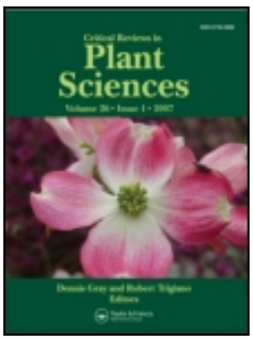

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Phytoremediation—An Overview

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The use of plants (directly or indirectly) to remediate contaminated soil or water is known as phytoremediation. This technology has emerged as a more cost effective, noninvasive, and publicly acceptable way to address the removal of environmental contaminants. Plants can be used to accumulate inorganic and organic contaminants, metabolize organic contaminants, and encourage microbial degradation of organic contaminants in the root zone. Widespread utilization of phytoremediation can be limited by the small habitat range or size of plants expressing remediation potential, and insufficient abilities of native plants to tolerate, detoxify, and accumulate contaminants. A better understanding and appreciation of the potential mechanisms for removing contaminants from the root zone and the interaction between plants, microorganisms, and contaminants will be useful in extending the application of phytoremediation to additional contaminated sites.

Keywords hyperaccumulation, phytodegradation, phytoextraction, phytofiltration, phytoimmobilization, phytostabilization, rhizodegradation, rhizofiltration

I. INTRODUCTION

Soil, surface water, and groundwater may become contaminated with hazardous compounds as a consequence of natural activities (e.g., geologic erosion and saline seeps) and human activities (e.g., industry, agriculture, wastewater treatment, construction, and mining). Pollutants may be traced to a particular source, point source, or result from a large area, nonpoint source. Contaminants of concern are both inorganic and organic compounds (heavy metals, radionuclides, nitrate, phosphate, inorganic acids, and organic chemicals) from sources including waste materials, explosives, pesticides, fertilizers, pharmaceuticals, acidic deposition, and radioactive fallout (Sparks, 1995). Both *in situ* and *ex situ* remediation methods have been employed to remove contamination, most relying on physical and chemical processes. *In situ* methods include volatilization via air venting, leaching with a surfactant, vitrification (contaminants are solidified with an electric current) and isolation and containment with physical barriers. *Ex situ* methods include excavation followed by thermal treatment, chemical extraction, and/or solidification (encapsulation) prior to disposal in a landfill. These remediation strategies are often very costly, depending on the contaminant of concern, extent of contamination, and the remediation strategy employed. Current price estimates for remediation of full-scale commercial sites begin at $200,000 plus an additional $40 to $70 per cubic yard of soil (Business Publishers Inc., 2004). An estimated 2 percent of the U.S. gross national product is spent on environmental remediation and pollution control (Sparks, 1995).

Phytoremediation, the use of plants to bioremediate contaminated soil, water, and air, has emerged as a more cost effective, noninvasive, and publicly acceptable way to address the removal of environmental contaminants (Boyajian and Carreira, 1997; Singh *et al*., 2003). An overview of phytoremediation, including phytofiltration and rhizofiltration, phytoextraction, phytoimmobilization, phytostabilization and phytodegradation, and rhizodegradation, is presented in this review. Biotechnological advances in phytoremediation are also discussed. These modern tools provide insight into processes important to phytoremediation and allow for the optimization of phytoremediation, improving its commercial viability.

II. PHYTOFILTRATION AND RHIZOFILTRATION

Phytofiltration or rhizofiltration is the use of plants to remove contaminants from water. The plant can take up contaminants into the biomass, thus removing the pollutant. Rhizofiltration is a form of phytoremediation, which refers to the approach of using hydroponically cultivated plant roots to remediate contaminated water through absorption, concentration, and precipitation of pollutants (Raskin and Kumar, 1994; Salt *et al*., 1995). Uptake and subsequent volatilization of contaminants can also occur. Aquatic plants have the ability to remove selenium (Se) from agricultural or industrial wastewater through Se accumulation and volatilization. Pilon-Smits *et al*. (1999) identified several species, including parrot's feather (*Myriophyllum brasiliense*), iris-leaved rush (*Juncus xiphioides*), cattail (*Typha latifolia*), saltmarsh bulrush, and Scirpus robustus, that showed great potential for Se phytoremediation in wetlands. De Souza *et al*. (1999) determined that bacteria in the rhizosphere of Indian mustard (*Brassica juncea*) were necessary to achieve the best rates of plant Se accumulation and volatilization of selenate.

Nonliving plant material can also serve as a biosorbent to remove contaminants. Plant material (living and nonliving) has been shown to bioaccumulate pollutants such as chromium (reducing the toxic form Cr(VI) to the nontoxic form Cr(III)). Heavy metals pose a serious health risk, and removal of metal ions using biosorption with plant-based materials has proven beneficial. Reviews on the phytoremediation of heavy metals are available in the literature (e.g., McGrath, Zhao, and Lombi, 2002; Lombi *et al*., 2001; Salt, Smith, and Raskin, 1998; Raskin, Smith, and Salt, 1997; Cunningham and Ow, 1996).

Phytofiltration and rhizofiltration are the topics of much research. Binding mechanisms within root and shoot tissue of plants allows for a very cost-effective approach to remove contaminants from the environment. Phytofiltration can also result in recovery and reuse of toxic, yet valuable, metals. While the volume of research on these topics is enormous, only a few representative examples will be provided here.

A novel phytofiltration technology has been proposed by Sekhar *et al*. (2004), which could be used to remove and recover lead (Pb) from wastewaters. This technology uses plant-based biomaterial from the bark of the plant commonly called Indian sarsaparilla (*Hemidesmus indicus*). The target of their research was polluted surface water and groundwater at industrially contaminated sites. Rhizofiltration of lead-contaminated water has also been investigated by Schulman, Salt, and Raskin (1999). They developed a screening method to look for mutants of *Brassica juncea* that had enhanced Cd or Pb accumulation

capabilities. The hyperaccumulating species were characterized by much smaller cell volumes, compared to the wild-types, which resulted in greater cellular surface area. Cell-wall binding and precipitation are the primary mechanisms of Pb accumulation in plants (Salt *et al*., 1995), thus the authors concluded that the hyperaccumulating characteristic of the mutant was due to the increased cell wall per unit of root weight.

