercury, using NADPH as the electron donor. Plants expressing the two bacterial genes, *merB* and *merA*, are resistant to extremely high levels of the environmental toxin methylmercury. They volatilize 100-1000 times more Hg than wild-type plants or controls expressing either gene alone. *MerB* enzyme levels appear to be rate limiting, but only account for 40% of the volatilization rate (Bizly *et al.*, 1999; Rugh *et al.*, 1996). The same *MerA* and *MerB* genes are now used to create mercury-volatilizing plants in other species. Enhanced mercury tolerance has already been shown in transgenic *MerA* and *MerB* tobacco and yellow poplar (Rugh *et al.*, 1998; Bizly *et al.*, in press).

The transgenic wetland plants can be generated by the insertion of *Mer* genes in plants such as cordgrass (*Spartina* Schreber spp.), cat-tail (*Typha* L. spp.) and bulrush (*Scirpus* L. spp.), as well as the water-tolerant trees poplar (*Populus* L. spp.) and willow (*Salix* L. spp.). These promising transgenic wet plants can be planted in contaminated aquatic ecosystems or in constructed wetlands to clean up mercury pollution.

Present and Future work

A better understanding of the biochemical processes involved in plant heavy metal uptake, transport, accumulation and resistance will help in systematic improvements in phytoremediation using molecular genetic approaches. Presently we are working on the identification and development of endemic heavy metal accumulator plants from Turkey for use in the phytoremediation process. The objectives of this research are as follows:

- 1) To find out heavy metal hyper-accumulator plants;
- 2) to identify genes involved in heavy metal tolerance and accumulation. For example, metallothioneins and phytochelatins (PC-synthase);
- 3) to overexpress these genes in hyperaccumulators to generate super-hyperaccumulator plants;
- 4) to use these plants for environmental clean-up. We have found a Cu accumulator, green alga *Dunaliella viridis* Teod.. At present we are working on the overexpression of the MT1 and MT2 genes in these organisms to generate super-accumulator algae and plants for the cleaning-up of contaminated waters and soils. Another approach for improving the high potential of phytoremediation is to introduce genes responsible for accumulation and resistance from wild slow growing plants to fast growing high biomass plant species. In the absence of known "phytoremediation" genes, this may be accomplished via somatic and sexual hybridization, followed by extensive screening and backcrossing of progenies. However, long-term efforts should be directed towards the development of a "molecular tool-box", composed of genes valuable for phytoremediation. Systematic screening of plant species and genotypes for metal accumulation and resistance will broaden the spectra of genetic material available for optimization and transfer. Mutagenesis of selected high biomass plant species may also produce improved phytoremediating cultivars.

Economic benefit

Clean-up of hazardous wastes by conventional

technologies is projected to cost at least \$400 billion in the US alone, based on estimates obtained from a variety of government and private sources. Clean-up of the US sites contaminated with heavy metals alone can cost \$7.1 billion, while mixtures of heavy metals and organics bear an additional \$35.4 billion price tag. The total clean-up of contaminated sites that have been identified and characterized to date will cost over \$10 billion using current treatment technologies.

This overwhelming cost burden has created an opening in the market for innovative technologies. There has been considerable interest in phytoremediation from both government and industry. The world phytoremediation market in 1999 was \$34-56 million, and is expected to grow tenfold between 2000 and 2005. The total world remediation market was \$18 billion in 1999. Heavy metal contamination in soils, a segment of the hazardous waste market suitable for phytoremediation, could constitute a \$400 million per year opportunity. Radionucleotide contamination represents another major opportunity for phytoremediation.

The biggest advantage of phytoremediation is its low cost. Phytoremediation can be up to 1000-fold cheaper than conventional remediation methods such as excavation and reburial. Moreover, it offers permanent in situ remediation rather than simply moving the pollution to a different site.

Phytoremediation is clearly a new field, and one which has great potential. It may one day become an established environmental clean-up method. Further development of phytoremediation requires integrated multidisciplinary research efforts that combine plant biology, genetic engineering, soil chemistry, and soil microbiology, as well as agricultural and environmental engineering. As a major renewable resource exploited by mankind, plants already give us food, energy, construction materials, natural fibres, and various chemical compounds. The use of plants in environmental clean-up may guarantee a greener and cleaner world for all of us to live in.

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