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Update on Biotechnology

Promises and Prospects of Phytoremediation

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We often think of plants primarily as a source of wood, food, and fiber. Secondarily we may also appreciate their presence for aesthetic reasons as well as for "altruistically" providing habitat for other species. Increasingly, however, their value as an environmental counterbalance to industrialization processes is being appreciated. These processes include the burning of fossil fuels, generation of wastes (sewage, inorganic and organic solids, and effluents), and general water flow and processing. Plants have long been recognized for their consumption of CO_2 and, more recently, of other gaseous industrial byproducts (Simonich and Hites, 1994). Recently, their role in slowing the rate of global warming has been further appreciated in both the scientific and popular press. Their use as a final water treatment and for disposal of sludge resulting from waste water treatment is centuries old (Hartman, 1975). The extensive literature concerning water and sludge treatment and the emerging field of air pollution abatement with plants will not be discussed here. Instead, we focus on an emerging concept, "phytoremediation," the use of plants to remediate contamination of soil with organic or inorganic wastes.

Remediation of soil contamination by conventional engineering techniques often costs between $50 and $500 per ton. Certain specialized techniques can exceed costs of $1000 per ton. With an acre of soil (to a 3-foot depth) weighing approximately 4500 tons, this translates to a minimum cost of about a quarter million dollars per acre (Cunningham et al., 1995). It is not surprising that the cleanup of contaminated sites has not been proceeding at a rapid pace.

There is an active effort to develop new, more cost-effective technologies to remediate contamination of such soils. For the most part these efforts are being led by engineers and microbiologists. More recently, however, green plant-based processes have begun receiving greater attention. It has long been known that the life cycle of a plant has profound effects on the chemical, physical, and biological processes that occur in its immediate vicinity. In the process of shoot and root growth, water and mineral acquisition, senescence, and eventual decay, plants can profoundly alter the surrounding soil. The effects of many of these processes are apparent on the restoration of land at physically and chemically altered sites, ranging from road cuts to the site of the Mount St. Helen's eruption. These same plant-driven processes also occur in areas heavily impacted by industrial, mining, and urban activities. One of the greatest forces driving increased emphasis on research in this area is the potential economic benefit of an agronomy-based technology. Growing a crop on an acre of land can be accomplished at a cost ranging from 2 to 4 orders of magnitude less than the current engineering cost of excavation and reburial. There have been perhaps two dozen field tests to date; however, in many ways phytoremediation is still at its initial stages of research and development. A comforting thought for plant biologists is that much of the research effort will be expected to center on a deeper understanding of basic plant processes.

So how do we envision phytoremediation working? The theory appears to be simple. Agronomic techniques will be used to ready the contaminated soil for planting and to ameliorate chemical and physical limitations to plant growth. Plants will then directly or indirectly absorb, sequester, and/or degrade the contaminant. Plants and irrigation, fertilization, and cropping schemes will be managed to maximize this remedial effect. By growing plants over a number of years, the aim is to either remove the pollutant from the contaminated matrix or to alter the chemical and physical nature of the contaminant within the soil so that it no longer presents a risk to human health and the environment.

As people who work in the remediation, herbicide development, and farming industries will attest, many weed species are remarkably tolerant of a wide range of organic and inorganic toxins. Plants can thrive in soil contaminated to levels that are often orders of magnitude higher than current regulatory limits. These limits are often set relatively independent of plant tolerance limits and are most often derived from human health and aquatic toxicology end points. Ironically, many remediation plans begin with the destruction of the existing vegetation.

REMEDIATION OF ORGANIC CONTAMINANTS

The movement of an organic contaminant in soil depends on the chemical's relative water solubility, vapor pressure, molecular size, and charge and on the presence

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of other organics in the soil. The ability of soil to absorb and sequester organics is directly associated with the organic matter content of soil, the type and amount of clay present, soil structure, and the pH as well as with the age of the spill and water flux through the profile. It is apparent from even a cursory overview of these parameters that the use of plants to successfully decontaminate soils is going to be site, contaminant, and timing specific. For example, the strong connection between soil organic matter and bioavailability is accounted for in the calculation of soil-applied herbicides. The bioavailability of a pesticide is reduced in soils with high organic matter contents and as a consequence application rates are increased. Furthermore, the bioavailability of many other organic contaminants decreases with time; hence, old "well-weathered" contaminants may be expected to be more difficult targets for phytoremediation than those present as a result of more recent contamination events.

