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Summary Report of a Workshop on
PHYTOREMEDIATION RESEARCH NEEDS

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EXECUTIVE SUMMARY

Phytoremediation encompasses the use of plants for the remediation of environments contaminated with hazardous wastes. Plants can be used in site remediation both through the mineralization of toxic organic compounds as well as through the bioaccumulation and concentration of heavy metals and other inorganic compounds. This report summarizes discussions from a workshop that brought together scientists and engineers involved in both basic and applied research in areas relevant to phytoremediation, including plant sciences, microbiology, and bioremediation. The workshop participants assessed the current status of phytoremediation, and focused on research needs and opportunities for using plants to clean-up soils polluted with toxic organic and inorganic chemicals.

At many industrial and mining locations throughout the world, including many DOE sites, surface soils are contaminated with radionuclides, heavy metals, and organic pollutants, as well as mixtures of these contaminants. Even though contamination levels are often relatively low, though still well above acceptable regulatory limits, such sites present a major clean-up challenge. Conventional technologies, developed for small, heavily contaminated sites are often not cost effective or sufficiently risk-reducing when applied to larger sites, where pollutants are widely dispersed and present at low concentrations. In contrast, specifically developed plant and plant-microbe systems could serve as a "green technology" to mineralize, degrade, or stabilize toxic organic pollutants. In addition, heavy metals and hazardous radionuclides may be extracted from soil by plants and concentrated in the harvested biomass, which can then be recovered and treated. Figure 1 presents a schematic of soil phytoremediation.

Plants have natural attributes that make them ideal candidates for cleansing contaminated soil environments. The root system represents an enormous surface area that enables plants to absorb and accumulate the water and nutrients essential for growth. Plants have remarkable metabolic and absorption capabilities and possess transport systems that can selectively take up many ions from soils. Plants have evolved a great diversity of genetic adaptations to handle potentially toxic levels of metals and other pollutants that occur in the environment. Most metal tolerant plants exclude toxic metal ions from uptake, while others, the so-called hyperaccumulators, actually tolerate and take up high amounts of toxic metal and other ions, up to several percent of their dry matter weight. Plants also excrete through their roots a variety of compounds that alter the root-soil environment by serving as nutrients and energy sources for soil microorganisms or by forming stable metal-chelates. Roots support a zone of increased microbial numbers and activity that may contribute to degradation of contaminants in soils. And finally, plant cultivation and management is a relatively inexpensive process, based on knowledge and practices developed in agriculture.

The use of vegetation to remediate contaminated soils offers the advantages of a photosynthetic, solar-energy driven process with a higher potential for public acceptance than many existing technologies, such as excavation with incineration or long-term storage. Degradation of toxic organic compounds in the root zone has the added advantage of avoiding the need of transfer of contaminants from one medium or