The ability to remove and recover heavy metals, including Pb(II), Cr(III), Zn(II), Cu(II) and Ni(II) from aqueous solutions was shown in experiments with *Medicago sativa* (alfalfa). Optimum binding was in aqueous solutions at pH 5 (compared to other pH levels tested), and tests showed that binding to alfalfa shoots occurred within five minutes. The heavy metals were recovered (up to 90%) by using 0.1 M HCl (Gardea-Torresdey *et al*., 1998). Alfalfa biomass also was effective in recovering Au(III) from aqueous solutions (Gamez *et al*., 2003). Gardea-Torresdey *et al*. (2000b) noted that the accumulation of Au by alfalfa increases with elevated temperature and at lower pH and involves reduction of Au(III) to Au(0) colloids. Biosorption with powdered dry alfalfa (*M. sativa*) roots immobilized in polysilicate efficiently removed Pb and zinc (Zn) from dyeing wastewater. Recovery of the metals was achieved by using 0.1M HCl (Sivakumar *et al*., 2002). Cassava waste biomass was effective in removing two divalent metal ions, Cd(II) and Zn(II), from aqueous solutions (Horsfall and Abia, 2003). Modification of the cassava waste biomass by treating it with thioglycollic acid resulted in increased adsorption rates for Cd, Cu, and Zn (Abia, Horsfall, and Didi, 2003).

Several species of *Sargassum* biomass (nonliving brown algae) was an effective biosorbent for heavy metals, such as Cd and Cu (Davis, Volesky, and Vieira, 2000). Additionally, *S. fluitans* nonliving biomass was effective in sequestering uranium (Yang and Volesky, 1999). Rhizofiltration of uranium (U) by terrestrial plants has been investigated by Dushenkov *et al*. (1997). They found that certain sunflower species had a high affinity for U and could concentrate it from water into the roots. Phosphoryl and dicarboxyl groups were the dominant functional groups responsible for the binding of uranyl ions on *Datura innoxia* cell walls (Ke and Rayson, 1992). Tomato and tobacco roots harvested from field-grown plants were highly effective bioadsorbents that could adsorb strontium (Sr) from an aqueous solution of $SrCl_2$. Metals were recovered by reduction in pH to less than 2.0 or by using a concentrated chloride solution. Tang and Willey (2003) investigated plant uptake of ^{134}Cs. Plants from the Asteraceae family accumulated higher concentrations of radiocesium than *Beta vulgaris* and provided a new candidate for phytoremediation of radiocesium-contaminated soils.

Phytofiltration of chromium-contaminated water has been studied, and it has been found that several plant species can uptake the toxic Cr(VI) species and reduce the pollutant to the nontoxic form, Cr(III). Water hyacinth (*Eichhornia crassipes*) supplied with Cr(VI) in nutrient culture accumulated Cr(III) in root and shoot tissues. Reduction to the nontoxic form appeared to occur in the fine lateral roots (Lytle *et al*., 1998). Additionally, an agricultural waste byproduct, oat biomass (*Avena monida*), was effective as a biosorbent for removing Cr(VI) and converting it to Cr(III) (Gardea-Torresdey *et al*., 2000a). Ion exchange plays a roll in the binding and reduction of Cr(VI) to Cr(III) in protonated *Sargassum* seaweed biomass (Kratochvil, Pimentel, and Volesky, 1998).

Zurayk *et al*. (2001) evaluated the role of wetland plants (*Nasturtium officinale*, *Veronica beccabunga*, *Mentha longifolia*, and *Cardamine uliginosa*) in aquatic phytoremediation of Cr and found that Cr was predominantly accumulated in roots with minimal shoot translocation. Accumulation reached 6700 mg Cr kg^{-1} in roots of *V. beccabunga*.

Phytofiltration of As from drinking water is being evaluated using the brake fern (*Pteris vittata*). This plant species has been shown to tolerate high concentrations of arsenic (As) and can hyperaccumulate the substance in its foliage (Elless, Poynton, and Blaylock, 2003). Additionally, the authors are investigating the effectiveness of a hydroponic system for removing trace levels of As from source water.

While plant materials may prove to have desirable characteristics for phytofiltration of toxic heavy metals from the environment, it is crucial to better understand the chemical mechanisms behind these capabilities before their usefulness can be exploited to the fullest extent in phytoremediation strategies. Much research is being conducted to elucidate the fundamental chemical interactions involved in sequestration of heavy metals. Rayson and coworkers (2000) have studied the mechanism of uptake of heavy metals by *D. innoxia*. In their research, involvement of carboxylate functional groups was indicated and binding of metals involved both electrostatic and complex formation. In a study investigating phytofiltration of toxic metal ions Cd(II), Cu(II), Cr(III), Cr(VI), Pb(II), and Zn(II) from solution by a perennial biniferous crop, Hops (*Humulus lupulus*), metal ion binding was rapid, indicating that adsorption to cell walls of the plant may be occurring. Binding of Cd(II), Cu(II), Cr(III), and Zn(II) was found to be pH dependent, suggesting involvement of carboxyl groups present on the cell walls.

Nitrates are a common contaminant of drinking water sources in agricultural areas; nitrate removal is expensive. Reverse osmosis is a commonly used method for removal of nitrates, resulting in a highly concentrated wastewater stream. Russelle *et al*. (2004) are conducting field experiments with alfalfa, smooth bromegrass, orchardgrass, and soybean, as well as laboratory tests with alfalfa, reed canarygrass, bermudagrass, and switchgrass to evaluate their ability to remediate nitrate contamination. They have found that all species remove nitrates effectively when the rate of water movement was not too rapid through the root zone.

III. PHYTOEXTRACTION AND HYPERACCUMULATION

Phytoextraction, the ability of plants to take up inorganic (primarily metal) contaminants from soil is becoming a

more widely-used remediation technology (McCutcheon and Schnoor, 2003). Disposal of vegetation containing the contaminant (especially after ashing) or recovery of the contaminant in the plant are both more attractive (financially and environmentally) than disposal of contaminated soil. Plants have a natural ability to uptake inorganic chemicals (including metals) from soil and sediment. Some of these materials are essential nutrients to the plant, while others have no known physiological function in plants. Several factors contribute to the success of phytoextraction as a remediation technology including the extent of contamination, metal bioavailability, and the plant's ability to intercept, absorb, and accumulate metals (Ernst, 1996).

