Chapter 1

# Phytoremediation of Contaminated Water and Soil


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Phytoremediation is the use of green plant-based systems to remediate contaminated soils, sediments, and water. Relative to many traditional remediation engineering techniques, phytoremediation is a fledgling technology intended to address a wide variety of surficial contaminants. Phytoremediation targets currently include contaminating metals, metalloids, petroleum hydrocarbons, pesticides, explosives, chlorinated solvents, and industrial by-products. The primary market driver for continued research in this area is the significant cost reduction these systems appear to afford. Phytoremediation, however has inherent limitations in that plants are living organisms with specific oxygen, water, nutrient and pH limits that must be maintained. In addition, significant depth, concentration, and time frame limitations also apply. Despite these limitations, many forms of phytoremediation have emerged from the laboratories and are currently in practice. Commercial phytoremediation systems for clean up of shallow aquifers and water born contaminants are now in place. Field tests for the phytoextraction of metals from contaminated soils are underway as well as advanced stabilization trials. For the most part, the current practices are technically sound, but far from optimized. Field tests have generally been met by good regulatory and public acceptance, yet improvements and extensions can and will be made on many of them. The biological resource for phytoremediation remains largely untapped. Bringing multi-disciplinary teams consisting of biologists, chemists, engineers, as well as lawyers, accountants, and public advocates should continue to yield additional solutions and possibilities for continued application of phytoremediation.


Significant quantities of air, water, and soil have been contaminated as a by-product of the industrial revolution and increased urbanization of the landscape. Increasingly stringent standards for water and air quality have propelled whole industries to re-engineer their fundamental




In Phytoremediation of Soil and Water Contaminants; Kruger, E., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1997.

processes and products. Through these types of change, contaminant loading to water and air has generally decreased. In addition to decreased input, the higher rates of mixing and dissipation of contaminants in air and water allow the environmental footprint of contaminants to rapidly fade. This is not the case in soil, a more static media where contaminants with low mobility (e.g. metals and lipophilic compounds) may cast longer shadows. Due to slow kinetics and reduced or absent dissipation processes, contaminated soils may show little inherent improvement over many decades. Unfortunately, contaminated soil is often the primary problem at many of the larger sites in need of remediation. Off site migration of the contaminants at such sites, if left unattended, can continue to further affect groundwater, neighboring areas, and bodies of surface water. Addressing these sites is proving litigious, time-consuming, and expensive.

The art of remediation begins with a site assessment, followed by containment of the identified problem, and theoretically ends with the clean-up of impacted areas. The term "remediation" often has more of a legal connotation than a technical one. Remediation can imply: a) "clean-up" where either the contaminant is removed from the matrix (leaching, bioremediation, etc.) or the entire contaminated matrix is removed from the site (excavation and landfilling at a second site), or b) "stabilization" where the physical or chemical form of the matrix or contaminant is transformed to a more inert condition. The intended remedy must be logistically and technically possible, accomplished within the required time frame, economically feasible, and in compliance with all legal requirements. The public and most site owners often prefer techniques that actually clean-up a site, as it provides the greatest degree of confidence and flexibility for future land use. At many sites, however, "clean-up" is not always possible or practical and "stabilization" techniques are then employed.

Traditional methods of remediating contaminated soils, sediments, and groundwater are often based on civil and chemical engineering technologies that have developed over the last 20 years. These include a wide variety of physical, thermal, and chemical treatments, as well as manipulations to accelerate or reduce mass transport in the contaminated matrix. In certain cases, however, biological (especially microbial) processes have shown some applicability. Recent flexibility in the legal requirements associated with environmental clean-up has increased the acceptability of such "passive" approaches to remediation. In spite of this, a majority of the plans developed for site remediation do not rely on "natural attenuation". The reasons for this are clear. Engineering technologies are often faster, relatively insensitive to heterogeneity in the contaminant matrix, and can function over a wide range of oxygen, pH, pressure, temperature, and osmotic potentials. Biological processes are at a significant disadvantage in most of these areas. The perceived advantage of bioremediation is the often prohibitive cost of effective engineering approaches. If remediation based on traditional technologies were inexpensive, there would appear to be no driving force for the development of alternative strategies based on biological activity.

In our experience, the total costs of remediation (calculated on a $m^3$ basis) ranges from \$10 to \$100 for remediation methods that can be performed *in-situ* and \$30 to \$300 for *ex-situ* processes. Specialized techniques such as *in-situ* vitrification can easily surpass \$1,000/ $m^3$. In comparison, the agronomic community can move and process large volumes of soil at a relatively low cost. Techniques for "land-farming" the top meter of soil are orders of magnitude less (e.g. as low as \$0.05/ $m^3$ per year). It is this cost differential, as well as the reduced capital requirements and lengthened expenditure schedule, that is the source of

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excitement over agriculturally-based remediation. Agricultural practices, developed for the management of soil and crops, are well understood and tested. As agriculture has as its primary goal the maintenance of soil quality, many of its techniques may have applicability for soil remediation. Most agricultural soil management strategies rely on the physical protection offered by a vegetative cover and on the biological activity of the plants and their associated microbial communities. These same components (stabilization and biological activity) are the basis of plant-based approaches to clean-up of inorganic and organic environmental contaminants.