PHYTOREMEDIATION

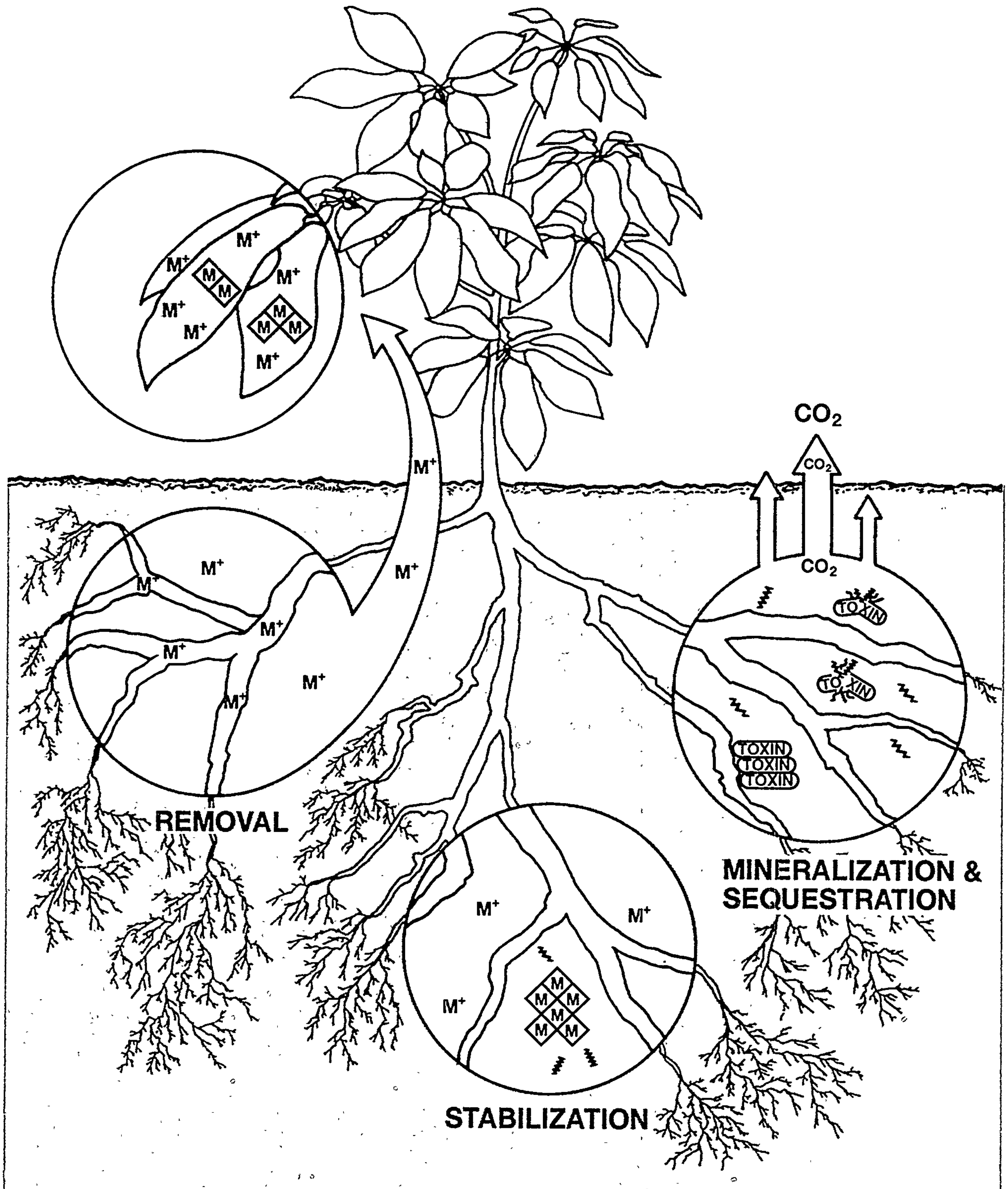

Figure 1. Plants are able to remove, stabilize or degrade toxic pollutants in soil. Plants can absorb, transport and concentrate metal ions (M^+) from soil into above ground shoots and thereby aid in the removal of heavy metals and radionuclides from soil. Plant roots and associated microorganisms can alter soil chemistry that stabilizes and reduces the bioavailability of pollutants in soil. Plant roots can provide adsorptive surfaces to sequester toxic pollutants in soil, and root exudates support microbial degradation of these organic compounds.

place to another. A better understanding of the interactions among plants, soil microorganisms and pollutants will improve our ability to manage vegetation for site remediation.

During the workshop a consensus developed that sufficient knowledge currently exists to project phytoremediation as a viable technology. Near-term applications will be restricted to favorable sites and easily remediated contaminants. However, as new fundamental and applied knowledge becomes available, the applications of phytoremediation should increase dramatically. The workshop participants identified four broad areas of research and development needs in phytoremediation:

- <u>Mechanisms of uptake, transport and accumulation</u> - Better understand and utilize physiological, biochemical, and genetic processes in plants that underlie the passive adsorption, active uptake, translocation, accumulation, tolerance and inactivation of pollutants.

Plants differ in their ability to satisfy the need for essential trace elements such as Fe, Zn, Mn, Ni, Cu, Cl, B, and Mo, whether these elements are present in soil in exceedingly small amounts or at excessive toxic levels. A number of mechanisms enable plants to mobilize and take up essential elements and these often effect the movement of other non-essential elements into plants. Powerful mechanisms including adsorption by cell walls; chelating agents; plant-induced pH changes and redox reactions; and specialized membrane channels, transporters, pumps and electrochemical gradients facilitate the movement of elements into roots. Research on these uptake and transport mechanisms are providing a better understanding of adaptability of these systems and how they might be used in phytoremediation. Similarly, improved understanding of how plants transport and isolate or inactivate near-toxic levels of elements and compounds in the cytoplasm is also important. Such fundamental research combined with the genetics, rhizosphere and field evaluations listed below are essential for selecting and developing plants for phytoremediation.

- <u>Genetic evaluation of hyperaccumulators</u> - Collect and screen plants growing in soils containing elevated levels of metals or other pollutants for traits useful in phytoremediation.