The most common route of chemical uptake into plants is through the root via an aqueous phase. Ions and organic molecules move to roots from soil and sediment through plant transpiration (ion transport from the soil water into the root occurs simultaneously with water transport), diffusive transport, and microbial facilitated transport (Committee on Bioavailability of Contaminants in Soils and Sediments, 2003). The plasma membrane serves as a barrier to uptake; chemicals need to cross the plasma membrane into the cytoplasm of the root cells.

Different mechanisms have been identified which control chemical uptake by plants. Some chemicals can enter root tissue by altering pH through efflux of hydrogen (H^+) ions, resulting in an electrochemical gradient that facilitates transport of cations and anions. This mechanism is termed a proton pump and requires cellular energy in the form of adenosine triphosphate (ATP). Most divalent cations are absorbed through ion channels. Ion channels can also mediate uptake and release potassium ions (K^+). There is also evidence for carrier-mediated active transport of K^+, SO_4^{2-}, NO_3^-, and Mg^{2+} that uses ATP as an energy source (Marschner, 1995). For metals, another possible mechanism of uptake is transport of metal-chelate complexes. Whenever there is a metal deficiency, plants produce and release chelating agents into the rhizosphere. The complexed metal form is then transported into the plant through a transport protein specific for that metal (Kochian, 1993; Von Wiren, Marschner, and Romheld, 1996). The selectivity of many of these mechanisms is limited; ions that have the same charge or same size can share the same carrier or channel with nutrients, resulting in an increased uptake of metal contaminants (Oliver *et al*., 1994; Fan *et al*., 2001).

While metal-tolerant plants are relatively common, most plants do not accumulate metals to significant levels in above-ground biomass. However, some plant species are capable of hyperaccumulation of metal ions, that is, they are able to take up and accumulate metals at concentrations of more than 0.1 percent (by dry weight of plant) or greater (Brooks, 1998). These hyperaccumulating plants are able to tolerate high concentrations of metals in above-ground biomass probably through the use of phytochelatins, sulfur-rich proteins similar in character to vertebrate metallothioneins (Grill, Winnacker, and Zenk, 1985). These plants have been used as candidates for a type of remediation known as phytoextraction due to their ability to uptake metals from soil and translocate those metals into harvested above-ground biomass (Kumar *et al*., 1995). Unfortunately, most plant species capable of hyperaccumulation of metals from soil do not possess the other desirable characteristics (adaptability, rapid growth, large above-ground biomass, etc.) relevant to their use in remediation systems (Brown *et al*., 1994, 1995).

While an exhaustive analysis of the literature on hyperaccumulation/phytoextraction is beyond the scope of this text, some common themes emerge from the excellent literature reviews available on the subject (for example, Lasat, 2002). A variety of terrestrial plant genera have been identified as possessing the ability to hyperaccumulate certain metals from soil including *Brassica, Thlaspi, Apocynum, Aeollanthus*, and *Paspalum* among others (Baker, 1995; Krämer *et al*., 1996). Overall, the number of taxonomic plant groups varies by metal; several plant groups (>20/metal) have been identified as having the ability to hyperaccumulate Co, Cu, and Zn, while only a few (<5/metal) have been identified as having the ability to hyperaccumulate Pb and Cd. Also relevant is the phytoextraction coefficient (plant:soil partition coefficient) for the various metals of concern. For some metals (Cr, Cd, and Ni) this value can be as high as 30 or greater, while for others (Pb) the partitioning is not very favorable (<2). In order to address current limitations to the use of plants in phytoextraction of metals from soil, research on the processes governing metal uptake in plants has focused on (1) enhancing metal availability in soil, (2) improving plant characteristics through breeding and biotechnology, and (3) exploration of plant hyperaccumulation mechanisms (Lasat, 2002 and references therein).

IV. PHYTOIMMOBILIZATION AND PHYTOSTABILIZATION

Phytoimmobilization is a remediation technology in which plants are used to remove contaminants from soil through plant uptake and subsequently the contaminants are released from decomposing plant materials and are then immobilized in either a mineral-amended soil or a geomat (mineral-containing mat). This strategy is being evaluated at the Savannah River Site in Aiken, S.C., by researchers who are investigating indigenous plants that have the natural ability to accumulate high concentrations of contaminants (Knox *et al*., 2001). Phytostabilization results in the elimination of the availability of toxic metals in soil through complexing with metals by certain plants (Gwozdz and Kopyra, 2003). This process does not remove the contaminant from the soil, but it does reduce the inherent hazard of the contaminant (Li *et al*., 2000). Remediation by removal of contaminants from a site is not always possible. In these instances, stabilization provides an alternative from a logistical and technical standpoint. The result is transformation of the hazardous chemical to an inert condition (Cunningham *et al*., 1997). A review on the topic of chemophytostabilization of metals in contaminated soils has been conducted (Knox *et al*., 2000).

V. PHYTODEGRADATION AND RHIZODEGRADATION

One of the most important phases in the process of remediation of organic pollutants is degradation of the contaminant. Degradation of a compound refers to its breakdown into smaller constituents, or its transformation to a metabolite. It is important to identify, quantify, and understand the significance of metabolites formed during remediation because of their potential unknown toxicities and availabilities to biota. Often, transformation products are less toxic and/or less available than parent compounds, but this is not always the case, making identification and characterization of metabolites important. In a phytoremediation setting, degradation can happen in the rhizosphere (soil surrounding plant roots), as well as within the plant itself. The latter, phytodegradation, occurs when a plant has taken up the contaminant into its tissues, and enzymes within the plant work to transform the compound, often into molecules that can be more readily broken down or released in root exudates. Rhizodegradation, or transformation of the contaminant in the rhizosphere, can occur in soil organisms such as fungi or bacteria, or via enzymes exuded from microorganisms or plants (for example, Schultz *et al*., 2001; Siciliano, Goldie, and Germida, 1998; Walton and Anderson, 1990). Additionally, microorganisms performing the degradation of organics may be supported by plants, because of the nutrient potential of plant root exudates. For example, Johnson *et al*. (2004) found enhanced degradation of the polycyclic aromatic hydrocarbon chrysene when nonsterile soil was planted with clover and inoculated with a rhizobia bacterium, but did not find increased degradation in the same treatment without the inoculum, or in any of the sterile soil treatments.

