PHOTOREMEDIATION

D. E. Salt¹, R. D. Smith², and I. Raskin
AgBiotech Center, Rutgers University, New Brunswick, New Jersey 08903-0231;
¹Present address: Chemistry Department, Northern Arizona University, Flagstaff, Arizona 86071-5698; ²Present address: DeKalb Genetics Corporation, 62 Maritime Drive, Mystic, Connecticut 06355-1958; e-mail: raskin@aesop.rutgers.edu

KEY WORDS: decontamination, hyperaccumulator, phytoextraction, phytodegradation, heavy metals

ABSTRACT
Contaminated soils and waters pose a major environmental and human health problem, which may be partially solved by the emerging phytoremediation technology. This cost-effective plant-based approach to remediation takes advantage of the remarkable ability of plants to concentrate elements and compounds from the environment and to metabolize various molecules in their tissues. Toxic heavy metals and organic pollutants are the major targets for phytoremediation. In recent years, knowledge of the physiological and molecular mechanisms of phytoremediation began to emerge together with biological and engineering strategies designed to optimize and improve phytoremediation. In addition, several field trials confirmed the feasibility of using plants for environmental cleanup. This review concentrates on the most developed subsets of phytoremediation technology and on the biological mechanisms that make phytoremediation work.

CONTENTS
INTRODUCTION ........................................................... 644

PHYTOEXTRACTION OF METALS ........................................ 645
Induced Phytoextraction ................................................. 645
Continuous Phytoextraction ............................................. 650
Metal Resistance Mechanisms ........................................... 652
Metal Bioavailability, Root Uptake, and Shoot Accumulation .......... 654

PHYTOVOLATILIZATION OF METALS ............................... 656

PHYTOREMEDICATION OF ORGANICS ............................... 657
Direct Uptake and Metabolism of Organics ............................ 658
Phytoremediation ex Planta .............................................. 660

CONCLUSIONS: FROM THE LABORATORY TO THE FIELD .............. 661

1040-2519/98/0601-0643$08.00
INTRODUCTION
Phytoremediation is defined as the use of green plants to remove pollutants from the environment or to render them harmless (42, 125). Several comprehensive reviews have been written on this subject, summarizing many important aspects of this novel plant-based technology (39, 41, 43, 44, 126, 136). The basic idea that plants can be used for environmental remediation is very old and cannot be traced to any particular source. However, a series of fascinating scientific discoveries combined with an interdisciplinary research approach have allowed the development of this idea into a promising, cost-effective, and environmentally friendly technology. Phytoremediation can be applied to both organic and inorganic pollutants, present in solid substrates (e.g. soil), liquid substrates (e.g. water), and the air. Phytoremediation is currently divided into the following areas:

- phytoextraction: the use of pollutant-accumulating plants to remove metals or organics from soil by concentrating them in the harvestable parts;
- phytodegradation: the use of plants and associated microorganisms to degrade organic pollutants;
- rhizofiltration: the use of plant roots to absorb and adsorb pollutants, mainly metals, from water and aqueous waste streams;
- phytostabilization: the use of plants to reduce the bioavailability of pollutants in the environment;
- phytovolatilization: the use of plants to volatilize pollutants; and
- the use of plants to remove pollutants from air.

Most of this review focuses on the phytoremediation of the metallic pollutants in soil, particularly the area of metal phytoextraction, which, arguably, is the area of major scientific and technological progress in the past years. This can be partially explained by the relative ease of detecting metals in various materials. Phytoremediation of metals is being developed as a potential cost-effective remediation solution for thousands of contaminated sites in the United States and abroad. Its development is driven by the prohibitively high cost of the available soil remediation methods, which mainly involve soil removal and burial at a price of about $1 million per acre. The metals of greatest importance as environmental pollutants and some of their regulatory limits are listed in Table 1. Elements in each category are ranked by authors according to their importance as environmental pollutants in the United States. This review focuses on phytoremediation technologies for removing toxins from the environment. We do not discuss rhizofiltration, which has been extensively reviewed (126).
Table 1  Currently found concentration ranges and regulatory guidelines for important metal and radionuclide contaminants in the order of relative importance

<table>
<thead>
<tr>
<th>Element</th>
<th>Concentration range</th>
<th>Regulatory limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metals</td>
<td>(µg kg(^{-1}))^a</td>
<td>(mg kg(^{-1}))^b</td>
</tr>
<tr>
<td>Lead</td>
<td>1000–6,900,000</td>
<td>600</td>
</tr>
<tr>
<td>Cadmium</td>
<td>100–345,000</td>
<td>100</td>
</tr>
<tr>
<td>Arsenic</td>
<td>100–102,000</td>
<td>20</td>
</tr>
<tr>
<td>Chromium</td>
<td>5.1–3,950,000</td>
<td>100</td>
</tr>
<tr>
<td>Mercury</td>
<td>0.1–1,800,000</td>
<td>270</td>
</tr>
<tr>
<td>Copper</td>
<td>30–550,000</td>
<td>600</td>
</tr>
<tr>
<td>Zinc</td>
<td>150–5,000,000</td>
<td>1500</td>
</tr>
<tr>
<td>Radionuclides</td>
<td>Units (see below)</td>
<td>pCi g(^{-1})</td>
</tr>
<tr>
<td>Uranium</td>
<td>0.2–16,000</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>0.06–18,700</td>
<td>250f</td>
</tr>
<tr>
<td>Strontium</td>
<td>0.03–540,000</td>
<td>—</td>
</tr>
<tr>
<td>Cesium</td>
<td>0.02–46,900</td>
<td>—</td>
</tr>
<tr>
<td>Plutonium</td>
<td>0.00011–3,500,000</td>
<td>—</td>
</tr>
</tbody>
</table>

*a Riley et al (130).  
*b Nonresidential direct contact soil cleanup criteria. In Cleanup Standards for Contaminated Sites, New Jersey Department of Environmental Protection (1996).  
*c Micrograms per gram (µg g\(^{-1}\)).  
*d Picocuries per gram (pCi g\(^{-1}\)).  
*e Picocuries per kilogram (pCi kg\(^{-1}\)).  
*f Stern et al (149).  