Plants that tolerate and colonize polluted environments are a valuable germplasm resource both as candidates for use in phytoremediation and as a source of genes for classical plant breeding and molecular genetics. Of particular interest are hyperaccumulators (plants that concentrate trace elements, heavy metals, or radionuclides at levels 100 fold or greater than normally found in plants). One of the objectives will be the determination of the mechanisms of specificity of uptake of ions. Continued collection efforts are needed for plant varieties that hyperaccumulate heavy metals and radionuclides. Similarly, areas contaminated by organic pollutants are useful habitats to search for plant-microbial combinations that can degrade the pollutants.

- Rhizosphere interactions - Better understand the interactive roles among plant roots, microbes, and other biota that make up the rhizosphere, and utilize their integrative capacity in contaminant accumulation, containment, degradation, and mineralization.

The soil rhizosphere is a very dynamic environment where the metabolism and growth of plant roots, microorganisms, and other soil biota alter the chemical and physical properties of soil as well as affect the growth of one another. Understanding rhizosphere chemistry, metabolism and ecology are key to managing the soil-plant-microbe-and other rhizosphere dimensions for successful development and application of phytoremediation. The potential exists to create "biased rhizospheres" where a plant root exudate favors the growth of one type of microorganism that can subsequently be selected or engineered to degrade specific organic pollutants.

- Field evaluations and validation - Employ early and frequent field testing to accelerate implementation of phytoremediation technologies and for feedback to basic and applied research programs. This includes standardized field-test protocols and model systems to validate laboratory results, as well as the subsequent application of test results to real world problems.

The large diversity of soil types and heterogeneity within soils compels a close linkage between laboratory research and field studies to gain a better understanding of the rhizosphere (soil-root zone) processes. Also, field tests can help address potential secondary impacts of phytoremediation such as biomagnification of toxic compounds in the food chain. Development of phytoremediation technology requires an interdisciplinary approach and cross-cutting research and development projects and programs, involving both basic and applied research.

Phytoremediation potentially offers unique, low cost, solutions to many currently intractable problems of soil contamination. Phytoremediation research can also contribute to the improvement of poor soils such as those with high aluminum or salt levels by providing a means of restoring arability for improved crop production.

A variety of new research approaches and tools are rapidly expanding our understanding of the molecular and cellular processes that can be employed in phytoremediation. Currently existing scientific and engineering infrastructure, and trained personnel could be rapidly deployed to address basic and applied research problems in phytoremediation. An integrated approach involving basic and applied research approaches along with consideration of safety, legal and policy issues will be needed to establish phytoremediation as a viable and attractive environmental restoration technology. It is hoped that this report will serve to highlight the exciting scientific and practical opportunities of this relatively undeveloped technology and that it will encourage the involvement of organizations and individuals with responsibilities and interests in this area.

INTRODUCTION: PHYTOREMEDIATION POTENTIAL AND OPPORTUNITIES

Plants are used extensively for food, fiber, fuel, and for sources of chemicals and many pharmaceuticals. However, the purposeful utilization of plants for clean-up of the environment has received relatively little attention despite the fact that plants, like microorganisms, play an important role in nature in sustaining and restoring environments. The capabilities of plants to absorb and accumulate varied ions, including many toxic heavy metals, and metabolize, directly or indirectly, organic compounds, suggests their utilization in the remediation of contaminated environments ("phytoremediation"). It should also be recognized that plants play a significant role in affecting aquatic and atmospheric environments, however, these components were not covered in the workshop.

The growing recognition of the potential use of plants for phytoremediation of soils led to the initiative to bring together researchers with expertise in both the basic and applied aspects of this field. The specific objectives of this workshop were to review the current status of phytoremediation and state the research needs, both basic and applied, that would form the scientific and technical foundation for the use of plants to remediate soils contaminated with hazardous organic and inorganic wastes.

There are currently virtually no commercial applications of phytoremediation, and only relatively few research projects and field trials have been carried out or are underway. A major impetus for the workshop was the realization that only a closely coordinated effort of basic and applied research could lead to rapid advances in this field.

At many DOE and other sites, surface soils are contaminated over relatively large areas with concentrations of heavy metals, other toxic elements and radionuclides that are often only a small factor above regulatory limits. At some sites organic pollutants, in particular chlorinated organic compounds, are present, often in combination with heavy metals and radionuclides. A listing of the most frequently observed contaminants in soils and sediments at DOE and other sites includes:

Heavy metals:
- Lead (Pb), Chromium (Cr), Arsenic (As), Zinc (Zn), Copper (Cu), Mercury (Hg), Cadmium (Cd), Aluminum (Al).

Radionuclides:
- Tritium (^3H), Uranium (U), Strontium (Sr), Cesium (Cs), Plutonium (Pu).