## Low-Input, Agronomic, Stabilization Techniques

The use of agronomic techniques to stabilize contaminated sites in-situ *(1)* is continuing to be advanced. Under this form of remediation, also called "phytostabilization", soil amendments are applied to contaminated soil to reduce the bioavailability of the contaminants. The site is then planted into vegetation, which reduces off site migration of the stabilized soil matrix and contaminant through water usage and erosion control. Plants are chosen to maximize root uptake of any small amounts of contaminant that would escape from the stabilizing mix and to sequester the material in the root tissue with little translocation to the shoot and potentially help catalyze the formation of insoluble contaminant species such as Pb-pyromorphite.

There are many examples that suggest this strategy is sound. It is known, for example, that Pb in soil and dust from natural sources is often less biologically available than similar concentrations of Pb from anthropomorphic sources. This is likely because many of the natural forms of Pb have low bioavailabilities. These natural forms do not dissolve well in either soil solution or mammalian gastro-intestinal tracts. Researchers involved with advanced stabilization techniques postulate that if anthropomorphic forms of Pb could be converted to more stable forms, it may be safe to leave these materials in place. Conversion of Pb to less toxic forms has been shown to take place in a natural environment with the application of low-cost amendments *(2-7)*. More importantly, these techniques are less likely to harm the soil's potential for sustaining plant growth than most engineering solutions. Similar processes for sequestration of organic contaminants are also beginning to be examined.

Work is ongoing to further develop and validate *in-situ* stabilization as a viable technology for eliminating the hazard of contaminated soils. The five primary goals of this effort are to: 1) understand the mechanisms by which these technologies work, 2) develop appropriate testing protocols and methodologies that illustrate their utility, 3) improve predictive capabilities, 4) facilitate validation of the effectiveness and persistence of the technique, and 5) prepare guidelines for its implementation.

## Phytoremediation

Phytoremediation is a word formed from the Greek prefix "phyto" meaning plant, and the Latin suffix "remedium" meaning to cure or restore. Although the term is a relatively recent invention, the practice is not. The use of plants to improve water quality in municipal and more recently industrial water treatment systems, is well documented *(8-9)*. Vegetation has long been used for the restoration of disturbed areas *(1)*, and tolerant vegetation is often found on or planted into contaminated soils *(10)*. There has also been the opportunity to study the plant-contaminant interactions that have resulted from the application of sewage sludge to land *(11)* and from our 50 years of pesticide use *(12)*. Given our strong agriculturally-based experience with planted soils and the more recent issues of environmental contamination, it is natural to explore the use of plants to remediate contaminated soils, aquifers, and wetlands.

The legal and technical process of soil remediation is complex and continually in flux. The guidelines are clearer when the contaminant is in water. In this media, there exists excellent toxicological data that allows a legal determination of when remediation and pollution prevention techniques are necessary. In soils, however, equivalent concentrations of contaminants often exhibit widely varying toxicity effects. Contaminant speciation, soil pH, other ions in solution, types of clays and oxide surfaces, presence of organic matter, vegetation and rainfall all affect the availability of the contaminant and its potential to cause harm. As a general rule, remediation appears to be necessary when anthropological activities result in an "unacceptable risk". As risk assessment is an imprecise science, and as the tolerance for risk and availability of economic resources vary widely at a community level, it is difficult to determine the exact extent of soils that need remediation. Despite this imprecision, the need for remediation at some sites is apparent even with a cursory overview. Sites that are devoid of vegetation, in proximity to residences, and those sites likely to represent a source of continued off site migration of contaminants are likely candidates for some type of remediation. Other cases are less apparent. A technical basis for decision making is of critical importance to the research and development community involved with phytoremediation.

The market for any remediation technology is entirely dependent on compliance with the law. Plants grown for phytoremediation purposes are not like an agronomic crop where 50 bu is half as good as 100 bu. Phytoremediation systems that do not result in site "closure" (reduction to a legally acceptable level) are failures. In addition, a phytoremediation technique that can reduce contaminant levels twice as low as the legally acceptable limit may have no increased value over a technology that simply meets that level. The marketplace for remediation does not necessarily have value for a "cleaner" clean. Phytoremediation, like all remediation technologies under development is sensitive to the ongoing evolution of regulations. If the regulations for allowable contaminant concentrations in soil or water are set high, the market for a technology is affected in two ways. First, the volume of contaminated soil and water to be remediated decreases dramatically. (There is much more low level contamination than high level contamination). Second, the technology must work at a still higher level and reduce it down to the new "acceptable" level. (Technologies that reduce Pb contamination in soil from 1,000 to 500 ppm have significantly different chemical and physical constraints than those that mitigate 5,000 ppm to a 3,000 ppm guideline).