PHYTOEXTRACTION OF METALS

A review of the phytoremediation literature reveals that, at present, there are two basic strategies of phytoextraction being developed: chelate-assisted phytoextraction (Figure 1), which we term induced phytoextraction; and long-term continuous phytoextraction (Figure 2). Of the two processes, chelate-assisted phytoextraction is the more developed and is presently being implemented commercially. Continuous phytoextraction is also being studied by several groups for the removal of metals such as zinc, cadmium, and nickel and oxianionic metals such as selenium, arsenic, and chromium. Field trials have been performed using both phytoextraction strategies. The results, though encouraging, suggest that further development of these technologies is needed (Table 2).

Induced Phytoextraction

THE CONCEPT OF CHELATE-ASSISTED PHYTOEXTRACTION  There are no reliable reports of plants capable of naturally accumulating the most environmentally important toxic metals such as lead, cadmium, arsenic, and radionuclides.
For example, vegetation growing on heavily lead-contaminated soil or solutions has been reported to contain only 0.01–0.06% of shoot dry biomass as lead (74, 81), levels well below that required for efficient phytoextraction. Early studies by Jøgensen (80) showed that application of synthetic metal chelates such as ethylenediaminetetraacetic acid (EDTA) to soils enhances lead accumulation by plants. Huang et al (74, 75) and Blaylock et al (18) were able to achieve rapid accumulation of lead in shoots to greater than 1% of shoot dry biomass. These discoveries paved the way to successful phytoremediation of lead and to defining strategies for the development of phytoextraction of other toxic metals using appropriate chelates.

The total amount of metal removed from a site is a product of metal concentration in the harvested plant material and the total harvested biomass. The observation that high biomass crop plants including Indian mustard, corn, and sunflower could be “induced” to accumulate high concentrations of lead (18, 74, 75) was another advance in the development of chelate-assisted phytoextraction.

The concept of chelate-assisted phytoextraction is applicable to other metals in addition to lead (18). The authors demonstrated the simultaneous accumulation of lead, cadmium, copper, nickel, and zinc in Indian mustard plants after
application of EDTA to soil contaminated with various heavy metals. Metal accumulation efficiency in these experiments was directly related to the affinity of the applied chelate for the metal. This suggests that for efficient phytoextraction synthetic chelates having a high affinity for the metal of interest should be used; for example, EDTA for lead, EGTA for cadmium (18), and possibly citrate for uranium.

Based on the above information, a hypothetical protocol for the chelate-assisted phytoextraction of a contaminated site can be outlined (Figure 1). 1. The site is evaluated and the appropriate chelate/crop combination is determined. 2. The site is prepared and planted, and the crop is cultivated. 3. Once optimal biomass is produced, the appropriate metal chelate is applied. 4. After a short metal-accumulation phase (several days or weeks), the crop is harvested. Depending on the crop and the season, the site could be replanted for further phytoextraction. Estimates suggest that plants can remove between 180 and 530 kg ha$^{-1}$ of lead per year (18, 75), making remediation of sites contaminated with up to 2500 mg kg$^{-1}$ lead possible in under 10 years. Following harvest, the weight and volume of contaminated material can be further reduced by ashing or composting. Metal-enriched plant residue can be disposed of as hazardous material or, if economically feasible, used for metal recovery.
Table 2  Examples of field trials for the phytoremediation of metals

<table>
<thead>
<tr>
<th>Metal</th>
<th>Plant</th>
<th>Location</th>
<th>Method(^a)</th>
<th>Comments</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pb</td>
<td><em>Brassica juncea</em></td>
<td>Trenton, N.J.</td>
<td>PE-CA</td>
<td>EDTA-enhanced uptake over one cropping season resulted in a 28% reduction in the Pb contamination area</td>
<td></td>
</tr>
<tr>
<td>Cd</td>
<td><em>Thlaspi caerulescens</em></td>
<td>Beltsville, Md.</td>
<td>PE-C</td>
<td>Phytoextraction of sludge-amended soils. Cd accumulation was similar in all three species. Zn accumulation in <em>T. caerulescens</em> was 10-fold higher than in other plants</td>
<td>26</td>
</tr>
<tr>
<td>Zn</td>
<td><em>Silene vulgaris</em></td>
<td></td>
<td>PE-C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td><em>Brassica oleracea</em></td>
<td>Rothamstead, U.K.</td>
<td>PE-C</td>
<td>Sludge-amended soil</td>
<td>12</td>
</tr>
<tr>
<td>Cd</td>
<td><em>Raphanus sativus</em></td>
<td></td>
<td>PE-C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ni</td>
<td><em>Thlaspi caerulescens</em></td>
<td></td>
<td>PE-C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td><em>Alyssum lesbiacum</em></td>
<td></td>
<td>PE-C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pb</td>
<td><em>Alyssum murale</em></td>
<td></td>
<td>PE-C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cr</td>
<td><em>Arabidopsis thaliana</em></td>
<td></td>
<td>PE-C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Se</td>
<td><em>Brassica juncea</em></td>
<td>Los Baños, Calif.</td>
<td>PE-C</td>
<td>Water-extractable B was reduced between 24–52% and total Se reduced between 13–48% by all species</td>
<td>13</td>
</tr>
<tr>
<td>B</td>
<td><em>Festuca arundinacea</em></td>
<td>Calif.</td>
<td>PV</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Hibiscus cannibus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Lotus corniculatus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>U</td>
<td><em>Helianthus annus</em></td>
<td>Asthabula, Ohio</td>
<td>RF</td>
<td>Removal of U from ground water(^b)</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Method of phytoremediation: PE, phytoextraction; PV, phytovolatilization; RF, rhizofiltration; CA, chelate-assisted phytoextraction; C, continuous phytoextraction.

\(^b\) Phytotech Inc., personal communication.

DEVELOPMENT OF CHELATE-ASSISTED PHYTOEXTRACTION  
Discovery of chelate-assisted metal uptake by plants is very recent, with only four publications appearing in the past four years. Chelate-assisted phytoextraction consists of two basic processes—release of bound metals into soil solution combined with transport of metals to the harvestable shoot. The role of chelates in increasing the soluble metal concentration in the soil solution can be explained using well-established equilibrium principles. However, the mechanisms involved in metal-chelate induced plant uptake and translocation of metals are not well understood.

Following EDTA application, lead accumulation in shoots is directly correlated with an accumulation of EDTA (A Vassel & D Salt, unpublished data). Thus, it is likely that lead is transported within the plant as a Pb-EDTA complex.
The presence of high levels of EDTA in plant tissues should increase soluble lead concentrations within the plant by formation of soluble Pb-EDTA, allowing its movement from roots to shoots where lead would likely accumulate as Pb-EDTA.