Chlorinated Solvents:
- Trichloroethylene (TCE), 1,1,1- Trichloroethane (TCA), 1,2-Dichloroethene (DCE), tetrachloroethylene (PCE), chloroform (CT).

Others:
- In addition, other inorganic and organic contaminants, such as nitrates and fuel hydrocarbons, are present at polluting levels.

The potential of phytoremediation for the clean-up of contaminated sites cannot at present be quantitated with any precision. Specific criteria for the selection of phytoremediation versus other soil remediation technologies are not yet available. Nor is there comprehensive information at most sites on the nature and concentration of the contaminants, the extent of the area and depth where pollutants occur, and specific clean-up goals. However, the high cost of alternative technologies, and the potential for vegetation-based systems to remediate extensive areas contaminated by relatively low levels of hazardous wastes, suggests that phytoremediation would have a large potential market.

Surface soils exhibiting relatively low concentrations of contaminants over large areas present a major technological challenge: conventional technologies, designed for smaller areas and higher levels of pollution, involve soil removal, use of large amounts of chemicals (e.g. surfactants), or heating and other expensive processes. Currently available technologies typically cost several hundred dollars per ton of soil treated. By contrast growing plants is relatively inexpensive and promises to be a cost effective approach to site remediation.

Perhaps the greatest opportunity, and need, for phytoremediation lies with the remediation of soils contaminated with heavy metals, radionuclides, and hazardous organic compounds found within surface soil. The chlorinated solvents listed above, being heavier than water tend to migrate and collect at the bottom of the water table, frequently below the rhizosphere. Thus they present relatively few opportunities for phytoremediation. However, other hazardous organic compounds held by soil particles are of concern and generally are retained within the rhizosphere zone and accessible for phytoremediation.

The effectiveness of phytoremediation is generally restricted to surface soils, within the rooting zone, or "rhizosphere". The most important limitation to phytoremediation is rooting depth, which can be 20, 50, or even over 100 cm depending on plant and soil type. Deeper root penetration can be achieved with perennial plants, such as shrubs and trees, which combine high productivity with well established root systems. Directed growth and development of root systems is desirable in phytoremediation and could be encouraged by careful control of watering and fertilization.

The potential for phytoremediation can be assessed by comparing the concentration of contaminants and volume of soil to be treated to the plant's seasonal productivity of biomass and ability to accumulate contaminants. For plants to be effective remediation devices one ton of plant biomass, costing from several hundreds to a few thousands of dollars to produce (depending on the scale of the site, plant productivity, remediation rates, location, etc.), must treat many tons of contaminated soil. For metals and other elements, which are removed from the soil and accumulated in the (generally) above-ground biomass, the ratio of biomass required to soil cleaned-up is the accumulation factor, the biomass:soil metal content ratio. This ratio, and the biomass productivity (tons/hectare/year), then determine the number of growing seasons (years) required for the process, a major determinant in the overall cost and feasibility of phytoremediation.

A major limitation in the phytoremediation of toxic elements is the maximal level that can be accumulated by plants. Plants with the highest levels of toxic metal contents, the so-called "hyperaccumulators", generally exhibit, on a dry weight basis, from about 2000 ppm (0.2%) for the more toxic elements (Cd, Pb, etc.) to above 2% for the less toxic ones (Zn, Ni, Cu, etc.). However, higher uptake values, about 1%, for the more toxic elements, are reported for a few plants and may be achievable in practice. Thus, the limits of phytoremediation are not yet well defined.

The second limitation is the amount of biomass that can be produced. Under the best climatic conditions, with irrigation, fertilization, etc., total biomass productivities can approach 100 t/ha/y. One of the unresolved issues is the trade-off between toxic element accumulation and productivity. In practice, a maximum harvestable biomass yield of 10 to 20 t/ha/y would be likely, particularly for heavy metal accumulating plants.

These values for productivity of biomass and heavy metal content would limit annual toxic element removal capacity to between about 10 to 400 kg/ha/y, depending on the pollutant, plant species, climatic and other factors. For a target soil depth of 30 cm (4,000 t/ha), this amounts to an annual reduction from 2.5 to 100 ppm in soil toxic element levels. This is often an acceptable rate of contaminant removal, allowing site remediation over a few years to a couple of decades, particularly where the concentration of the contaminant can be lowered sufficiently to meet regulatory criteria.

For soils contaminated with radionuclides or low mass amounts of the more toxic elements, even lower removal rates could be acceptable, allowing the use of plants with only moderate uptake capabilities. This could allow early applications of this technology at sites of particular interest to DOE.