## Phytoremediation of Inorganic Contamination

The elemental composition of normal soils is dependent on the geological and physical processes that occurred during its formation. Soils derived from marine sediments vary from those derived from rock outcroppings abundant in heavy metals. In addition to this inherent variability, anthropomorphic activities have increased soil heterogeneity. The most commonly cited sources of anthropogenic inorganic contamination are the mining and smelting of metalliferous ore, fossil fuel handling and use, industrial manufacturing, and the application of fertilizers and municipal sludges to land.

Decontamination of heavy metals in soils and sediments is among the most technically awkward clean-ups. Many inorganic contaminants bind tightly to the soil matrix. At older contaminated sites this is nearly always true, since more mobile contaminants have already migrated off site in areas of even moderate rainfall. Few remediation options exists for the contaminants that remain. Some advanced treatments include soil washing or electroosmosis, but for the most part many site clean-ups involve excavation and removal to a secure landfill area. Alternatively, some of these areas have also been simply covered in place in a process known as "capping". For still larger areas, particularly in the mining and smelting regions, site stabilization with some soil modification is done and revegetation occurs where possible.

**Phytoextraction.** Phytoextraction involves the use of plants to extract contaminants from the environment. The term was originally applied almost exclusively to heavy metals in soils but has since come to apply to many other materials in other media as well. The phytoextraction R & D community involved with inorganics has gradually evolved into two groups. The first group uses phytoextraction for remediation purposes (primarily targeting Pb and radionuclides with some efforts on Cr, As, and Hg). The second group targets inorganics with intrinsic economic value (primarily Ni, some Cu and a few with precious metals). This latter technical area (also known as "bio" or "phyto"- mining) is still in its infancy, but significant progress is being made with Ni *(13)*.

*Water.* The initial research on phytoextraction of inorganic contaminants in the environmental field began with the use of wetlands (constructed, natural, and reed beds) for water purification *(8)*. In these systems the inorganic contaminants often precipitated out of the water into the sediments making them difficult to recover. Floating plant systems followed *(14)* in which the contaminants could be removed in the harvested biomass. Unfortunately, these systems are not particularly efficient or economical, especially in temperate zones. More recently, greenhouse based hydroponic designs using terrestrial plants *(15)* have been field tested for heavy metals and radionuclides. These latter systems have been developed with plants selected for high contaminant root uptake/affinity and poor translocation to the shoots. These terrestrial plant (and more recently seedling) based applications were developed to take the place of synthetic resin chelates for water purification. Again the contaminants are removed from the system by harvesting root biomass. Rhizofiltration fits well with many of the biological limitations inherent in phytoextraction (e.g. poor translocation of many contaminants from root to shoot). These systems appear technically promising and require less R & D innovation than the soil remediation systems. Unfortunately, there currently exists much competition from other waste water purification technologies. Many excellent, non-plant based technologies exist to reduce water borne contaminant to meet regulatory guideline levels (e.g., alkali precipitation and cation exchange). For sites that are currently in compliance with local regulations the impetus for adopting new technology may be low. This is particularly true when the site has capital investments in water purification equipment already in place. Barring a change in water quality standards, an additional capital outlay for another technique (even if it were proven technically superior at a lower unit cost) is unlikely. New sites, additional polishing steps or large scale uses would appear to be the best target for "rhizofiltration"

*Soils.* In the soil environment, phytoextraction has fewer competing technologies for the clean-up of inorganic contaminants. The few that exist have significant technical and economic disadvantages. Stabilization technologies, however, may at times be in direct competition with this technology.

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ACS Symposium Series; American Chemical Society: Washington, DC, 1997.

At the beginning of this decade, most researchers believed the success of phytoextraction of inorganic contaminants from soils centered on four requirements: 1) the bioavailability of the contaminant in the environmental matrix, 2) root uptake, 3) translocation internal to the plant, and 4) plant tolerance *(16)*. This set of minimum requirements has since changed, as will be addressed later in this review, although the original set of requirement remains a long-term goal of many active research programs.

The fundamental paradigm of phytoextraction is that most inorganic contaminants in soils are difficult to extract with engineering technologies such as thermal, chemical, and physical techniques. Soil is a complex matrix consisting primarily of Si, Al, O and Fe based materials that are difficult to separate from contaminating inorganics. The premise behind phytoextraction is that plants can be used to extract inorganic contaminants from the soil matrix and transfer them into a primarily carbon-based matrix, the plant material. Engineering techniques that were ineffective on the soil matrix are more readily employed on the plant material. Chemical, thermal, microbial and leaching processes separate many of these inorganics from the plant matrix. The most cited example is the smelter analogy where an energy source and an ore are used to produce a product. In soil, the contaminating materials are often at too low a level and the energy requirements in smelting soil are too large to be effective. When plants are used to transfer and concentrate soil-borne contaminants, however, the process becomes feasible. Plant material can have both significant stored energy potential and metal concentrations and can act simultaneously as both energy source and ore for a smelting process.