Clearly, transport of metal-chelate complexes within plants plays a pivotal role in chelate-assisted metal accumulation in plants. What are the mechanisms involved in transport of metal-chelate complexes in plants? A good place to start looking for answers to this question is the mineral nutrition literature. In the 1950s, Fe³⁺ chelates were introduced as a way to correct iron deficiency in plants. Since that time, the mechanism by which plant roots use iron from stable Fe³⁺ chelates has been debated. It appears that the roots of dicotyledonous plants acquire iron from Fe³⁺ chelate complexes either as Fe²⁺ after chelate splitting by a root Fe³⁺ chelate reductase (38) or as an intact Fe³⁺ chelate complex (72, 86, 133). The uptake mechanism depends on the iron nutritional status of the plant, with Fe²⁺ uptake predominating in iron-deficient plants (38). Thus, the highly stable Pb-EDTA complex, which cannot be split by the root Fe³⁺ chelate reductase, may be acquired in the same way as Fe³⁺ chelates. However, optimal “induction” of metal uptake occurs at chelate concentrations at least two orders of magnitude higher than those used in hydroponic nutrient solution (18). Is the mechanism of chelate uptake different at these elevated concentrations? An intriguing report by Jeffreys & Wallace (79) suggests that it is. Using the red iron chelate Fe-EDDHA, these authors showed that a threshold chelate concentration exists above which accumulation of iron chelate in shoots is induced and below which only low levels of iron chelate accumulate. This report predates the first observation of chelate-assisted metal accumulation by 25 years and suggests that there are at least two mechanisms involved in metal chelate uptake functioning at low and high chelate concentrations. Induction of metal chelate uptake by plants is correlated with severe plant stress and ultimately plant death; however, it is not clear if stress is necessary for induction or simply reflects the accumulation of high concentrations of synthetic chelate in the plant. More recently the biphasic nature of chelate uptake has been confirmed by the direct measurement of plant movement and distribution of ¹⁴C-labeled EDTA and Pb-EDTA (A Vassel & D Salt, unpublished data).

Chelate-assisted transport of metal to shoots appears to occur in the xylem (74) via the transpiration stream (18). The metal appears to move to shoots as a metal-chelate complex (A Vassel & D Salt, unpublished) where water evaporates and the metal-chelate complex remains. In this way, after chelate-assisted induction the plant becomes a wick, which drives chelated metal from the soil solution into the leaves. The operation of the wick relies on a high-surface-area collection system provided by the roots and by the efficient capillary plumbing system inside the plant. Although it may be possible to design and
Continuous Phytoextraction

An alternative approach to chelate-assisted metal accumulation is the reliance on the specialized physiological processes that allow plants to accumulate metals over the complete growth cycle. This type of metal uptake is epitomized by hyperaccumulating plants that grow on soils rich in heavy metals (9). These plants are naturally able to accumulate >1% of shoot dry biomass as Zn, Ni, Mn, or Se. It was the existence of this hyperaccumulation phenomenon that inspired Chaney in 1983 to formulate the concept of phytoextraction (37). Unlike induced metal uptake, continuous phytoextraction is based on the genetic and physiological capacity of specialized plants to accumulate, translocate, and resist high amounts of metals. Major disadvantages of using naturally occurring metal hyperaccumulators for continuous phytoextraction are their relatively low biomass, slow growth rates, and the lack of any hyperaccumulators for the most environmentally important metallic pollutants (e.g., lead, cadmium, arsenic, and uranium). However, understanding the biological mechanisms of hyperaccumulation may help in the development of superior plants for the phytoremediation of metals.

The Hyperaccumulation Concept

As early as 1885, A. Baumann, a German botanist working near the border of Germany and Belgium, had observed that leaves of certain plant species growing on soils naturally enriched in zinc contained extraordinarily high levels of this element (14). Two species of particular note were the violet *Viola calaminaria* and the mustard *Thlaspi calaminare*, more recently classified as *Thlaspi caerulescens* (77), which contained about 1% and 1.7% zinc in dry leaves, respectively. This can be compared with zinc levels between 0.001% and 0.02% in dried leaves of plants growing on unmineralized soils. Fifty years later, studies in the United States implicated selenium as the plant component responsible for alkali disease in range animals in South Dakota. This observation led to the discovery of plants, notably of the genus *Astragalus*, capable of accumulating up to 0.6% selenium in dry shoot biomass (31, 32). Shortly thereafter, two Italian botanists (107) discovered plants that accumulate nickel. They observed that dried leaves of *Alyssum bertolonii* growing on nickel-enriched serpentenitic soils near Florence, Italy, contained about 1% nickel, over 100–1000 times higher than other plants growing nearby.

Since these early observations, plants that accumulate elevated levels of cobalt, copper, manganese, and possibly lead have also been described (9). However, the existence of hyperaccumulators for metals other than Ni, Zn, and
Se has been continuously questioned and requires further substantiation. The first hyperaccumulators characterized were members of the Brassicaceae and Fabaceae families. Presently, at least 45 plant families are known to contain metal-accumulating species. The number of metal-accumulating taxa identified to date has now grown to 397. This number is likely to change in the future. As more of the metal-enriched environments are investigated, new hyperaccumulators will be identified, and plants initially classified as hyperaccumulators from herbarium and field specimens may be reclassified as nonaccumulators after closer scrutiny.

The ecological role of metal hyperaccumulation is still not entirely clear. It has been suggested that metal accumulation provides protection against fungal and insect attack (21, 129). Recent evidence has confirmed the protective function of nickel hyperaccumulation against fungal and bacterial pathogens in *Streptanthus polygaloides* (23), and insect herbivory in *S. polygaloides* and *T. montanum* (22, 105). The antiherbivory effect of zinc has been also demonstrated in the zinc hyperaccumulator *T. caerulescens* (122).

**DEVELOPMENT OF CONTINUOUS PHYTOEXTRACTION** The unique capacity of hyperaccumulators to accumulate high foliar metal concentrations makes these plants suitable for the development of phytoremediation crops for continuous phytoextraction. This idea was first introduced by Chaney (37) and Baker and coworkers (10). The ideal plant for continuous phytoextraction should grow on metal-polluted soils to high biomass and accumulate and resist high concentrations of metal in shoots. The first reported field trials of continuous phytoextraction were performed in 1991 with moderate success (12). Most known hyperaccumulator plants have low biomass and/or slow growth rates, whereas rapidly growing high-biomass crop plants are sensitive to metals and accumulate only low concentrations in shoots. To overcome these limitations, a two-component long-term strategy needs to be developed for continuous phytoextraction to succeed.