Plant roots absorb organics in nearly direct relationship to their relative lipophilicity. Once absorbed, these compounds can have multiple fates; however, many compounds are substantially bound into plant tissues in a form that is less biologically available and may be unavailable to normal chemical extraction. In sandy soils with little organic matter, schemes for root absorption and harvesting may prove useful. One patented process uses carrots to absorb dichloro-diphenyl-trichloroethane. The carrots are then harvested, solar dried, and incinerated to destroy the contaminant. In this process a lipophilic contaminant partitions out of the soil substrate and into the high-lipid content carrot roots (McMullin, 1993). Other root-harvesting techniques have been proposed. Another use of plants for direct extraction of an organic contaminant from soil is root accumulation, xylem translocation, and subsequent volatilization from leaf surfaces. Both of these scenarios have significant potential logistical and physical limitations that would be expected to reduce their applicability to only a relatively few situations.

Unlike inorganic pollutants, which are immutable at an elemental level, organic pollutants can be degraded or even mineralized by plants or their associated microorganisms. Plants have significant metabolic activities in both the root and the shoot (Hathway, 1989). Some of these metabolic enzymes may also be useful in remediation, even outside of the plant root/rhizosphere itself (Schnoor et al., 1995). These inherent abilities of plants can be further augmented by active microbial communities around their roots, in their root tissue, in the xylem stream, in shoot and leaf tissue, and on the surfaces of leaves. Progress in the area of degradation in the rhizosphere has been reviewed recently (Anderson and Coats, 1994). Accelerated degradation has been obtained for certain pesticides, trichloroethylene, and petroleum hydrocarbons, but the overall rate and quantity of degradation has been relatively slow.

Soil or rhizospheric microorganisms can play a major role in the decomposition of many organic contaminants. Recently, however, remediation research originally thought to center on microbial activities has provided additional appreciation of the degradative capacities of plants. The isolation of enzymes from sediment that had trinitrotoluene degradation potential led to the discovery that the enzyme was of plant and not bacterial origin. This led to further testing and eventually to a system design based on a nitroreductase in plant roots (Schnoor et al., 1995). In addition, these researchers reported that similar research pathways have led to a plant source of a dehalogenase and a laccase that can be used to degrade other contaminants.

REMEDIATION OF INORGANIC CONTAMINANTS

Unlike the case with organic compounds that can be mineralized, the remediation of contamination with an inorganic contaminant must either physically remove the contaminant from the system or convert it into a biologically inert form. Removal can be accomplished by removing the biomass or, with certain inorganic contaminants, by contaminant volatilization. In the case of Se, research on certain western U.S. soils has led to proposed vegetation management systems that encourage Se volatilization through what appears to be a plant and/or plant-microbe interaction (Banuelos et al., 1993; Zayed and Terry, 1994). More recently, a bacterial mercuric ion reductase has been engineered into *Arabidopsis thaliana*, and the resulting transformant is capable of tolerating and volatilizing mercuric ions (Rugh et al., 1996). The toxic cation is absorbed by the root and reduced to volatile Hg(O) by the introduced mercuric ion reductase. Biovolatilization, however, is generally not applicable for most inorganic ions, thus leaving biomass removal as the only alternative for the extraction of most of these contaminants.

Certain bacterial, fungal, algal, and plant systems are capable of concentrating some toxic inorganics. Extraction of inorganic contaminants from soil is theoretically possible by all of these organisms; however, no cost-effective way currently exists to remove many of these small organisms from the matrix after they sequester inorganic ions. Harvesting plants, on the other hand, is a familiar technology. Plants in the course of growth acquire perhaps two dozen elements in their shoots. For the most part, plants take up large amounts of elements required for growth and only small amounts of elements that may harm them. Some relatively benign nonessential elements (e.g. Si and Na) may appear in larger amounts, but generally, the levels of target pollutants are only 0.1 to 100 mg kg^{-1} dry weight in a plant (Jeffery, 1987). Finding or developing plants that acquire high levels of metal contaminants in harvestable tissue was thought impossible until the (re)discovery of a small group of remarkable plants called hyperaccumulators (Brooks et al., 1977). Although uncommon, these plants are taxonomically widespread in the plant kingdom (Baker and Brooks, 1989). Some of the hyperaccumulators and their metal accumulation capabilities are listed in Table I.