In certain cases, inorganic contaminants have volatile forms and remediation strategies that end with the volatilization of the contaminant have been proposed and field tested. Selenium phytoremediation as phytoextraction *(17)* and "phytovolatilization" *(18)* have both been explored. More recently, transgenic plants have been shown to reduce Hg from the more hazardous ionic and methylated forms to Hg(0), which is then volatilized *(19)*. In most remediation strategies, however, phytoextraction involves biomass removal and processing.

## Exploiting Botanical Variation

All plants accumulate a wide variety of mineral elements but, for the most part, plants are quite adept at excluding those elements that are non-essential for their growth and survival. This general rule has notable exceptions. The first exception is that certain classes of plants take up large amounts of some non-essential, and relatively non-toxic elements. The clearest example of this is the element Si, which is taken up by many plants (particularly grasses) to levels that may exceed 1% of their dry weight. From this researchers have learned that if an element does not interfere with normal cellular metabolic processes and is appropriately sequestered, plants can tolerate relatively large loadings of inorganics. The second exception is that to a small degree all plants reflect the environment in which they grow. Many geologists have recognized this fact and have used plant-tissue analysis to locate buried ore bodies. The use of plants in this manner is referred "geobotanical prospecting" and is used to locate ore bodies containing elements such as uranium. The third exception to the text book rule is perhaps the most remarkable. There exists a small group of plants known as hyperaccumulators that can take up, translocate, and tolerate levels of certain heavy metals that would be toxic to any other known organism *(20)*.

**Hyperaccumulators.** Despite widely varying soil concentrations of most elements, with rare exceptions almost all plants exist within a narrow spectrum of relative concentration of elements *(21)*. Hyperaccumulating plants, on the other hand can take up, translocate and tolerate shoot concentrations of heavy metals in excess of 0.1% Ni, Co, Cu, Cr, Pb or 1% Zn on a dry weight basis *(22)*. These plants, which often evolved on metalliferous outcroppings, are remarkable not only for their high levels of accumulation and tolerance, but also for their nearly insatiable desire to concentrate these elements from even "normal" soil. Although taxonomically widespread, this hyperaccumulating trait is relatively rare, indicating a rather late appearance in the evolution of modern species *(22)*. Although the study of these plants still represents a focus of many labs, practical phytoremediation with these plants has remained elusive. Many of these species seem to hyperaccumulate only one metal while most sites have mixed metal contaminants. In addition, like any "weed" species little is known about their management. Many of these species are slow growing and, although they have high metal concentrations, produce low biomass. This is particularly problematic as the measure of phytoextraction success is often based on the amount of contaminant removed/hectare -y. High metal concentrations alone are insufficient. Slow-growing, low-biomass hyperaccumulating plants, no matter how fascinating biologically, are insufficient to form the backbone of a technology useful for remediation or biomining.

Hyperaccumulators, however, remain a significant motivational factor to the agronomic and molecular biology communities. These plants prove that biological systems can be developed with plants maintaining up to 4% metal in their tissues without significant yield decreases. Additional effort will be required to engineer, breed or adapt plants to obtain this goal, but some efforts are now under way. Progress is being made on a number of fronts and the area has recently been reviewed *(23)*.

**Induced Hyperaccumulation.** Not long after hyperaccumulators showed the inherent botanical potential for phytoextraction *(24)*, many additional laboratories began active research programs with these plants. It soon became apparent that the existing populations of hyperaccumulators were not ideal for commercial level phytoextraction. In 1991, screening projects began in a number of labs to identify higher biomass crop or weed species which might also accomplish the same feat. Various species were proposed by different groups ranging from Indian mustard *(25)*, through ragweed *(16)*. All of these plants showed improvement in biomass yet reduced metal uptake compared to hyperaccumulators. These experiments have continued with cultivar / ecotype screening in both Brassica juncea and Arabidopsis as they afford two ways to directly obtain or to engineer future high biomass hyperaccumulators. It is well known that there is much heterogeneity in many germplasm sources and screening these for metal uptake, translocation, and tolerance has proven no exception *(26)*.

Paralleling these efforts, however, the fundamental limiting factors of phytoextraction are being explored. Much of this work has been done with Pb, the most common heavy metal contaminant in the environment. In the case of this element, three limitations were uncovered. The first was that Pb soil solution levels were low even when total Pb concentrations were high. The second was that although most plant roots took up the Pb well, translocation from roots to shoots was poor. Lastly, tolerance to Pb in plant tissue at high rates severely reduced biomass *(27)*. In retrospect, this troubling lack of translocation from root to shoot is not surprising given what is known about the chemistry of Pb under cellular conditions of near neutral pH and in the presence of cytoplasmic concentrations of phosphate, proteins and carbonate anions.