First, attempts to improve existing lines of phytoextracting plants should be continued (48, 85, 91) as well as the search for new high-biomass metal hyperaccumulators. The usefulness of this search was recently demonstrated by the identification of *Berkheya coddii* (Asteraceae), a tall, high-biomass plant from the northeastern Transvaal, South Africa, capable of accumulating up to 3.7% nickel in its shoot dry biomass (109). This (and related) species may have significant phytoremediation potential, due to strong hyperaccumulation, relatively high biomass production, and the ability to grow in dense stands. Biotechnological approaches to the production of high-biomass metal hyperaccumulators should also be considered. Modern genetics can be used to transfer hyperaccumulating genes to nonaccumulating plants.
Second, we need to understand and exploit the biological processes involved in metal acquisition, transport, and shoot accumulation in both hyperaccumulating and nonaccumulating plants. The plant mineral nutrition literature is rich in information relating to metal tolerance, metal ion uptake, transport, and accumulation (for recent reviews, see 86, 103, 169), and we will therefore only review here areas that have particular relevance to phytoextraction.

**Metal Resistance Mechanisms**

Continuous phytoextraction relies on the ability of plants to accumulate metals in their shoots, over extended periods. To achieve this, plants must possess efficient mechanisms for the detoxification of the accumulated metal. The recent observation that nickel resistance in *Thlaspi goesingense* is a primary determinant of nickel hyperaccumulation when plants are grown hydroponically (89) supports this conclusion. Therefore, the ability to manipulate metal tolerance in plants will be key to the development of efficient phytoremediation crops. As an elegant demonstration of this principle, Hg$^{2+}$-resistant *Arabidopsis thaliana* overexpressing bacterial mercury reductase was recently shown to remove Hg$^{2+}$ efficiently from solution (134).

In order to develop hypertolerant plants capable of accumulating high concentrations of metals it will be vital to understand the existing molecular and biochemical strategies plants adopt to resist metal toxicity. The processes involved in intracellular detoxification of heavy metals have been extensively reviewed (56, 78, 152). Thus, in the interests of brevity, we only cover those processes that could potentially be manipulated to improve the metal resistance of phytoextraction crops. These mechanisms include chelation, compartmentalization, biotransformation, and cellular repair mechanisms.

**CHELATION**

Chelation of metal ions by specific high-affinity ligands reduces the solution concentration of free metal ions, thereby reducing their phytotoxicity. Two major classes of heavy metal chelating peptides are known to exist in plants—metallothioneins and phytochelatins. Metallothioneins are gene-encoded, low-molecular-weight, cysteine-rich polypeptides (131). Plant metallothioneins are induced by Cu and have high affinity for this metal (111, 178). Recent investigations of metallothionein (MT) expression levels in *A. thaliana* demonstrated that expression levels of MT2 mRNA strongly correlated with Cu resistance (112), suggesting that metallothioneins are involved in Cu resistance. Phytochelatins are low molecular weight, enzymatically synthesized cysteine-rich peptides known to bind cadmium and copper in plants (127, 128, 146). These peptides are essential for cadmium detoxification in *A. thaliana* (73). Although not strictly defined as chelation, precipitation of zinc as Zn-phytate has also been suggested as a zinc detoxification mechanism (156–158).
It is also likely that intra- and extracellular precipitation of lead as carbonates, sulfates, and phosphates plays a role in the detoxification of this metal in plant tissues.

COMPARTMENTALIZATION Within cells, cadmium and phytochelatins accumulate in the vacuole (162), and this accumulation appears to be driven by a Cd/H antiport and an ATP-dependent PC-transporter (138, 139). A similar system of cadmium detoxification also exists in the fission yeast, *Schizosaccharomyces pombe*. Mutants lacking the ability to accumulate Cd-PC complex in the vacuole are Cd-sensitive and have a defect in *hmt1*, a gene encoding an ATP-binding cassette-type transport protein (117). The *hmt1* gene product is responsible for transporting Cd-PC complex into the vacuole (118). Once inside the vacuole, sulfide is added to the Cd-PC complex, forming a more stable high-molecular-weight Cd-PC-sulfide complex that may be essential for Cd resistance in the yeast (117, 145).

Intact vacuoles isolated from tobacco and barley exposed to Zn have also been shown to accumulate this metal (27, 90). Vacuolar Zn accumulation has been confirmed in roots and shoots of the Zn hyperaccumulator *Thlaspi caerulescens* (160, 161). Zinc accumulation within the vacuole, as a Zn detoxification mechanism, is also supported by the observation that the vacuolar volume fraction of meristematic cells of *Festuca rubra* increases during Zn exposure (46). Leaf trichomes also appear to provide a site for the sequestration of Cd (137), Mn (16), and Pb (104).

BIOTRANSFORMATION The toxicity of such metals and metalloids as chromium, selenium, and arsenic can be reduced in plants by chemical reduction of the element and/or by its incorporation into organic compounds. Excess selenium is toxic to most plants because it is metabolized to selenocysteine and selenomethionine, which replace cysteine and methionine residues in proteins. By funneling selenium into the nonprotein amino acids methylselenocysteine and selenocystathionine, selenium accumulator species of *Astragalus* are able to reduce the amount of selenium incorporated into proteins, thereby tolerating elevated concentrations of selenium in shoots (93). Recently the enzyme responsible for the methylation of selenocysteine in the selenium accumulator *Astragalus bisculatus* has been isolated and characterized, a first step in determining the molecular basis of selenium resistance in plants (113). It also appears that several selenium-accumulating species are able to selectively exclude selenium from the methionine biosynthetic pathway, thereby avoiding the synthesis of selenomethionine, a toxic seleno-derivative of methionine (30). Selenium is also volatilized by plants by as yet uncharacterized mechanisms (see section on “Phytovolatilization of Metals”).
Arsenic is toxic to plants, as demonstrated by the use of organoarsenical as herbicides, though little is known about arsenic detoxification in terrestrial plants. However, in marine macroalgae, arsenic is incorporated into various dimethylarsinylribosides and certain lipids (63), and it is likely that terrestrial plants also biotransform arsenic. Chromium is also toxic to plants, and there is limited evidence that plants, like certain bacteria and animals, can reduce Cr(VI) to Cr(III) as part of a detoxification mechanism (53).