A. Phytodegradation

Methods for studying phytodegradation can involve growing plants in soils, nutrient media in hydroponic systems, or utilization of tissue preparations (Bhadra *et al*., 2001; Roeth and Lavy, 1971; Larson *et al*., 1999; Schmidt *et al*., 1997). In a hydroponic system, the solution is treated with a known concentration of the contaminant in question, and plant uptake, metabolism, and exudation are studied. While this system is helpful for delineating the plant's role in the remediation process, it is less useful in determining the availability of the contaminant to the plant. Additionally, some compounds may not be readily soluble in the nutrient solution, also affecting availability to the plant in that system. Related methods involve a similar system, except that the plants are grown in deactivated silica sand treated with a nutrient medium containing the contaminant (Sun *et al*., 2000). This provides a more realistic medium for plant growth, as well as for assessing the availability of the compound. Radiotracers (^{14}C-labeled contaminants) are very useful in these types of studies, particularly for identification of metabolites, and for mass balance, which can include measurement of CO_2 ($^{14}CO_2$) evolution and other volatiles.

To determine if the plants are absorbing parent compound and performing metabolism unaided by microbial metabolism, a variety of studies can be performed. Radiolabeled contaminant may be applied to foliar tissue, or injected into the stem of the plant, and distribution and metabolism monitored over time. Alternatively, sterilized hydroponic systems may be used to evaluate root uptake of the contaminant by the plant. Detection of radiolabel in plant biomass can indicate uptake of the contaminant by the plant, particularly if live plant results are compared with killed (control) plants; detection of radiolabel within dead tissue would indicate passive movement of the contaminant into the plant, rather than active uptake. Additional analytical work could resolve identity of metabolites within plant biomass. In a sterile system, it would be likely that metabolites detected within the plant were a result of metabolic activity within the plant (Bhadra *et al*., 1999, 2001).

The most realistic laboratory-type study should involve a relevant soil and appropriately aged residues of the contaminant. Recent research has attempted to resolve some of these issues through the development of closed systems for study of mass balance and metabolism in plants and soil (Figure 1). Plant uptake and phytodegradation of herbicides, atrazine and metolachlor, were evaluated in this system with prairie grass. Both parent compounds and their metabolites were detected in root and leaf tissues (0.5 to 7% applied radioactivity, respectively) (Henderson, 2004). Concentrations of parent atrazine and metabolites in the grasses were different than those described in other model plant systems (Mathew, Nelson, and Khan, 1996; Raveton *et al*., 1997). Raveton *et al*. (1997) found hydroxyatrazine, an atrazine degradate, was the dominant compound in both roots and shoots of corn plants. Schmidt *et al*. (1997) reported N-dealkylation reactions resulting in the didealkylated atrazine metabolite in soybean (*Glycine max*) cell cultures only, in a study examining several plant species. In the aforementioned study by Henderson (2004), didealkylatrazine was a prominent metabolite detected, which may indicate different metabolism

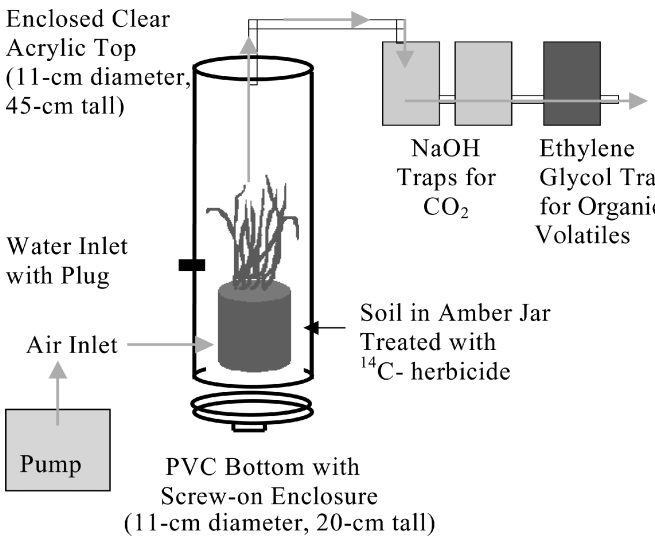

FIG. 1. Example of a mass balance chamber.

and/or uptake of atrazine or metabolites by the grasses used in that study. In the study of metolachlor metabolism and uptake, metolachlor ethane sulfonic acid, a glutathione conjugation product, was the major metabolite found in soil and plant tissue (Henderson, 2004). Crops, such as corn, soybeans, and sorghum, have developed tolerance of some herbicides through utilization of a glutathione conjugation pathway (Al-Khatib *et al*., 2002). In a similar flow-through chamber system, Orchard *et al*. (2000) evaluated uptake of trichloroethylene (TCE) by hydroponically-grown poplar trees in a variety of plant-stress situations including high concentrations of TCE and low oxygen levels. They found that transpiration and TCE concentration were the most important factors dictating uptake. Low concentrations of metabolites were detected in poplar roots (Orchard *et al*., 2000).

Closed incubation systems have also been utilized to evaluate the ability of aquatic plants to remediate herbicide-contaminated surface water. *Ceratophyllum demersum* (coontail) significantly reduced levels of atrazine and metolachlor by at least 44 percent relative to surface water systems void of vegetation. Parent compounds extracted from the plants were less than 1 percent of the applied herbicide, whereas metabolites accounted for 5 and 21 percent. After 16 days, surface waters without aquatic plants contained 61 and 85 percent of the applied metolachlor and atrazine, respectively, compared to 1 percent (metolachlor) and 41 percent (atrazine) remaining in the systems containing *C. demersum* (Rice, Anderson, and Coats, 1997a).