Plant mediated mineralization of toxic organic compounds, involving in most cases microbial activities, may not be directly dependent on biomass productivity. Thus, a biomass:soil ratio is not clearly defined in the phytoremediation of organic compounds. However, the time required for site clean-up of organic compounds is the major determinant of feasibility for using vegetation to mineralize organic compounds.

Only a deeper knowledge of the underlying physiological, genetic, and biochemical processes can elucidate the limits of phytoremediation. These mechanisms are considered below. However, even with only partial understanding of these mechanisms, enough information is already available to allow confident predictions of practical applications, even in the short-term.

MECHANISMS OF PLANT UPTAKE, TRANSLOCATION, AND STORAGE OF TOXIC ELEMENTS

Plants have evolved highly specific and very efficient mechanisms to obtain essential micronutrients ("trace elements") from the environment, even when present at sub ppm, levels. Essential micronutrient elements required by plants are Fe, Zn, Mn, Ni, Cu, Cl, B and Mo. (V, Si, and Se are also possibly beneficial but have not been shown to be generally essential.) Plant roots, aided by plant-produced chelating agents and plant-induced pH changes and redox reactions, are able to solubilize and take up micronutrients from very low levels in the soil, even from nearly insoluble precipitates. Plants have also evolved highly specific mechanisms to translocate and store micronutrients. These same mechanisms are also involved in the uptake, translocation and storage of toxic elements, whose chemical properties mimic those of essential elements. Thus, micronutrient uptake mechanisms are of central interest to phytoremediation.

Basic Mechanisms

The range of known transport mechanisms or specialized proteins embedded in the plant cell plasma membrane and involved in ion uptake and translocation include:

- proton pumps (H^+-ATPases that consume energy and generate electrochemical gradients),
- co-and anti-transporters (proteins that use the electrochemical gradients generated by H^+-ATPases to drive the active uptake of ions), and
- channels (proteins that facilitate the transport of ions into the cell).

While the uptake-transport processes for some macronutrients (e.g. K^+ and Ca^{2+}) are fairly well understood, very little is certain about the corresponding processes for micronutrients. There is evidence that micronutrients may be transported via Ca^{2+} and other divalent cation channels and that these channels may function in the uptake of several micronutrients into plants. Both high and low affinity systems are known for macronutrients and suspected for micronutrients.

Most of the research in micronutrient uptake and transport has focused on Fe uptake (as Fe is often a limiting nutrient in agriculture). Thus much of what we know, and speculate, regarding trace and toxic element uptake is based on Fe metabolism. For example, it appears that accumulation of some metals in dicots may be correlated with a membrane surface Fe-reductase activity. Many species of grass produce iron chelators ("plant siderophores") that bind to the cell surface with high affinity only when charged with iron. Chelators may also facilitate uptake of other metal ions.

Many heavy metals of concern are either micronutrients (e.g. Zn) or analogues of micronutrients (e.g. Cd), as deduced from competitive inhibition studies that indicate the sharing of common transport functions. Each transport mechanism is likely to take up a range of ions. A basic problem is the interaction of ionic species during uptake of various heavy metal contaminants. This is also a practical issue, as multi-metal soil contamination is the rule. Also, for phytoremediation to work, relatively low concentrations of toxic elements in soil must be accumulated to high levels in the above ground biomass. Thus, energy-driven active uptake systems are likely to be required.

There are no data on mechanisms of metal uptake in any of the hyperaccumulators. A central issue is whether hyperaccumulators have higher rates of metal ion influx into the roots or, alternatively, whether they achieve high levels of accumulation by taking up and sequestering such ions over longer periods than normal plants.

After uptake by roots, translocation into shoots is desirable because the harvest of root biomass is generally not feasible. Little is known regarding the forms in which metal ions are transported ("translocated") from the roots to the shoots. Both complexed and free metal ion transport have been demonstrated, although the information is too sparse to draw many conclusions. The mechanisms of translocation may be the most critical step in phytoremediation, representing the greatest challenge in the evolution or development of hyperaccumulation characteristics. There is a need for detailed investigations of metal species and chelators (citrate, malate, amino acids, phytochelatins, peptides, etc.) translocated by the xylem and phloem systems.

Plant uptake-translocation mechanisms are likely to be closely regulated and self-limiting; plants generally do not accumulate trace elements beyond near-term metabolic needs. And these requirements are small: 10 to 15 ppm of most trace elements suffice for most needs. The exceptions are "hyperaccumulator" plants, which can take up toxic metal ions at levels in the thousands of ppm. They are discussed in the next section.