**CELLULAR REPAIR MECHANISMS** A primary component of cellular resistance to elevated Cu concentrations appears to be enhanced plasma membrane resistance to, or repair of, Cu-induced membrane damage (50, 111, 150). The intriguing observation that plant metallothioneins may be prenylated and targeted to the plasma membrane (111) suggests a possible mechanism whereby metallothioneins may be involved in plasma membrane repair. The involvement of membrane repair mechanisms in Cu resistance is also strongly supported by the recent observation that an acyl carrier protein (ACP) and an AcylCoA binding protein (ACBP), two proteins known to be involved in lipid metabolism, are induced in Cu-exposed *A. thaliana* (A Murphy & L Taiz, personal communication). These authors also showed that antisense down-regulation of ACBP expression caused increased sensitivity to Cu, supporting the role of membrane repair in Cu resistance (A Murphy & L Taiz, personal communication).

Metal resistance will clearly be an important characteristic of a phytoremediation crop. However, metal resistance alone may not be sufficient to allow plants to accumulate high concentrations of metals. Metal bioavailability, root uptake, and translocation are also essential for successful phytoextraction.

**Metal Bioavailability, Root Uptake, and Shoot Accumulation**

The enhancement of metal ion bioavailability in soil by addition of metal chelates is an essential component of chelate-assisted phytoextraction and may also be important for continuous phytoextraction. This is illustrated by the mechanism(s) involved in the acquisition of iron and other micronutrients by plants. Because of the high binding capacity for metallic micronutrients by soil particles, plants have evolved several strategies for increasing their soil bioavailability. These strategies include the production of metal-chelating compounds (phytosiderophores) such as mugenic and avenic acids (84), which are synthesized in response to iron (69, 70, 83) and possibly zinc (33, 34) deficiencies. In the rhizosphere, phytosiderophores chelate and mobilize Fe, Cu, Zn, and Mn (132). Once chelated to phytosiderophores, metal ions can be transported across the plasma membrane as a metal-phytosiderophore complex via specialized transporters (163–165). By reducing chelated Fe(III) with a root ferric
chelate reductase (108), plants are also able to release soluble Fe(II) for root uptake (174). There is also some evidence that this ferric chelate reductase may play a more general role in Cu and Mn uptake (168). Plants can also solubilize iron and other metals by exuding protons from roots to acidify the rhizosphere (40). It may, therefore, be possible to enhance the bioavailability of metal pollutants by manipulating these root processes. Reliance on plant-produced chelating agents should also reduce the need for addition of synthetic chelates, thus reducing the cost of phytoextraction.

Possibly with the exception of Fe, little is known about the molecular mechanisms of metal entry into root cells. However, recently putative plasma membrane copper (COPT1) and iron (II) (IRT1) transporters have been cloned from A. thaliana using functional complementation in yeast (55, 82). Several genes have been also recently isolated from A. thaliana that appear to encode plasma membrane zinc transporters (65). Using a metal uptake screen in yeast, a wheat root gene has been identified that enhances both Cd and Pb uptake in transgenic yeast expressing the gene (7). It was suggested that this gene may encode a putative plasma membrane metal transporter. These data provide important molecular insight into plasma membrane metal ion transport in plants and suggest that it may soon be possible to manipulate metal ion transport systems in order to promote phytoextraction of toxic metals.

Once metal ions have entered the roots, they can either be stored or exported to the shoot. Metal transport to the shoot primarily takes place through the xylem. Cadmium loading into the xylem sap of Brassica juncea displays biphasic saturation kinetics (137), suggesting that xylem loading of metal ions is facilitated by specialized membrane transport processes. Recent evidence from work with Ni hyperaccumulators from the genus Alyssum suggests that xylem loading of Ni may be facilitated by the binding of Ni to free histidine (88). Movement of metal ions, particularly Cd, in xylem vessels appears to be mainly dependant on transpiration-driven mass flow (137).

Because xylem cell walls have a high cation exchange capacity, they are expected to retard severely the upward movement of metal cations. Therefore, noncationic metal-chelate complexes, such as Cd-citrate, should be transported more efficiently in the transpiration stream (143). Theoretical studies have predicted that the majority of the Fe(II) and Zn(II) in xylem sap should be chelated by citrate, whereas Cu(II) should be chelated by various amino acids including histidine and asparagine (170). Isolation of a citratonickelate (II) complex from the latex of the Ni hyperaccumulator Sebertia acuminata supports the role of organic acids in metal transport (94). X-ray absorbance fine structure (EXAFS) analysis showed that Cd in the xylem sap of B. juncea was chelated by oxygen or nitrogen atoms, suggesting the involvement of organic acids in Cd translocation (137). EXAFS analysis produced no evidence for sulfur
coordination of Cd, confirming that phytochelatins and other thiol-containing ligands play no direct role in Cd transport in the xylem. X-ray spectroscopy also demonstrated that a portion of the Ni and Zn transported to the shoots of the Ni hyperaccumulator *T. goesingense* and the Zn hyperaccumulator *T. caerulescens* is coordinated with organic acids (D Salt, I Pickering & R Prince, unpublished data). However, this analysis also revealed that substantial amounts of Ni and Zn are transported in the xylem sap as hydrated cations. A similar speciation of Ni in the xylem sap of the Ni hyperaccumulator *Alyssum lesbiacum* was established by mathematical modeling (88).

Other chelating compounds may also play a role in metal ion mobility in plants. The nonproteinaceous amino acid nicotianamine is ubiquitous among plants and has the ability to form complexes with various divalent metal ions including Cu, Ni, Co, Zn, Fe, and Mn (147, 148). Investigations of the tomato mutant *chloronerva*, which lacks the ability to synthesize nicotianamine (71), demonstrated that nicotianamine is possibly involved in distributing Fe(II), Zn, and Mn in young growing tissues via the phloem (148) and in Cu(II) transport within the xylem (121). Recent evidence also suggests that in *A. thaliana* cellular Cu is transported chelated to a functional analogue of the yeast low-molecular-weight Cu-binding protein (ATX1) (R Amasino, personal communication). In addition, metals may be transported in the phloem chelated to other low-molecular-weight metabolites or proteins (102).