White (2000, 2001) described uptake of a DDT metabolite (DDE) by several plant types in agricultural soils, with cucurbits (i.e., pumpkin and zucchini) having the highest concentrations of DDE in their tissues. These plants were also successful with aged residues of DDE.

Uptake and conjugation of other contaminants and their metabolites, including sequestration of the compounds within plant tissues, have been reported. Bhadra *et al*. (1999) observed reductive and oxidative metabolism of trinitrotoluene (TNT) in an aquatic plant species, as well as accumulation of the compound throughout the plant. In studies with the explosives hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), Bhadra *et al*. (2001) found uptake, metabolism, and sequestration of RDX, with a small fraction ($<10\%$) of RDX being "bound" in plant tissue after 65 days. Differences in the ability of aquatic plant species to remove HMX were observed. Lust and Schnoor (2004) found that sunlight induced the transformation of RDX to potentially toxic metabolites in a phytoremediation situation using reed canarygrasses. Each of these findings illustrates the need for further research on absorption and metabolism of different compounds in various plant species, and in varying environmental conditions.

B. Rhizodegradation

The rhizosphere, with myriad species of microorganisms from many taxa, is a perfect example of biodiversity. While any individual species is usually capable of producing one or more enzymes that can carry out a biotransformation reaction, a consortium of microbes in the root zone of a plant can carry out many and varied types of enzymatic transformations (Anderson, Guthrie, and Walton, 1993; Walton *et al*., 1994). The evolutionary significance of a plant's nourishing of microbes in the rhizosphere is at least partially based on the protective value of the microbes in the root zone. Biotransformations accomplished by microorganisms can detoxify chemicals that may be toxic to the plant, e.g., allelopathic compounds released by neighboring plants that are competing for the same space, nutrients, water, and light. Herbicides, such as atrazine and metolachlor, are detoxified by the microbial consortium in root zone soils from prairie grasses; this leads to less detrimental effect on the plants than in a soil with less microbial activity, where herbicide concentrations remain high for a relatively long time. Likewise, naturally occurring herbicidal substances (allelochemicals, such as phenylethyl propionate or eugenol) are also likely to be subjected to much more enzymatic detoxification in a rhizosphere soil than in a comparable soil devoid of living microorganisms. Arthur *et al*. (1997) reported enhanced degradation of the atrazine metabolite deethylatrazine in soils with long atrazine histories, compared to soils never receiving atrazine; this indicates a potential selection of contaminant-degrading microorganisms in soils with long histories of contamination. Plants also benefit from microbes in their rhizosphere because microbes can secrete enzymes that can solubilize certain crucial nutrients from the soil (Perkovich *et al*., 1996; Qiu *et al*., 1997; Anhalt *et al*., 2000). Additionally, rhizobial bacteria capable of nitrogen fixation often associate with plant roots (i.e., legumes), to provide a nitrogen source for the plant. In turn, the plant likely provides other nutrients to root zone bacteria through root exudates or in root nodules.

Biotransformations achieved by microorganisms in the rhizosphere can be the result of cells secreting enzymes into the soil matrix or from enzymatic action on compounds that have been taken into the cells. These reactions are numerous and include oxidation, reduction, hydrolysis, conjugation, and rearrangement (Coats, 1991). Any particular microbial species may be capable of several reaction types, and when a multitude of species are present, that group may be able to collectively degrade an organic compound completely. Similarly, an assemblage of several species of plants, growing together, may support an even more diverse rhizosphere, which may be capable of an even wider array of biochemical transformations (Belden and Coats, 2004).

The methods for studying rhizodegradation have traditionally included (1) soil metabolism studies and (2) isolation and culturing of individual species of organisms, typically bacteria or fungi. The former method provides largely realistic data on the overall fate of a chemical in the soil with opportunities for controlling variables such as temperature, moisture, amendments, etc. The single-species experiments can generate information on the specific reactions and products formed by that organism, including rates, biodegradation pathways, and biochemical

strategies (cometabolism and catabolism). A few examples are provided below. Bollag and Liu (1972) and Bollag, Czaplicki, and Minard (1975) isolated a soil fungus and bacterial isolates from river water; these organisms were capable of metabolizing, and in some cases mineralizing 1-napthol, a metabolite of the insecticide carbaryl. Enhanced mineralization of ethylene glycol, active ingredient of aircraft deicers, was reported in rhizosphere soils of alfalfa (*M. sativa*) and Kentucky bluegrass (*Poa pratensis*), and increased with greater soil temperatures (Rice, Anderson, and Coats, 1997b). Zablotowicz, Locke, and Hoagland (1997) reported rapid metabolism of acifluorfen, a nitrodiphenyl ether herbicide, to aminoacifluorfen in rhizosphere suspensions relative to soil suspensions, and identified strains of *Pseudomonas fluorescens* displaying nitroreductase activity, an important catabolic pathway for initial degradation of nitroaromatic compounds. Chen, Banks, and Schwab (2003) found substantial increases in the mineralization of pyrene in rhizospheric soils of fescue and switchgrass compared to nonrhizospheric soil. Each of these examples demonstrates the potential importance of the rhizosphere and its residents in the degradation of organic contaminants.

C. Transformation Pathways

Transformation of contaminants can occur through a variety of pathways. In this case, we will discuss the metabolism and cometabolism of pesticides, as example compounds. Plants and soil microorganisms, including bacteria and fungi, contain many similar enzymes for detoxification or transformation of xenobiotics. One major difference between microorganisms and higher plants is that microbes are much more likely to mineralize a contaminant (Hoagland, Zablotowicz, and Hall, 2001) or use it as a nitrogen source (Assaf and Turco, 1994).

During mineralization processes, hydrolysis or reduction reactions may occur, in addition to oxidation of aromatic rings. These reactions may occur in the soil or in the rhizosphere via enzymes from a consortium of bacteria. Microbes may also release extracellular enzymes to hydrolyze or reduce a contaminant prior to absorption (Barkovskii, 2001; Crowley, Luepromchai, and Singer, 2001). During cometabolism, or incidental biotransformation, contaminants may be hydrolyzed with amidases, esterases, or nitrilases. Oxidation reactions can occur with peroxidases or mixed function oxidases. Reductive dehalogenases and transferases may also play roles in detoxification of a compound. These enzymes may be intracellular or extracellular (Hoagland *et al*., 2001; Schultz *et al*., 2001).