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The lack of availability of the contaminant and poor translocation and tolerance in plants were disappointing to many and led to the testing of potential techniques to circumvent these limitations.

Much is known about the soil chemical processes that influence the solubility and availability of many inorganic contaminants. Many researchers working in the phytoextraction of inorganics sought to alter such factors as soil pH, organic matter, phosphate level, etc. in an attempt to increase metal concentrations in plant tissue. To some degree this worked, and a doubling of Pb concentration in tissue was reported. This 200 to 1000 ppm level, however, was far short of the hyperaccumulator levels reported in some plants as well as the phytoextraction goal of 1%, calculated from engineering and economic parameters. Other remediation hybrid technologies were used, including electrokinetics, with only marginal increases in plant tissue concentrations of heavy metals. It was only when phytoextraction was combined with techniques learned from soil washing experiments that tissue concentrations began to approach target levels.

Chelating solutions have been used in soil washing experiments for some time. They increase the solution concentration of many heavy metals in soils and have also been used in agronomic and horticultural environments to deliver micronutrients to plants (most notably Fe-EDTA). Initial trials with chelates in many labs were too tentative. Many researchers were afraid of injuring the plants with high levels of chelates or chelate-Pb complexes. It was only when a "non-botano-centric" rethinking of the problem occurred that high enough doses of chelates were used to obtain the desired Pb level in plants *(27)*. In this case, chelates are applied to the soil and, as expected, Pb soil solution levels are increased. A remarkable, and serendipitous, benefit occurs, however, when the chelate Pb complex enters the plant and prevents Pb from precipitating in the root. The Pb-chelate complex continues up into the plant shoot through the xylem *(28-29)*. In this scenario, plants are grown in soil, chelates and other amendments are added, and Pb complexes are taken into the plant under the plant transpiration gradient. In this case plant tolerance is more or less irrelevant as the plant lives most of its life without much tissue Pb. The amendments are applied to the soil and within one to two weeks the plants are harvested. The plant sustains significant damage; however, the Pb-laden plant can be readily harvested regardless of its physiological state. Chelates thus act to eliminate limitations in Pb solubility in the soil, root to shoot translocation and tolerance.

The use of chelates is not, however, without a significant risk management and cost penalty. The technology is currently in its first field testing season with commercial customers slated for 1997. In conjunction with regulators and with local and state oversight, these techniques are being field tested by Phytotech, Inc. a New Jersey based firm. Chelates, albeit even ones used in foods like EDTA, raise safety concerns. Chelates increase the mobility of Pb in the soil. Downward movement must be monitored. Pb and EDTA have approximately the same molecular weights, so that if a 1-1 ratio exists, removing a ton of Pb may require a ton of chelate. Mechanisms to reduce this requirement are being explored along with chelates with significantly different properties. Given the value inherent in clean (vs. contaminated) soil, ton quantities of chelates and chelated-assisted phytoextraction may be a viable remediation technology, but most view this as only a first phase in its development. Chelate assisted phytoextraction may not be applicable for all areas, but with appropriate irrigation management techniques and site management, it may eventually become a viable remediation alternative. It is not, however, the best or probably even the ultimate answer for Pb phytoextraction.

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**Future of Phytoextraction**

To date, no inorganic contaminated site has been remediated using phytoextraction. To these authors' knowledge perhaps a dozen field tests of phytoextraction as a remediation technique have been conducted with an additional four geared toward phyto-mining. The largest economic opportunity identified to date is in Pb phytoextraction. Unfortunately, the chemical and biological constraints are among the most difficult for this contaminant. Zinc or Ni would have been preferable targets from a biological and chemical perspective. It is clear that at least for Pb, where most work has been done at a field/practical level, some mechanism of increasing Pb solubility around the root zone will be required. Plant or plant derived chelating complexes and pH shifts have been proposed but these hypotheses remain untested. Internal chelating mechanisms will also be needed to prevent Pb sequestration in the root tissue and to allow it to move from root to shoot tissue. Lastly, the ultimate Pb-phytoextraction technique, unlike the current chelate-assisted one, will probably also require Pb tolerant plants.

This review represents only a brief snapshot in time of a field arguably only a decade old. Significant breakthroughs in many laboratories are continuing with reports, papers, and patent activity dramatically increasing for the phytoextraction of a wide range of elements and contaminated environments. To date, relatively few plants, people, and years have been spent on the development of phytoextraction as either a mining or remediation technique. Given that no plant in the history of the world was ever selected for maximum metal yield, the biological potential remains largely unplumbed. Futurists remark on a time when we may need to recycle most of our inorganics from our waste streams. Biomining and plant phytoextraction may provide one mechanism to do so.