Enhanced rates of metal ion translocation from roots to shoots appear to be important for zinc hyperaccumulation in *T. caerulescens* (92), suggesting that modifications in the transport processes described above may allow development of plants with enhanced root to shoot transport of pollutant metal ions, an important development in the creation of effective phytoextraction crops.

**PHYTOVOLATILIZATION OF METALS**

Volatile selenium from plant tissues may provide a mechanism of selenium detoxification. As early as 1894, Hofmeister proposed that selenium in animals is detoxified by releasing volatile dimethyl selenide from the lungs. He based this proposal on the fact that the odor of dimethyl telluride was detected in the breath of dogs injected with sodium tellurite (referenced in 95). Using the same logic, it was suggested that the garlicky odor of plants that accumulate selenium may indicate the release of volatile selenium compounds. Lewis (97) was the first to show that both selenium nonaccumulator and accumulator species volatilize selenium. This was later confirmed by other authors (52, 57, 176, 177, 179). The volatile selenium compound released from the selenium accumulator *Astragalus racemosus* was identified as dimethyl diselenide (57). Selenium released from alfalfa, a selenium nonaccumulator, was different from the accumulator species and was identified as dimethyl selenide (96).
However, it is not clear whether plants are able to take inorganic selenium (as selenate or selenite) and reduce and methylate it to the volatile methyl forms. Recent work by Zayed & Terry (177) demonstrated that addition of the antibiotic penicillin to hydroponically grown Indian mustard (Brassica juncea) inhibited selenium volatilization by approximately 90% when selenium was provided as selenate. However, plants may still volatilize selenium in the absence of rhizobacteria when it is supplied as selenomethionine (151). This suggests that root-associated bacteria play an important role in reducing and assimilating selenium into organic forms. However, more work is needed to clarify the role of microbial and plant biochemical processes in selenium volatilization by plants.

Volatilization of arsenic as dimethylarsenite has also been postulated as a resistance mechanism in marine algae. However, it is not known whether terrestrial plants also volatilize arsenic in significant quantities. Studies on arsenic uptake and distribution in higher plants indicate that arsenic predominantly accumulates in roots and that only small quantities are transported to shoots. However, plants may enhance the biotransformation of arsenic by rhizospheric bacteria, thus increasing rates of volatilization.

More recently, a modified bacterial mercuric ion reductase has been introduced into transgenic A. thaliana, which converts Hg\(^{2+}\) into elemental mercury (Hg\(^0\)). In addition to being more tolerant, these transgenic plants are very effective at volatilizing mercury (134). Phytovolatilization of metals may have unique advantages over phytoextraction, because it bypasses harvesting and disposal of metal-rich biomass. However, the environmental implications of metal volatilization have to be considered before this approach becomes accepted by regulators and the public.

**PHYTOREMEDIATION OF ORGANICS**

The use of plants to cleanse waters contaminated with organic and inorganic pollutants dates back hundreds of years and has been the basis for the present use of constructed wetlands in treating municipal and industrial waste streams (67). The concept of using plants to remediate soils contaminated with organic pollutants is a more recent development, based on observations that disappearance of organic chemicals is accelerated in vegetated soils compared with surrounding nonvegetated bulk soils (8, 29, 42, 45, 59, 68, 166). Subsequent metabolic studies have established the ability of plants to take up and metabolize a range of environmentally problematic organic pollutants, including ammunition wastes (e.g. TNT and GTN), polychlorinated phenols (PCBs), and trichloroethylene (TCE) (64, 76, 114, 141).

In addition to the direct uptake and metabolism of organics, plants release exudates from their roots that enhance microbial bioremediation in the
rhizosphere, which has been termed phytoremediation *ex planta* (3, 41). A brief review of these two basic phytoremediation strategies is presented below. Additional reviews on this subject have been published recently (3, 42, 44, 141), including a timely review by Cunningham et al (41) that provides an in-depth discussion of the technical, logistical, and economic considerations and strategies for the phytoremediation of soils contaminated with organic pollutants.

**Direct Uptake and Metabolism of Organics**

**BIOAVAILABILITY AND UPTAKE** By analogy with the phytoextraction of metals, direct uptake of organic contaminants is primarily limited by the availability of the target compound and uptake mechanisms. Plants can take up chemicals from three distinct soil phases: vapor, liquid, and solid (41). With a few notable exceptions [i.e. uptake of some polyaromatic hydrocarbons (PAHs) and herbicides from the vapor phase], movement of organics into plants occurs via the liquid phase, which has been extensively investigated in plants for uptake of pesticides and herbicides (25, 120, 153). A major criterion in assessing the probability that a target chemical will be taken up by plants is its lipophilicity. This governs its movement across plant membranes as well as its solubility in the water phase. Chemicals most likely to be taken up are moderately hydrophobic compounds with octanol-water partition coefficients ranging from 0.5 to 3.0 (25, 135).

In addition to the physicochemical properties of the target compound, other factors including soil conditions (e.g. pH, pKa, organic and water content, texture; 2, 19, 54, 135, 153) and plant physiology (100, 101) influence solubility and uptake of target compounds. Differences in uptake of organics among plant species and varieties are well recognized (66, 98) and should be a primary consideration in the development of effective phytoremediation strategies. Only limited screening of plants for uptake and metabolism of priority organic pollutants has been carried out by a few research groups (141). More exhaustive screening in the future may yield novel species or varieties that have enhanced phytoremediation capabilities for environmental pollutants, in the same way that screening for metal accumulators identified members of the Brassicaceae as superior phytoextractors (91).

Current strategies for remediating organics rely on long-term continuous phytoextraction approaches analogous to continuous phytoextraction of heavy metals (described above). Bioavailability has been found to be a major limiting step in the phytoextraction of metals. Similarly, availability of organics in soils appears to be a primary restriction for effective phytoremediation of organic pollutants (41, 141). While the application of soil amendments (e.g. EDTA) is considered a major breakthrough in the development of induced phytoextraction strategies, similar attempts to identify soil amendments that can induce the
uptake and accumulation of organics in plants have not been made. The use of synthetic (e.g. triton X-100, SDS) and naturally produced biosurfactants (e.g. rhamnolipids) to enhance the apparent water solubility and bacterial degradation of organic contaminants is well documented (24, 49, 123, 155, 175). A recent study has also shown the beneficial use of cyclodextrins to increase the solubilities of both organics and heavy metals (28). Whether these or other chemical agents could be applied as amendments to enhance the availability and uptake of organic pollutants remains to be investigated. Potential advantages of using biosurfactants or cyclodextrins—aside from increasing the bioavailability of organics—include their rapid biodegradation in the environment, the capability of synthesizing these compounds in engineered plants (116) and rhizospheric microorganisms, and the ability of these compounds to solubilize both organics and metals (106, 115), which could be instrumental in remediation soils with mixed contaminants.