In plants, the activity of oxidative enzymes often results in hydroxylated metabolites of aromatic rings; these metabolites can then be conjugated to sugars, amino acids, or glutathione, via glutathione-S-transferase. These are often called phase II transformations, while the initial hydrolysis reactions are termed phase I transformations. Additional reactions, phase III (Sandermann, 1992), may result in further conjugations, sequestration of the metabolite in organelles, or incorporation into plant tissues ("bound" residues) (Manahan, 1992).

D. Variables Influencing Degradation

While phytodegradation and rhizodegradation proceed via enzymatic activity, there are numerous other variables that influence the process, including soil temperature, moisture, pH, organic matter content, and aeration, all of which can affect the proliferation of microorganisms in the soil, which in turn will affect biodegradation. Some of the factors affect degradation processes directly; others impact degradation by altering the bioavailability of the substrate (pollutant). Overall, there are numerous soil and environmental parameters that influence the fate of a contaminant in the rhizosphere. There are also factors from the biological perspective that can modify the contaminant and its degradates in a phytoremediation setting. It is crucial to understand the physical, chemical, and biological processes, as well as interactions between them, before we can optimize conditions for phytoremediation or have confidence in making predictions about the potential extent or rate of cleanup from a phytoremediation approach to a given soil-contamination situation.

VI. IMPROVING PHYTOREMEDIATION WITH BIOTECHNOLOGY

Widespread utilization of phytoremediation can be limited by the small habitat range or size of plants expressing remediation potential, and insufficient abilities of native plants to tolerate, detoxify, and accumulate contaminants (Krämer and Chardonnens, 2001). Selective breeding has been utilized to improve plant performance (Kopp *et al*., 2001; Bert *et al*., 2003), whereas current research focuses on a transgenic approach (Pilon-Smits and Pilon, 2002; Berken *et al*., 2002; Tong, Kneer, and Zhe, 2004). Genetic engineering of plants involves inserting foreign DNA into the genome of plant cells producing a transgenic plant that exhibits a desired trait. Biotechnology has been utilized to elucidate biochemical and genetic mechanisms for processes important to phytoremediation (root uptake, translocation from roots to shoots, sequestration, and chemical modification), to exploit natural characteristics and optimize rate-limiting processes, or enhance plants with novel traits from other organisms (Bai and Mebra, 1997; Heaton *et al*., 1998; Arazi *et al*., 1999; Hirschi *et al*., 2000; Pilon-Smits and Pilon, 2002; Cohen, Garvin, and Kochian, 2004).

A. Transfer of Metabolic Functions from Microorganisms to Plants

The transfer of genes and their unique metabolic capabilities from microorganisms to plants is one of several transgenic remediation strategies to enhance the environmental decontamination functions of plants (Table 1). Bioremediation of contaminated soils and sediments with contaminant-resistant microorganisms has been reported (for example, Bollag, Mertz, and Otjen, 1994; Sheehan, 1997); however this approach is restricted to a narrow range of environmental conditions as bacteria and fungi inhabit limited niches (Rugh, 2001). Plants have wider habitat ranges and deeper soil interaction because of extensive root

TABLE 1
Transgenic plants and phytoremediation

Pollutant(s)[1]	Transgenic plant[2]	Gene source[3]	Foreign gene or DNA	Gene product[4]	Maximum observed effect[5]	Medium[6]	Reference
Transfer of metabolic functions from microorganisms to plants							
As	*B. napus*	*E. cloacae*		ACC-D	T, A (4-fold)	PRMV	Nie *et al.*, 2002
Cd	*B. juncea*	*E. coli*	*gshI*	$\gamma-$ECS	T&A (90%)	HYDR	Zhu *et al.*, 1999b
Cd	*B. juncea*	*E. coli*	*gshII*	GS	T	Agar	Zhu *et al.*, 1999a
					A: shoot (25% increase)	HYDR	
					A: total (3-fold)	HYDR	
Cd, Pb	*A. thaliana*	*S. cerevisiae*	*YCF1*	YCF1	T&A: Cd&Pb	Agar	Song *et al.*, 2003
Cd, Cu, Pb	*B. Juncea*	*E. coli*	*gshI*	γ-ECS	γ-ECS plants	Soil	Bennett *et al.*, 2003
			gshII	GS	A: Cd, Cu, Zn (2.4- to 3-fold)		
					γ-ECS plants and GS plants		
					A: Cd (1.5-fold)		
					A: Zn (1.5- to 2-fold)		
Hg(II)	*Arabidopsis*	Bacteria	*merA*	MIR	V: Hg(II) ($\geq$3-fold)	HYDR	Heaton *et al.*, 1998
					R: Hg(II) (>3-fold)		
Hg (II)	*L. tulipifer*	Bacteria	*merA*	MIR	V: Hg(0) (10-fold)	IM	Rugh *et al.*, 1998
MeHg	*A. thaliana*	*E. coli*	*merApe9*	MIR	T: $HgCl_2$ (20 ppm)		Rugh *et al.*, 1996
					V: Hg(0)		
MeHg	*A. thaliana*	Bacteria	*merBpe*	OL	Catalyzes carbon-mercury bond	Agar	Bizily *et al.*, 1999
MeHg	*A. thaliana*	Bacteria	*merA, merB*	MIR, OL	T: (50-fold)	Agar	Bizily *et al.*, 2000
MeHg	*Arabidopsis*	Bacteria	*merB*	OL	T, D (70-fold)	Agar	Bizily *et al.*, 2003
PMA	*N. tabaccum*	Bacteria	*merA, merB*	MIR, OL	T: (400 μM)	Soil	Ruiz *et al.*, 2003
ACET, MET	*Populus*	Bacteria		γ-ECS	T	Soil	Gullner *et al.*, 2001
GTN, TNT	*N. tabaccum*	Bacteria		PTR	T&D: GTN(1 μM)	HYDR	French *et al.*, 1999
					T: TNT (0.05 μM)		
PCP	*N. tabaccum*	*C. versicolor*		MnP	Activity (54-fold)	HYDR	Iimura *et al.*, 2002
					R: 2-fold (250 μM)		