**Phytoremediation of Organic Contaminants**

Unlike inorganic pollutants which are immutable at an elemental level, most organic pollutants can be altered by biological systems. This increases the inherent economic advantage of such techniques by potentially eliminating the needs for harvesting plant material.

**Direct Plant Effects- Uptake of Organics.** Plant uptake of organic compounds is an important component which must be considered in the evaluation of phytoremediation. The majority of the data on plant uptake of nonnutritive substances comes from studies of agricultural chemicals, primarily herbicides. The effectiveness of many herbicides depends on their ability to enter the target plant. Since these compounds were commercially designed with this goal in mind, the principles regarding their uptake provide a strong basis for understanding that of other chemicals *(30-31)*.

Root uptake of organic compounds from soil is affected by three factors: (1) physicochemical properties of the compound, (2) environmental conditions, and (3) plant characteristics *(32-33)*. Plant characteristics such as root surface area can substantially alter absorption. Surface area may be increased in plants with large root morphologies, or in those with a high number of fine root hairs. As water mediates the transfer of solutes to the root, a plant characteristic which affects evapotranspiration could also influence the potential for uptake of organic contaminants. A systematic approach to selecting plant species and varieties for maximizing traits such as these has rarely been attempted, but would be warranted in the development of phytoremediation technology. The bioavailability of organic contaminants for plant uptake is primarily under the control of environmental soil factors such as organic matter content, pH, and moisture. Even in this aspect, however, species and families of plants with traits that allow them to modify the environment (such as pH) surrounding their roots, could be investigated.

Assuming constant plant and environmental characteristics, root uptake has been shown to be directly proportional to the n-octanol/water partition coefficient (Kow) for the chemical. More lipophilic compounds can better partition into roots and this structure-activity relationship has been used to develop empirical models of the uptake of different classes of organic compounds (primarily pesticides) by plants. Unlike root absorption, translocation to aboveground plant tissues via the transpiration stream (measured as the transpiration concentration factor or TSCF) is most efficient for compounds with intermediate polarity (e.g. log Kow = 1.8) *(34)*. Briggs and co-workers found that this relationship (maximum TSCF = log Kow of 1.8) held true for literature data despite a variety of plant species, compounds, and experimental techniques. Plant uptake of organic contaminants from soil has been primarily investigated using hybrid poplar trees by a number of groups *(35-36)*. These authors now report extending the model proposed by Briggs et al. *(34)* for organics ranging from pesticides to volatile organic compounds (VOCs) *(37)*. Results of these studies indicate that VOCs with log Kow from 1.0 to 3.0 are taken up and translocated by rooted poplar cuttings in hydroponic solutions. It has been noted, however, that the modeling parameters developed for plants in hydroponic systems can be compromised in soil where fairly strong sorption to soil occurs. In soil system, it seems that the log Kow for TSCF maximum may be shifted down by two units *(38)*.

Many of the field tests currently underway with the phytoremediation of organics involve plant uptake of the contaminant from a water phase. These water borne contaminants include surface applied water, wetland areas, ponds and impoundments as well as shallow aquifers *(35)*.

**Direct Plant Effects- Fate of Contaminants in the Plant.** Much is known about the general fate of xenobiotics in plants. Unfortunately most of the literature base has evolved around increased understanding of the fate of pesticides with specific information on many of the priority pollutants lacking. In general, however, plants have a wide array of metabolic capacities which can effect the fate of a chemical once it enters the plant. General plant metabolism of many xenobiotics often appears remarkably similar to metabolic detoxification processes that occur in mammalian livers *(39)*. In addition to herbicides, much metabolic work in plants has also been done with PCB's and more recently TCE. In many cases much of the contaminant entering plants get incorporated into cell biomass that is chemically difficult to extract and characterize. The rate and extent of these metabolic processes, relative to total contaminant uptake rate by the plant remain poorly characterized. It now appears, that transpiration rate, xylem mobility of the contaminant, and volatility may play a large fate in determining contaminant fate/partitioning. Evidence appears to be gathering that our understanding of the fate of contaminants may be greatly influenced by how these experiments are carried out. Sealed jars for mass balance studies may accentuate metabolism, studies under high transpiration streams found in a laboratory hood may accelerate volatilization of the contaminant through the plant transpiration stream. It is for this reason that carefully controlled field studies are needed.

Preliminary results from one such study are reported in this volume *(36)*. In this report, laboratory and field tests on phytoremediation of TCE-contaminated groundwater

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are reported. Initial studies in the laboratory indicated that a hybrid poplar clone, H11-11, was able to absorb trichloroethylene (TCE) from groundwater and that cell cultures of H11-11 could metabolize $^{14}$C-TCE as well as incorporate $^{14}$C into the cells.