The processes involved in phytoextraction are shown in Figure 1. The contaminant must be in a biologically accessible form. Root absorption must be possible and must occur. Translocation of the contaminant from root to shoot makes tissue harvesting easier and lessens worker exposure to the contaminant. The actual rate of removal for a contaminant is dependent on the biomass gathered during harvesting, the number of harvests per year, and the metal concentration in the harvested portion of the plants. Decontaminating a site in a "reasonable number" of harvests requires plants that produce both a high yield of biomass and metal accumulation of 1 to 3% metal by dry weight. Thus, even for plants that accumulate relatively high concentrations of metals, low biomass production can limit their utility. For example, shoots of *Thlaspi rotundifolium* reportedly can contain up to 8200 mg Pb kg^{-1} dry weight (Reeves and Brooks, 1983), but these plants amass only 5 to 50 mg/plant of dry tissue after 5 months of growth. Although many of the properties desired for phytoremediation are present in currently recognized hyperaccumulators, their limitations include low biomass and restricted element selectivity in hyperaccumulation and the fact that there is knowledge about the agronomics, genetics, breeding potential, and disease spectrum of these plants.

After harvesting, a biomass-processing step is believed to be practical to recover most metal contaminants. Alternatively, the harvested biomass could be reduced in volume and/or weight by thermal, microbial, physical, or chemical means. This step would decrease handling, processing, and potential subsequent landfilling costs. With some metals (e.g. Ni, Zn, and Cu), the value of the reclaimed metal may provide an additional incentive for remediation.

AGRONOMIC ENHANCEMENT OF PHYTOREMEDIATION

The use of plants to remediate environmental contaminants can be aided by the proper use of soil amendments and agronomic practices. Soil amendments can be chosen to either increase or decrease the biological availability of the contaminant for plant uptake. Many plant roots absorb metal ions well if they are available in the soil solution, but long-distance translocation into the shoot is often limiting. In addition, soil amendments can greatly increase the tilth of the soil and benefit plant establishment and growth. For example, increasing bioavailability and plant uptake for many metals can be accomplished by lowering soil pH, adding chelating agents, using appropriate fertilizers, and altering soil ion composition. At the DuPont laboratory, the most successful amendments to date have been the addition of chelates such as EDTA and hydroxyethylethylene diaminetriacetic acid. These chelates can increase ambient soil solution levels of certain heavy metals (e.g. Pb) greater than 1000-fold and simultaneously alter root/shoot partitioning in a wide variety of crop plants. In laboratory pot trials with chelate flooding, shoot Pb concentrations have reached 1% Pb (on a dry weight basis) in plants such as corn and peas (J.W. Huang and S.D. Cunningham, unpublished results). The result of this action is often plant death; however, such plants remain heavily saturated with the metal and eminently harvestable as a metal removal strategy.

Table I. *Metal concentrations (on a dry weight basis) in known hyperaccumulators*

For reasons of logistics and potential "worker exposure," root tissue (in which concentrations can be significantly higher in some species) are not considered as "harvestable" here.

Metal	Plant Species	Concentrations in "Harvestable" Material from Plants Grown in Contaminated Soil (dry wt basis)
Cd	*Thlaspi caerulenscens*	1,800 mg kg^{-1} in shoots[a]
Cu	*Ipomoea alpina*	12,300 mg kg^{-1} in shoots[a]
Co	*Haumaniastrum robertii*	10,200 mg kg^{-1} in shoots[a]
Pb	*T. rotundifolium*	8,200 mg kg^{-1} in shoots[a]
Mn	*Macadamia neurophylla*	51,800 mg kg^{-1} in shoots[a]
Ni	*Psychotria douarrei*	47,500 mg kg^{-1} in shoots[a]
	Sebertia acuminata	25% by wt of dried sap[b]
Zn	*T. caerulenscens*	51,600 mg kg^{-1} in shoots[c]

[a] Baker and Walker (1990). [b] Jaffre et al. (1994). [c] Brown et al. (1994).