BIOTRANSFORMATION AND COMPARTMENTALIZATION Following uptake, organic compounds may have multiple fates: They may be translocated to other plant tissues (36, 58, 142) and subsequently volatilized, they may undergo partial or complete degradation (64, 114, 141, 172), or they may be transformed to less toxic compounds and bound in plant tissues in nonavailable forms (60). Biotransformation and sequestration of herbicides and pesticides, in particular, have been extensively investigated in plants (reviewed by 60). More recently, metabolism of nonagricultural xenobiotics such as TCE, TNT, and nitroglycerin (GTN) has been studied using axenic cell cultures and whole plants (64, 114, 141). In general, most organics appear to undergo some degree of transformation in plant cells before being sequestered in vacuoles or bound to insoluble cellular structures, such as lignin. Metabolism of chloroacetanilide herbicides, for instance, results in the production of reduced and oxidized sulfur-containing compounds following conjugation to glutathione (60). The nitrate ester, GTN, is degraded to glycerol dinitrate and glycerol mononitrate in sugar beet cell cultures (64), and TCE metabolism in poplars generates trichloroethanol and di- and trichloroacetic acid (114). However, few chemicals appear to be fully mineralized by plants to water and CO₂, and where this does occur, it only represents a small percentage of the total parent compound (114). This property puts plants at a relative disadvantage compared with bacteria in degrading organic pollutants. In addition, the possibility that plant metabolites of pollutants may be more toxic than the original pollutants creates a difficult regulatory environment for phytoremediation of organics.

An important consideration in developing phytoremediation strategies for organics is the short- and long-term fate and potential toxicity of the metabolic end products of biodegradation. For classes of chemicals such as pesticides and
herbicides, these questions have, in most cases, been thoroughly addressed, and
the evidence indicates that most compounds are bound irreversibly with plant
materials (18a, 60, 87, 154). Information on the metabolism of priority organic
pollutants is limited, however, and will require further investigation given re-
sults from recent studies. For instance, the majority of the TNT transformation
products in *Myriophyllum spicatum* could not be identified, and a significant
fraction of these products were either released into the culture medium or asso-
ciated with water-extractable cellular fractions (76). Studies by other groups,
however, suggest that TNT degradation products in plants are not available
(119, 141, 172). Plant differences in the partitioning of organics between roots
and shoots as well as differences in metabolism of organic pollutants should
also be considered when choosing specific plant species for phytoremediation.

**Phytoremediation ex Planta**

Plants may secrete 10–20% of their photosynthate in root exudates, which sup-
port the growth and metabolic activities of diverse fungal and bacterial com-
unities in the rhizosphere (4, 99, 110, 144). Densities of rhizospheric bacteria
can be as much as two to four orders of magnitude greater than populations
in the surrounding bulk soils and display a greater range of metabolic capa-
bilities, including the ability to degrade a number of recalcitrant xenobiotics
(4, 167). It is not surprising, therefore, to find accelerated rates of biodegra-
dation of organic pollutants in vegetated soils compared with nonvegetated
soils (5, 29, 45, 59, 68). Some organic compounds in root exudates (i.e. pheno-
lcs, organic acids, alcohols, proteins) may serve as carbon and nitrogen
sources for the growth and long-term survival of microorganisms that are ca-
pable of degrading organic pollutants. For instance, plant phenolics such as
catechin and coumarin may serve as co-metabolites for PCB-degrading bacte-
ria (15, 51, 61, 68).

The chemical composition of root exudates and rates of exudation differ
considerably among plant species (124). This has led some research groups
to screen for plant species that exude phenols capable of supporting PCB-
degrading bacteria (61). Although studies directed at understanding mecha-
nisms of plant-enhanced microbial degradation of organics are only begin-
ing to emerge, several field and pilot studies have examined the use of specific
plants for rhizospheric degradation of organic pollutants. Soils planted with
crested wheat grass (*Agropyron desertorum*) showed enhanced mineralization
of PCBs (59), while accelerated removal of PAHs was achieved using prairie
grasses (5, 62). Similar studies have examined the degradation of TCE (5, 62)
and TNT (172).

Rhizospheric microorganisms may also accelerate remediation processes by
volatilizing organics such as PAHs or by increasing the humification of or-
ganic pollutants (41, 47). In particular, the release of oxidoreductase enzymes
(e.g. peroxidase) by microbes, as well as by plant roots, can catalyze the polymerization of contaminants onto the soil humic fraction and root surfaces (1, 47). Armoracia rusticana (horseradish) has received particular attention with regard to the production of root peroxidases and its potential use for the remediation of polluted soils and water streams (47).

PLANT-DERIVED ENZYMES In addition to secreting organic compounds that support the growth and activities of rhizospheric microorganisms, plants also release enzymes capable of degrading organic contaminants in soils. Soil enzymes derived from plant sources, based on immunological assays, include laccases, dehalogenases, nitroreductases, nitrilases, and peroxidases (20, 35, 141). Degradation of ammunition wastes (e.g. TNT, dinitromono-aminotoluene, and mononitrodiaminotoluene) and triaminotoluene is catalyzed by nitroreductases and laccases, respectively (141, 172). Whether release of plant enzymes from abscized plant tissue, root exudates, or guttation fluid can provide a cost-effective phytoremediation strategy for organic contaminants remains to be determined. The presence of plant-derived enzymes capable of degrading environmentally problematic xenobiotics (e.g. TNT and TCE) will no doubt be exploited for the development of future phytoremediation strategies.

CONCLUSIONS: FROM THE LABORATORY TO THE FIELD

At present, phytoremediation of metals and organics may be approaching commercialization. Additional, short-term advances in phytoremediation are likely to come from the selection of more efficient plant varieties and soil amendments and from optimizing agronomic practices used for plant cultivation. Major long-term improvements in phytoremediation should come when scientists isolate genes from various plant, bacterial, and animal sources, which can enhance the metal accumulation or degradation of organics. In addition, manipulating rhizospheric bacteria to enhance their role in phytoremediation can increase the efficiency of the future phytoremediation efforts.