There is also increasing evidence that plants can directly affect xenobiotic concentrations outside the plant itself. Root enzymes, such as those involved with oxidative coupling. have long been suspected of influencing concentrations of xenobiotics both internal and external to the plant upon release during normal or accelerated plant turn over *(40)*. This process has been extended into sediment remediation by plant produced enzymes as well *(35)*. The nature and extent of this process is under active investigation by a number of groups.

**Indirect Plant Effects: Plant-Microbe Interactions.** The previous sections described ways in which the plant may act directly on organic contaminants through plant based uptake and/or transformation. Although the plant may often metabolize or sequester environmental toxins, plants are at a significant disadvantage in two ways. Plants are primarily autotrophic and derive their living primarily by construction of cell materials from $CO_2$, light, water, and minerals. As such, unlike microbial systems, they have not had to evolve with the necessity to degrade chemically intransigent materials. The result of this is that plants metabolize a more restrictive set of chemical structures than do their microbial counterparts. Secondly, plants often detoxify xenobiotics by chemically altering them (e.g., by hydroxylation and glycosilation) to more water soluble forms in the plant tissue. In many cases the parent material, or chemically altered form, is then sequestered in the cell wall matrix or cytoplasm. In contrast, microbial metabolism often ends with the compound being reduced to CO2, water, and cellular biomass. In many ways, combining the plant structural functions (water transpiration, root surfaces and soil penetration) with microbial degradative processes is technically, economically and regulatorially attractive.

In soils contaminated with lipophilic organic compounds, the plant may play a less direct role - perhaps only that of a support system for degradative microorganisms and microbial communities *(41)*. Since the late 1970s, numerous studies have shown that plants (or planting) may enhance the degradation of selected compounds, including organo-phosphates *(42)*, parathion *(43)* polyaromatic hydrocarbons *(44)*, and chlorinated organics *(45-46)*. To date, much of the research has been descriptive. Observed increases in degradation have been generally attributed to microbial activity in the rhizosphere, however, a mechanistic understanding of the process, is lacking. Successful application of plant-microbial systems for bioremediation of a wide range of contaminants will require that we understand how microorganisms that bring about chemical transformations are influenced by plant roots. The plant-contaminant-soil interactions are already exceedingly complex. Adding to this a microbial community component which varies in time and space along the length of a root and in the bulk soil adds another technical dimension (and required skill base) and dramatically increases complexity of the system.

Plants might influence microorganisms that degrade organic contaminants by providing substrates for microbial growth or cometabolism, by allowing the assemblage of unique communities (analogous to biofilms) on root surfaces, and by alteration of soil chemical and physical conditions such as redox, pH and inorganic nutrient availability. These plant-microbial interactions are not necessarily distinct from the more direct activities of the plants themselves. For example, contaminants which are taken up by the roots may be transformed by the plant and subsequently redeposited into the rhizosphere in an altered chemical form.

One of the first steps in dissecting how plants might accelerate biodegradation of organic soil contaminants is to consider the factors that influence the survival and activity of bacteria along a root. During growth, plants release significant quantities of carbon into the rhizosphere as amino acids, organic acids, sugars, and numerous other structurally diverse materials, which can comprise from 15 to 40% of the carbon assimilated through photosynthesis. Most of these materials are released in the zone of elongation, just behind the root tip, and support a diverse microbial community. In older root zones, carbon becomes limiting and the rhizosphere community includes nematode and protozoa predators that graze on bacteria associated with the roots. This bacterial turnover leads to selection for microorganisms that are adapted to coexistence in a crowded, oligotrophic environment. Subsets of the oligotrophic community undergo further succession as new carbon substrates become available when soil animals feed on the roots, or at sites where lateral roots emerge and rupture through the cortex tissue. Mycorrhizae formation with symbiotic root-colonizing fungi in the older root zones further changes the quantity and composition of root exudates to the rhizosphere microbiota. Eventually, as plant roots die and decompose, all of the compounds produced by plants and their associated microbiota, are released into the soil where they are degraded by yet another microbial community that includes specialized degrader organisms capable of growth on cellulose, chitin, and lignin.

The above concerns ways in which plant roots and their depositions determine the basic structure and makeup of the rhizosphere microbial community. The plant may also foster the development of degradative activity in the community by bringing together dense populations of diverse microorganisms that contain catabolic plasmids or genetic material. Following recombination, this proximity may lead to new pathways for degradation. Since bacterial mating and genetic recombination is dependent on the active growth of microorganisms, plants may accelerate the evolution of new catabolic pathways for degradation of xenobiotics by providing a mating surface. In addition to plasmids carrying catabolic genes, other plasmids may confer the genes for chemoattractant sensors, or antibiotic or heavy metal resistance that are beneficial to growth and survival of introduced degrader organisms. Much of the latter remains speculative, but it is well known that genes for catabolism of a variety of substrates are plasmid borne, and recent work has shown that plasmid stability for degradation of a normally recalcitrant substrate, 2,5 dichlorobenzoate, is increased in the rhizosphere *(47)*. In this manner, bioaugmentation of indigenous microbial communities using bacterial vectors that contain specific plasmids may be more easily accomplished in planted soils.