Another issue is the form in which toxic metal ions are stored in plants, particularly in hyperaccumulating plants, and how these plants avoid metal toxicity. Multiple mechanisms are involved. Storage in the vacuole appears to be a major one. The molecular mechanisms may involve transport processes mediated by chelators such as the phytochelatin peptides which have been demonstrated in fission yeasts and confirmed in higher plants. Other mechanisms involve the binding of metals by organic acids such as citrate and malate. Deposition in cell walls has been demonstrated *in vitro* but its significance with whole plants is uncertain. The very high levels of metal storage required in phytoremediation suggests this area as a prime target for research and development.

Genetic Approaches

Central to all these studies are genetic techniques, from isolation of specific mutations to gene cloning and functional analysis. Mutants can be used to identify and study important genes involved in heavy metal hyperaccumulation. A few such genes have already been identified. For example, in peas the brz mutation increases, by an unknown mechanism, the foliar concentration of Fe, and also somewhat elevates Cu and Zn levels.

The issue arises whether to study ion uptake, transport and accumulation in well established model systems such as fission yeast, *Arabidopsis*, and plant cell cultures, or whether to work with actual hyperaccumulator plants of practical use in phytoremediation. Both approaches have validity. For example, a metal accumulation gene first identified in fission yeast appears to be present also in higher plants. Mechanisms involved in root to shoot translocation would not be discovered in such model systems, however. Working with both model systems and candidate phytoremediation species is therefore desirable.

Phytoremediation R&D can use the tools of genetic and protein engineering to create new gene combinations and even novel catalysts. Genetic engineering was first applied in this field over a decade ago by transferring the genes for the metal-binding metallothionein proteins between animals and plants. Although, initial reports of enhanced metal tolerance and accumulation by such genetically engineered plants were often not reproducible, more recent studies have demonstrated metallothionein expression and increased metal tolerance in plant cell culture, although not in regenerated plants.

In a recent example, the enzyme mercuric reductase was transferred from bacteria into *Arabidopsis*, resulting in decreased mercury toxicity. Such a system may not be practical, as root expression of the enzyme would not allow mercury recovery, while translocation into the canopy may result in volatilization. However, the study of microbial genes and proteins involved in heavy metal binding, reduction, and resistance, and their transfer into and function in higher plants, would be a very fruitful area of basic research. For example, it was suggested that protein engineering of bacterial metal reductase genes could alter their specificity to allow reduction of other toxic metals.

Hyperaccumulation of metals into high-yielding above-ground biomass involves many processes and genes, and single-gene based approaches may not be sufficient. Techniques for working with complex genetic traits, such as the development of stable hybrids between low productivity hyperaccumulators with related, high yield, crop plants, may be promising.

It is clear that the area of trace and toxic element accumulation by plants is rich in research needs, from the fundamentals of ion transport for specific trace elements by hyperaccumulating plants, to the roles of rhizosphere microbes in facilitating metal transport and accumulation; a subject addressed in the next section.

The Soil-Microbe-Plant Complex in Toxic Element Accumulation

The microbial populations associated with roots play an important, though at present little studied, role in ion uptake processes. The function of the mycorrhizal fungi in increasing availability of macronutrients to plants is well documented and there is evidence for increased uptake of micronutrients, such as Zn and Cu, due to mycorrhizal infections. Also, such plant-microbe associations have been reported to result in increased metal tolerances. Microbial populations can affect trace metal mobility and availability to the plant, through release of chelators, acidification, and redox changes. The role of microbial populations in the root zone (rhizosphere) and their effect on the uptake of toxic elements by plant roots is still largely unexplored.

In addition to microbial populations, many abiotic soil factors affect plant ion uptake, and might be managed to increase toxic element removal from soils into plants. The basic chemistry of metal interaction with soil solid phases (clay minerals, sesquioxides, and organic matter) is relatively well known. Since most of the elements of interest partition fairly effectively to the solid phase, a phytoremediation strategy could involve desorption from surfaces or the dissolution of the surfaces themselves. However, in actual soil systems involving interacting complex organic and mineral phases, including microbes and roots, metal bioavailability becomes more unpredictable. Contaminant chemical speciation and dynamics are a major gap in our understanding of the accumulation of toxic ions by plants.