Exploitation of plant detoxification mechanisms							
Cd, Pb	*N. glauca*	Wheat	*TaPCS1*	PS	T: Pb, Cd A: Pb (2-fold)	Agar Soil	Gisbert *et al*., 2003
LN, ISP, CT	*N. tabaccum*	*H. tuberosus*	*HTR1*	CYP76B1	T: LN (20-fold) T: ISP & CT (10-fold)	FT	Didierjean *et al*., 2002
Transfer of metabolic functions from mammals to plants							
Herbicides	*S. tuberosum*	Human	cDNA	CYP1A1 CYP2B6 CYP2C19	T	FT	Inui *et al*., 2000
Herbicides	*O. sativa*	Human	cDNA	CYP2C9 CYP2C19	T&D	MS HYDR	Inui *et al*., 2001
ATR, CT	*S. tuberosum*	Rat	cDNA	CYP1A1	D	MS	Yamada *et al*., 2001
TCE, EDB	tobacco	mammal	cDNA	CYP2E1	D (increased rate)	HYDR	Doty *et al*., 2000

[1]Pollutant(s): MeHg = methyl mercury, ACET = acetochlor, MET = metolachlor, GTN = glycerol trinitrate, TNT = trinitrotoluene, PCP = pentachlorophenol, LN = linuron, ISP = isoproturon, CT = chlortoluron, ATR = atrazine.

[2]Plant: *Brassica napus, Brassica juncea, Arabidopsis thaliana, Liriodendron tulipifer, Nicotiana tabaccum, Nicotiana glauca, Solanum tuberosum, Oryza sativ*.

[3]Gene source: *Enterobacter cloacae, Escherichia coli, Saccharomyces cerevisiae, Coriolus versicolor, Helianthus tuberosus.*

[4]Gene product: ACC-D = 1-aminocyclopropane-1-carboxylate deaminase, γ-ECS = γ-glutamylcysteine synthetase, GS = glutathione synthetase, YCF1 = yeast vacuolar transporter, MIR = mercuric ion reductase, OL = organomercurial lyase, PTR = pentaerythritol tetranitrate reductase, MnP = Mn-peroxidase, PS = phytochelatin synthase; CYPxxx = cytochrome P450 enzyme.

[5]Observed Effect: A = accumulation, D = degradation, R = removal of contaminant from media, T = tolerance, V = volatilization.

[6]Medium: PRMV = Pro-mix and vermiculite, HYDR = hydroponics (liquid medium), IM = initiation-maintenance medium, FT = plants grown in soil followed by foliar treatment of herbicide, MS = MS medium.

systems and influence of the rhizosphere. Engineered plants containing novel traits from contaminant-resistant microorganisms have demonstrated enhanced tolerance, growth, and degradation of both inorganic and organic contaminants.

A successful example of this engineering strategy was demonstrated in transgenic plants that convert hazardous organomercurial compounds and toxic ionic mercury (Hg(II)) into less toxic and volatile elemental mercury (Hg(0)) through degradation pathways that occur naturally in some bacteria and not at all in plants (Heaton *et al*., 1998; Krämer and Chardonnens, 2001; Rugh, 2001). Microorganisms isolated from mercury-contaminated environments have evolved mercury resistance, which is genetically encoded by the *mer* operon containing a cluster of genes involved in the detection, mobilization, and enzymatic detoxification of mercury, and specifically contains the *merB* and *merA* genes that code for the mercury-processing enzymes organomercurial lyase and mercuric ion reductase, respectively (Summers, 1986; Rugh *et al*., 1998). Initial research, transferring mercury detoxifying abilities of bacteria into plants, focused on developing plants expressing the *merA* gene that could survive on media spiked with Hg(II) and transform Hg(II) to Hg(0) (Rugh *et al*., 1996). Later research focused on engineering plants to express the bacterial enzyme organomercurial lyase to degrade hazardous compounds such as methylmercury. Subsequent research further improved the efficiency of this process in transgenic plants by modifying the bacterial *merB* gene to target the MerB protein for accumulation in the endoplasmic reticulum and for secretion to the cell wall (Bizily *et al*., 1999; Bizily, Rugh, and Meagher, 2000). Ruiz *et al*. (2003) reported the integration of both *merA* and *merB* genes into chloroplast genome of tobacco plants (*Nicotiana tabacum*) produced transgenic plants able to tolerate high levels of phenylmercuric acetate, an organomercurial compound. Results of these studies suggest that transgenic plants engineered to express bacterial *merB* gene or coupled *merB* and *merA* genes can remediate organomercurial-contaminated sites by degradation of the organomercurial compound to Hg(II) followed by sequestration for later removal or further conversion of Hg(II) to relatively inert and volatile Hg(0).

Naturally occurring hyperaccumulating plants tend to produce little biomass and exhibit slow growth (Zhu *et al*., 1999b; Tong *et al*., 2004). Transgenic approaches are being utilized to improve the tolerance and accumulation of metals in fast-growing plants with large biomass production. Transgenic canola plants (*Brassica napus*) expressing *Enterobacter cloacae* UW4 1-aminocyclopropane-1-carboxylate deaminase, a bacterial deaminase, displayed better seed germination, greater biomass in roots and shoots, higher concentrations of leaf chlorophyll and protein, and arsenate accumulation approximately four times that of nontransformed canola when exposed to arsenate (Nie *et al*., 2002). The transfer of genes, from *Escherichia coli* into Indian mustard (*B. juncea*), encoding for overexpression of glutathione synthestase (GS) in the cytosol and γ-glutamylcysteine synthetase (γ-ECS) targeted to the plastids of Indian mustard resulted in greater tolerance and accumulation of Cd relative to wild-type Indian mustard (Zhu *et al*., 1999a,b). GS and γ-ECS transgenic Indian mustard and *B. juncea* overexpressing adenosine triphosphate sulfurylase reduced the concentration of metals (Cd, Cr, Cu, Mn, Pb, and Zn) by up to 25 percent from soil collected at a USEPA Superfund site (Bennett *et al*., 2003). Greater tolerance and accumulation of Cd and Pb were also observed in transgenic *Arabidopsis thaliana* plants overexpressing YCF1, producing a yeast protein (YCF1) that detoxifies cadmium by vacuolar compartmentalization (Song *et al*., 2003). Phytoremediation of metals using transgenic plants is reviewed further by Pilon-Smits and Pilon (2002).