Although the effect of plants on the physicochemical environment is a large and separate topic, one particular note should be made in regard to biodegradation of highly chlorinated compounds. Plant roots and their associated microflora alter soil redox through respiration. This could increase rates of reductive dehalogenation as electrons are generated during metabolism of root exudates. Plants can deplete soil oxygen while also removing the nitrate which may serve as an alternate microbial electron acceptor in lieu of oxygen. Depending on water management of the soil, or the presence of plants with aerenchyma to transport oxygen into the rhizosphere, it may be possible to generate conditions favorable to both aerobic and anaerobic microorganisms that could transform chlorinated compounds through sequential anaerobic and aerobic metabolism. The microbial consortium would be analogous to a biofilm through which the contaminant is delivered during bulk flow of water. This idea is supported by reports of dechlorinating microorganisms being found in the rhizosphere. Many primary root colonizers (Pseudomonas, Arthrobacter, Achromobacter) are known to degrade various chlorinated hydrocarbons (see review- *48*). In addition to bacteria, a rhizosphere fungal species, Aspergillus niger, degrades PCBs (49) and 2,4-D, as well as carboxin fungicides *(50)*. Still other rhizosphere fungi have been investigated for their ability to degrade chloroaniline-based pesticides *(51)*.

Given the dynamic nature of the rhizosphere, it is an enormous challenge to figure out the influence of the plant on the microbial community. Methods for examining microbial activity in the rhizosphere may involve the use of reporter genes in which the promoter for selected degradative genes is coupled to a reporter system such as bioluminescence *(47)*, or may employ PCR to amplify genes lifted directly from soil or root sites *(52)*. Other more general approaches have involved the plating of microorganisms associated with plant roots to determine their degradative abilities, or by simply sampling rhizosphere soil which contains microorganisms that have been previously enriched in soil associated with plant roots. Regardless, the growing number of techniques available are now allowing the exploration of the rhizosphere and providing the information needed to assess the potential for phytoremediation of organic contamination. This research area is receiving much current attention *(53)*.

**Future**

This overview has only touched on our current understanding and use of plants for stabilization or clean-up of inorganic and organic contamination. Increasingly phytoremediation literature and reviews are appearing in many venues *(54)*. All indications to date suggest that multiple challenges and opportunities remain in the development and application of this as a viable technology. Perhaps the greatest hurdle is the elucidation of the mechanisms involved in all forms of phytoextraction and phytoremediation. Without a better understanding of many of these processes, it is difficult to exploit the selection and engineering of plant and microbial populations for process optimization. The research presented in the remainder of this volume is aimed at closing the gap between our current position and where we need to be; often by identifying key areas which require further investigation to advance this new technology.

While the above suggests that this is still largely in the research and development phase, many scientific, economic, and societal factors support its development. The pace of research in this area is quickening, as evidenced by this volume. In a field that was of little interest 10 years ago, research is now on-going in dozens of labs. Because of the potential for significant economic return on these endeavors, groups conducting and funding research extend beyond the traditional academic and environmental sectors. Small entrepreneurial companies and spin-off technologies have recently emerged with names like: Phytotech, Phytokinetics, PhytoWorks, Ecolotree and Treemediation.

Even as scientific progress is made, the societal view of environmental contamination and remediation is changing. The number of sites and volume of materials being listed in the U.S. alone under CERCLA (ie. superfund) and RCRA is staggering and unmanageable given the technologies and resources now available. While as a society we identify cleanup of environmental contamination as a national priority, there is a growing awareness of the economic realities associated with addressing this problem. This awareness is reflected in changes in legal standards and methods and measurements used to assess and prioritize remediation. In the past, cleanup was based on the total loading of a contaminant - regardless of its biological and chemical availability or the intended future use of the site. At all levels the regulatory community is now leaning towards a more risk-based decision making process, where corrective action at a given site would be based on qualitative and quantitative assessment of the hazardous compounds available for interaction with living systems as well as future land use. This "bioavailable" pool may be only a small percentage of the total amount of the compounds present. One of the pressing needs in environmental risk assessment, therefore, is the establishment of methods and approaches to evaluate the status of contaminants at a site. With these methods, environmentally acceptable (treatment) endpoints could be established as targets for traditional and alternate remediation technologies. With treatment guidelines based on biologically available quantities of contaminants, and a treatment technology which, almost by definition, remediates the biologically available fraction phytoremediation would have increased and renewed interest. These changes in technology, societal views on remediation, as well as increasing flexibility in the legal system would appear to bode well for the development of phytoremediation as a viable remediation technology of the future.

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In Phytoremediation of Soil and Water Contaminants; Kruger, E., et al.;
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