Altering the organic matter content of metal contaminated soils would alter their bioavailability. For metals occurring in the cationic form, sorption to soil organic matter and hydrous oxide surfaces reduces their activity. In soils containing variable charge surfaces, increasing pH increases the sorption of heavy metals. Increasing pH also forms hydroxy ions of certain heavy metals, such as $ZnOH^+$, which are more strongly sorbed than the uncomplexed ions. In such cases, soil acidification would tend to increase the solubility of many metals such as Zn, Ni, Cu, Mn and Cd. It also decreases the cation exchange capacity available for initial sorption sites. For example, depending on soil and pollutant conditions, phytoremediation might be more effective if the plant species selected are tolerant of soil acidity.

Another factor is the excretion by plants of more or less specific chelating agents for iron (termed plant siderophores) that, when having sequestered a ferric ion, are taken into the cell by highly specific and active transport systems. Siderophores may be induced by zinc as well as by iron deficiency stress, and may increase availability and uptake of Cu, Zn, Cd and other ions, in addition to Fe, to the root. Less specific metal binding exudates are also produced by plants. Again, this is an area deserving more investigation.

A variety of soil amendments and manipulations can be considered to maximize uptake of elements in phytoremediation. The goals would be to increase mobility of the soil contaminant(s) and to improve the kinetics of root transport. There are many possible soil manipulations and amendments, from deep tilling to the addition of dilute acids or organic matter (e.g. straw). EDTA and other chelators increase metal movement in soil, as can acidification using weak acids such as $(NH_4)_2SO_4$ or S (slowly oxidized to sulfuric acid). Chloride (as $CaCl_2$ or NaCl) can increase the mobility of Cd and even increase the rate of Cd uptake relative to Zn. Soil liming can be used to make other toxic elements such as Se and As more available.

Although such soil manipulation techniques can be useful, the major research issues in phytoremediation of toxic elements relate to the use of hyperaccumulating plants, addressed next.

HYPERACCUMULATION OF TOXIC ELEMENTS BY PLANTS

It has been suggested that so-called "hyperaccumulator" plants could remove large quantities of toxic elements, such as heavy metals and radionuclides, from soils. Plants are generally considered hyperaccumulators if they contain about a 100-fold higher trace and toxic element level than found in normal plants. By this definition, Cd hyperaccumulators would contain only about 100 ppm (dry weight basis), while hyperaccumulators for Zn or Mn would be over 10,000 ppm, with other metals such as Co, Cu, Pb, and Ni, being in the few thousand ppm range. However, there are no exact definitions and there is a broad range in metal concentrations among plants, from the low (1 - 10 ppm) levels for many micronutrients in crop plants, to the astonishing, such as over 20% Ni (200,000 ppm), on a dry weight basis, measured in the latex of a tree (*Sebertia acuminata*) from New Caledonia.

Hyperaccumulator Plants

Hyperaccumulating plants are often, but not always, associated with serpentine, calamine, cupriferous or other soils with relatively high metal contents, generally in the parts per thousand range. In such natural or anthropogenically contaminated soils, plant species that grow in surrounding low-metal soils are stunted or absent, but metal tolerant plants are able to colonize such areas.

Hyperaccumulators are a small subset of the metal tolerant plants, most of which simply exclude the toxic elements. Even among hyperaccumulators most known species are limited to the accumulation of a few metals: several hundred Ni, but only a few dozen Cu, Zn, and Co hyperaccumulating species are known. Cd and Pb hyperaccumulator species are counted in single digits.

Hyperaccumulation of U, Pb, and some other target toxic metals has not been well studied. Some older, but unconfirmed, literature reports U hyperaccumulation exceeding 1%, and recent studies suggest even higher levels of Pb hyperaccumulation. Thus, at present the upper limits of hyperaccumulation, even for highly toxic elements, are not well defined, but appear to be potentially relatively high.

Besides being rare, hyperaccumulating plants, particularly from temperate regions, are mostly small, and, thus, of low productivity. However, many Zn and Ni hyperaccumulators found in the tropics are woody shrubs or trees. Only a small sample of naturally occurring hyperaccumulator species are likely to have been discovered thus far.

The combination of properties in hyperaccumulator plants, including rarity, restriction to highly contaminated soils, hyperaccumulation of only a few (generally not the most toxic) elements, and low biomass productivity, raises the issue of the feasibility of using such plants in phytoremediation processes. For soil clean-up the interest is, generally, in hyperaccumulation of the more toxic elements from low mass levels in soil into high productivity plants. Meeting these goals is the central challenge of phytoremediation.

It can be speculated that the biochemical compromises required for metal hyperaccumulation may decrease biomass production. However, tropical hyperaccumulating plants of relatively large size and projected high productivity are known, such as *Berkheya coddii* for which yields of 18 t/ha/y have been estimated. Thus, biomass productivity may not be an inherent limitation in phytoremediation.