However, biology alone cannot make phytoremediation work. The highly integrated nature of phytoremediation requires synergy with many other disciplines. For example, parallel developments in environmental and agricultural engineering should have a major impact on the efficiency of plant cultivation, amendment application, and disposal of metal-enriched biomass.

Only the future can tell whether phytoremediation will become a widely accepted technology. However, it is clear that the utilization of the remarkable potential of green plants to accumulate elements and compounds from the environment and to perform biochemical transformations is becoming a new frontier of plant biology.
ACKNOWLEDGMENTS

We thank the US Department of Agriculture (grant #96-35102-3838 to DES), US Department of Energy (grant #DE-FG07-96ER20251 to DES and RDS), Phytotech Inc., New Jersey Commission on Science and Technology, and Peter Day for comments.


Literature Cited


6. Deleted in proof


11. Deleted in proof


17. Deleted in proof


75. Huang JW, Cunningham SD. 1996. Lead phytoextraction: species variation in lead
uptake and translocation. New Phytol. 134:75–84
100. MacFarlane JC, Pfleeger T, Fletcher J. 1987. Transpiration effect on the uptake and distribution of bromacil,
nitrobenzene, and phenol in soybean plants. J. Environ. Qual. 16:372–76
129. Reeves RD, Brooks RR, MacFarlane RM. 1981. Nickel uptake by Californian Strep-
tanthus and Caulanthus with particu-
lar reference to the hyperaccumulator S.
polygaloides Gray (Brassicaceae). Am. J. 
Bot. 68:708–12
Chemical Contaminants on Doe Lands and 
Selection of Contaminant Mixtures for 
Subsurface Science Research. Wash-
131. Robinson NJ, Tommey AM, Kuske C, 
Biochem. J. 295:1–10
132. Römheld V. 1991. The role of phyto-
 siderophores in acquisition of iron and 
other micronutrients in graminaceous 
species: an ecological approach. Plant 
Soil 130:127–34
133. Römheld V, Marschner H. 1981. Effect 
of Fe stress on utilization of Fe chelates 
between efficient and inefficient plant stress. J. 
Plant Nutr. 3:551–60
134. Rugh CL, Wilde HD, Stack NM, Thom-
son DM, Summers AO, Meagher RB. 
1996. Mercuric ion reduction and resis-
tance in transgenic Arabidopsis thaliana 
plants expressing a modified bacterial 
93:3182–87
135. Ryan JA, Bell RM, Davidson JM, 
O’Connor GA. 1988. Plant uptake of non-
ionic chemicals from soils. Chemosphere 
17:2299–323
136. Salt DE, Blaylock M, Kumar NPBA, Vi-
atcheslav D, Enshey BD, et al. 1995. Phyto-
remediation: a novel strategy for the 
removal of toxic metals from the envi-
ronment using plants. Bio-Technology 13: 
468–74
137. Salt DE, Prince RC, Pickering JJ, Raskin 
I. 1995. Mechanisms of cadmium mobil-
ity and accumulation in Indian mustard. 
Plant Physiol. 109:427–33
138. Salt DE, Rauser WE. 1995. MgATP-
dependent transport of phytochelatins 
across the tonoplast of oat roots. Plant 
Physiol. 107:1293–301
transport across tonoplast of vesicles from 
avt roots. Evidence for a Cd$^{2+}$/H$^+$ Antipo-
rt activity. J. Biol. Chem. 268:12297–302
140. Deleted in proof
141. Schnoor JL, Lichten LA, McCutcheon SC, 
Wofle NL, Carrera LH. 1995. Phyto-
remediation of organic and nutrient contam-
142. Schrock R, Bierling B, Cao G, Dörfler U, 
of organic chemicals from soil by agricul-
tural plants. Chemosphere 28:297–303
143. Senden MHHM, Van Paassen FJM, Van 
Der Meer AJGM, Wolterbeek TH. 1990. 
Cadmium-citric acid-xylem cell wall in-
teractions in tomato plants. Plant Cell En-
viron. 15:71–79
144. Shimp JF, Tracy JC, Davis LC, Lee E, 
of plants in the remediation of soil and 
groundwater contaminated with organic 
145. Speiser DM, Ortiz DF, Kreppel L, Scheel 
biosynthetic genes are required for cad-
mium tolerance in Schizosaccharomyces 
146. Steffens JC. 1990. The heavy metal-bind-
ing peptides of plants. Annu. Rev. Plant 
Physiol. Mol. Biol. 41:553–75
147. Stephan UW, Schmidke I, Stephan VW, 
Scholz G. 1996. The nicotianamin molecule 
is made-to-measure for complexa-
tion of metal micronutrients in plants. 
Biometals 9:84–90
148. Stephan UW, Scholz G. 1993. Nicotia-
namine: mediator of transport of iron and 
heavy metals in the phloem? Physiol. 
Plant. 88:522–29
149. Stern RJ, Moon J, Key T, Amidon T, Sick-
els F, et al. 1996. A pathway analysis ap-
proach for determining generic cleanup 
standards for radioactive materials. Draft 
Rep. Comment. Trenton, NJ: NJ Dep. En-
Sci. Technol. 29:318A-23
for a role in the cell membrane in copper 
tolerance of Mimulus guttatus Fisher ex 
151. Terry N, Zayed A. 1997. Remediation of 
selenium-contaminated soils and wa-
ters by phytovolatilization. In Proc. Ex-
tended Abstr. 4th Int. Conf. Biogeochem-
istry of Trace Elements, ed. IK Iskandar, 
SE Hardy, AC Chang, GM Pierzynski, pp. 
Off. 785 pp.
152. Tomsett AB, Thurman DA. 1988. Mol-
ecular biology of metal tolerances of plants. 
Plant Cell Environ. 11:383–94
153. Topp E, Scheunert I, Attar A, Korte F. 
1986. Factors affecting the uptake of 14C-
labeled organic chemicals by plants from 
154. Trapp S, MacFarlane JC. 1995. Plant con-
tamination: modeling and simulation of 
organic chemical process. Boca Raton, 
FL: Lewis. 254 pp.
155. Van Dyke MI, Gulley SL, Lee H, Trevors 
JT. 1993. Evaluation of microbial sur-
factants for recovery of hydrophobic


159. Deleted in proof


171. Deleted in proof


173. Deleted in proof