Biotechnology has also been used to transfer foreign genes from microorganisms into plants to enhance phytoremediation of organic contaminants. An extensive root system, high water uptake, rapid growth, and large biomass production make poplar trees (*Populus*) a good candidate for phytoremedation. Attempts have been made to increase their tolerance of chloroacetanilide herbicides by overexpression of bacterial γ-ECS in the cytosol or chloroplast. Transgenic poplars were significantly more tolerant to chloroacetanilide herbicides than wild-type poplars; however, both exhibited decreased shoot and root weights showing a need for further improvement of the detoxification capacity (Gullner, Kömives, and Rennenberg, 2001). Introduction of genes from an explosive-degrading bacterium into tobacco plants (*N. tabacum*) produced transgenic plants that expressed pentaerythritol tetranitrate reductase and were capable of enhanced degradation of nitrate ester and nitroaromatic explosives (French *et al*., 1999). In addition, transgenic tobacco plants containing a gene for Mn-peroxidase (MnP) from the fungi *Coriolus versicolor* expressed MnP activity at levels 54-fold higher than in control lines. Expression of this gene and production of the peroxidase oxidatively degrades halogenated hydrocarbons. Roots of transgenic plants exposed to liquid medium containing 250 μM pentachlorophenol (PCP) showed a 2-fold reduction of PCP relative to control plants and no obvious adverse effects on vegetative and sexual growth (Iimura *et al*., 2002).

B. Exploitation of the Inherent Detoxification Mechanisms of Plants

The expression of plant genes in yeast (*Saccharomyces cerevisiae*) has resulted in enhanced degradation of phenylurea herbicides (Robineau *et al*., 1998) and increased cadmium tolerance (Clemens *et al*., 1999). This suggests the transfer of these plant genes into vegetation exhibiting fast growth and large above-ground biomass, may produce transgenic plants with an exceptional ability for phytoremediation. Transgenic tobacco (*N. tabacum*) and Arabidopsis, engineered to express a xenobiotic inducible cytochrome P450 enzyme (CYP76B1) from the Jerusalem artichoke (*Helianthus tuberosus*), were 10- and 20-fold more tolerant to phenylurea herbicides compared to nontransgenic plants (Didierjean *et al*., 2002). Shrub tobacco (*Nicotiana glauca*), a fast-growing high-biomass plant tolerant

of a wide range of environmental contaminants, was genetically modified to overexpress *TaPCS1*, a wheat gene encoding phytochelatin synthase. Transgenic seedlings exhibited greater tolerance to cadmium and developed roots up to 160 percent longer than nontransformed plants. Genetically modified plants, grown for 6 weeks in mine waste-contaminated soil, were found to contain 50% and 85% more lead (Pb) in aerial tissues and root tissues, respectively, than wild-type plants. Although the shrub tobacco plant had already shown the ability to develop resistance to metals (Cd, Cu, Pb, Zn) and survive on mine soils, incorporation and expression of a wheat gene multiplied the tolerance and accumulation of metals in the transgenic plant (Gisbert *et al*., 2003) (Table 1).

C. Transfer of Metabolic Functions from Mammals to Plants

Transgenic plants expressing mammalian genes have been evaluated as a means to increase the remediation efficiency of plants (Table 1). Initial attempts to engineer herbicide-tolerant plants utilized P450 cDNA from bacteria and mammals because of a lack of relevant plant genes (Morant *et al*., 2003). Transgenic rice (*Oryza sativa*) expressing human cytochrome P450 monooxygenases and transgenic potato (*Solanum tuberosum*) expressing either human or rat cytochrome P450 monooxygenases have shown enhanced metabolism and tolerance to a variety of herbicides (acetochlor, atrazine, chlorsulfuron, chlortoluron, imazosulfuron, methabenzthiazuron, metolachlor, norflurazon, pyributicarb), relative to nontransformed plants (Inui *et al*., 2000, 2001; Ohkawa *et al*., 2001; Yamada *et al*., 2002). Introduction of human cytochrome P450 2E1, an enzyme that oxidizes a wide range of important halogenated hydrocarbon pollutants, into tobacco plants resulted in enhanced metabolism of trichloroethylene (TCE) and ethylene dibromide, widespread groundwater contaminants. The largest increase in TCE metabolism, measured by the presence of the metabolite trichloroethanol, was found in the roots (642-fold increase) followed by the stems (171-fold increase) and leaves (140-fold increase) as compared with the nontransgenic plants (Doty *et al*., 2000). To date, evaluation of transgenic plants expressing mammalian genes has been limited to laboratory assessments. Results of these studies suggest that transgenic plants containing genes for mammalian cytochrome P450 may be useful for phytoremediation of both soil and groundwater polluted with organic contaminants. For a recent review on cytochrome P450s and their use in engineering plants for phytoremediation see Morant *et al*. (2003).

VII. CONCLUSIONS

The use of vegetation directly or indirectly to remove contaminants from water or soil is an important innovative remediation technology potentially applicable to a variety of contaminated sites. Selection of the appropriate plant species is a critical process for the success of this technology. There are several potential mechanisms for contaminant removal including hyperaccumulation, phytodegradation, phytoextraction, phytofiltration, phytoimmobilization, phytostabilization, rhizodegradation, rhizofiltration. A better understanding and appreciation of these potential mechanisms for removing contaminants from the root zone and the interaction between plants, microorganisms, and contaminants will be useful in extending the application of phytoremediation to additional contaminated sites and will help in limiting phytoremediation to situations where there is a good chance of success.

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