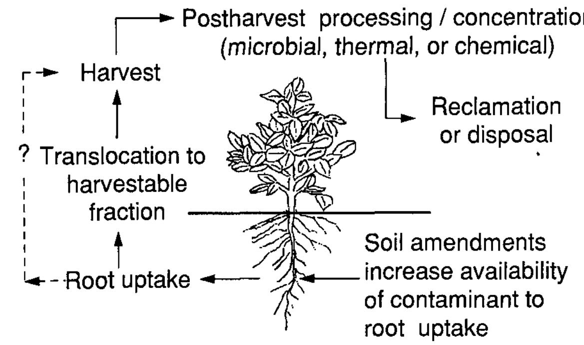

Figure 1. Processes involved in phytoextraction.

In stabilization schemes, soil amendments can be chosen that precipitate, bind, or absorb contaminants to eliminate off-site movement of the contaminant and decrease its bioavailability to plants and animals and even in mammalian gastrointestinal tracts (Berti and Cunningham, 1994). The choice of soil amendments to decrease this chemical and biological availability is made in relation to the chemical nature of the contaminant (including oxidation state, alkylation, precipitant, and adsorbing phase) as well as its interaction with the soil/sludge matrix.

Soil amendment choice borrows heavily from mine spoil, sludge, and traditional engineering reclamation efforts (Bradshaw and Chadwick, 1980). Contaminated dredged materials amended with lime, coarse limestone gravel, and horse manure and then planted with tolerant grasses show significant improvement over untreated soils. Untreated control plots were barren even after 6 years and produced an acid (pH 3.5) and metal-rich surface runoff that exceeded water quality standards (Brandon et al., 1991).

Some exciting possibilities for the development of new remediation schemes include hybrid technologies combining phytoremediation and traditional engineering techniques. For example, at the DuPont laboratories, tests have shown that combining electrokinetics (movement of soil ions under a direct current), in situ soil washing with chelates, and phytoextraction may be more effective than any single technique alone. Chelates bring more ions into solution, electrokinetics speeds the migration of relatively immobile ions, and properly selected plants provide enhanced surface area for absorption of the ions and their chelated forms. Other hybrid technologies such as phyto-vapor extraction and phytoland farming are being actively investigated for technical and economic feasibility. The use of plant roots as "biocurtains" or "biofilters" for the passive remediation of shallow groundwater is also an active area of research. These hybrid technologies will undoubtedly provide both research opportunities and potential field applications in the short term. In the longer term, use of these hybrid technologies still entails significantly greater costs than might possibly be achieved through advanced plant selection and creation techniques.

OPPORTUNITIES FOR ENHANCEMENT OF PHYTOREMEDIATION BY PLANT GENETIC MODIFICATION

The growing knowledge of the factors important to phytoremediation can provide a basis for genetic modification of plants for improved performance. Breeders have been modifying agronomically important plant traits for years. However, yield and aesthetics were often the criteria for selection. Phytoremediation requires a new paradigm in which plants are valued based on what they absorb, sequester, destroy, and tolerate. All of these traits can be specifically targeted by traditional breeding as well as molecular biology. Several aspects of plant root structure could be improved. These include root depth, penetration into anaerobic zones, and root density. Deeper roots would increase the depth from which a contaminant could be retrieved for phytoremediation. Improved penetration into anaerobic zones would allow phytoremediation to be used on sites contaminated with biodegradable organic contaminants. Increasing the quantity of plant degradative enzymes (e.g. peroxidases, laccases, oxygenases), both within the root tissue and excreted into the soil, should increase their utility. Increased root densities should make extraction more efficient. Perhaps the extensive root proliferation caused by *Agrobacterium rhizogenes*, normally considered an undesirable characteristic, may find appreciation in phytoremediation efforts.

With respect to the possible phytoextraction of inorganic ions, breeding programs could be envisioned whereby slow-growing, low-biomass hyperaccumulator plants are bred into fast-growing, high-biomass varieties. Alternatively, metal-hyperaccumulation traits might be introduced into fast-growing, high-biomass plants. Genetic analysis of mutants affected in metal biology could be a promising start to understanding mechanisms that govern metal accumulation. For example, chemical mutagenesis has produced a large variety of mutants. In pea (*Pisum sativum*) a recessive mutation that causes 10- to 100-fold higher accumulation of Fe was found associated with higher ferric-chelate reductase activity (Welch and LaRue, 1990; Grusak, 1994). This trait seems to extend beyond simply the Fe accumulation phenotype because shoot Pb accumulation is also increased by 50% over that of the parent genotype in recent hydroponic experiments (J.W. Huang and S.D. Cunningham, unpublished results). It is surprising that a recessive mutation resulting in an 8-fold greater concentration of Mn in *A. thaliana* also showed a positive correlation in ferric-chelate reductase activity (Delhaize et al., 1994; E. Delhaize, personal communication).

In *A. thaliana*, mutants that exhibit hypersensitivity to various combinations of Cd, Cu, Hg, or other heavy metals have been reported (Howden et al., 1995; Murphy and Taiz, 1995). Identifying the gene functions affected by these mutations may provide clues to increasing metal hyperaccumulation. In the fission yeast *Schizosaccharomyces pombe*, a model system for phytochelatin (metal-binding peptides found in plants and some fungi) research, investigators have cloned the gene for a vacuolar membrane transport pump that facilitates vacuolar sequestrating of the peptide-Cd complex (Ortiz et al., 1992, 1995). Hyperproduction of this protein in *S. pombe* enhances tolerance to and accumulation of Cd, suggesting that hyperexpression of this yeast protein, or a plant homolog, may yield similar results in a higher plant (Ow, 1993). There have also been numerous attempts to engineer the production of animal metallothioneins in plants (Misra and Gedamu, 1989; Yeargan et al., 1992; Elmayan and Tepfar, 1994; Hattori et al., 1994; Pan et al., 1994). Varying degrees of enhanced tolerance were reported in the resulting transgenic lines; however, metal uptake levels were not dramatically enhanced. It is not yet clear whether metal hyperaccumulation can be achieved through this approach.

As for organic contaminants, the prevalence of microbial mineralization of many organic compounds begs the question of whether plants can play an active role in the remediation of soil or merely provide a rhizospheric environment to promote microbial growth. One possible future direction for plant-assisted decontamination of organics may be in the use of genetically engineered plants that exude specific molecules that induce rhizospheric bacteria to degrade anthropogenic toxins. Plant-fungal interactions would also seem ripe for exploitation in this area, specifically mycorrhizal associations. Alternatively, transgenic plants can harbor microbial genes for biodegradation. This is already routine practice in the engineering of many herbicide-resistance plants and such field testing, product development, and registration are well advanced (Dale, 1995). The concept could easily be extended to address additional xenobiotics. The advantage of moving genes of microbial origin into a higher plant could lie in greater control over the biodegradative process and organism. Releases of biodegradative microbial strains are notoriously unreliable because of poor survival of the strain in the natural environment. In addition, such releases are prone to public opposition. Conversely, agriculture has great abilities to influence both the competitiveness and spread of an introduced plant. Permits for field testing of genetically altered plants now vastly outstrip permits for genetically altered microbes.

LIMITS OF PHYTOREMEDIATION

Phytoremediation has a number of inherent technical limitations. The contaminant must be within (or must be drawn toward) the root zones of plants that are actively growing. This implies water, depth, nutrient, atmospheric, physical, and chemical limitations. In addition, the site must be large enough to make farming techniques appropriate. It must not present an eminent danger to human health or further environmental harm. There may also be a considerable time differential between phytoremediation techniques and "dig and dump" techniques.

Research in this area is spurred by current engineering technologies that tend to be clumsy, costly, and disruptive. The research community, both basic and applied, working in multidisciplinary teams has a unique opportunity to produce a needed technology that is low cost, low impact, visually benign, and environmentally sound. Plant biologists could play a pivotal role in providing knowledge of basic plant processes on which genetic modification and breeding efforts depend.

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