A second possible limitation is that hyperaccumulation of an element might only occur at high concentration of that element in soil. However, the biochemical mechanisms for trace element uptake are high affinity processes. Thus they should operate at near maximal rates until contaminant concentrations are quite low. At least in principle, plants should be able to reduce the heavy metal content in soils to low levels. Indeed, there is some experimental evidence for this (see below).

In conclusion, plant elemental content need not be limited by the uptake mechanisms per se, but possibly by other factors, such as translocation, storage, and toxicity. Another limiting factor could be the low mobility of ions in soils, resulting in mass transfer limitations to the root surfaces. It may be possible to increase metal ion mobility and bioavailability through the manipulation of the soil environment (see preceding and following sections).

Perhaps the most important limitation of hyperaccumulators is the rarity of Pb, Cd, and other toxic metal hyperaccumulators, when compared to Zn, Ni, and Cu hyperaccumulators. There may be an evolutionary basis for this preference: Ni and Zn are likely the most frequently encountered toxic metals in nature, while for Cd and Pb soil phytotoxicity is less frequent. For some elements, hyperaccumulator plants may need to be developed by directed genetic selection, rather than relying on collections from natural sites.

A general conclusion of this workshop is that the genetic traits present in hyperaccumulator plants offer potential for the development of practical phytoremediation processes.

Collection and Selection of Hyperaccumulator Plants

Hyperaccumulator plants have been discovered through a combination of field collections at sites known to contain high concentrations of heavy metals and by analysis of herbarium specimens. A few were discovered accidentally, others by broader screening efforts. Hyperaccumulators represent only a small fraction of the metal resistant flora because most plants survive such conditions by excluding toxic metals. The search for hyperaccumulators has not been exhaustive and it is likely that only a fraction of the hyperaccumulators in nature have been found.

A recurring theme at the workshop was the urgent need to establish a seed and germplasm bank for the collection and preservation of hyperaccumulator plants. Such a germplasm bank could help sponsor specific collection efforts into new areas as well as previously visited sites, and collaborate with broader plant collection projects.

Most work has been focused on finding new hyperaccumulator species through collections at remote sites. However, the benefits of exploiting intra-species variability among plants already at hand should not be overlooked. High genetic variability is expected in natural populations, particularly those surviving in an extreme environment. This variability could allow rather rapid selection for desired characteristics, without need for mutagenesis and screening of large numbers for novel mutations.

Accumulator plants (intermediate in metal contents between normal and hyperaccumulator plants) appear to be more widespread, particularly in plant families where hyperaccumulators are already widely represented (such as the *Brassicaceae* and *Euphorbiaceae*). Accumulator plants could provide a starting point for future genetic selection and development of novel hyperaccumulator plants, as well as near-term applications of phytoremediation. It should be possible to greatly accelerate such processes using traditional plant breeding and modern genetic techniques.

These various approaches to increasing metal accumulation in plants, from the study of field specimens to selection through plant breeding, requires suitable screening protocols. One possibility would be to select for metal tolerance and then screen for hyperaccumulation in tissue culture. Screening for mutants strains of *Arabidopsis* by analyzing single leaves has been successfully used to find a novel mutant which exhibits significant accumulation of Zn, Cu, and Mn. Even though this mutant constitutively expressed ferric reductase it, somewhat surprisingly, did not exhibit increased Fe accumulation. In any event, it is clear that such mutants can be used to identify and study important genes involved in heavy metal accumulation and tolerance.

The major objectives in selecting hyperaccumulator plants can be summarized as:

- high rates of toxic ion influx even at relatively low concentrations in soil;
- hyperaccumulation to very high levels and large accumulation factors;
- hyperaccumulation of multiple metal ions;
- fast growth and high biomass productivity;
- deep proliferating root system for maximal root-soil contact;
- growth habits suitable for easy cultivation and harvesting; and
- disease and pest resistance.

One key issue for phytoremediation is the first one: hyperaccumulation from soils with relatively low contamination levels. In that regards, experiments both in England and the U.S. demonstrated uptake of Zn from soils containing a few hundred ppm into plant biomass containing several thousand ppm of this metal, with accumulation factors of over 10. This is an acceptable goal for many practical applications. Although not achieving true hyperaccumulation levels, such experiments demonstrate a potential for plants to accumulate metals from relatively low concentrations.

However, much more work is required. Concerns exist, for example, about the rate of contaminant uptake achievable with active energy-driven uptake systems (e.g. H^+-ATPases), which operate at low external ion concentrations, compared to the rates of ion uptake observed with ion channels, operating at higher external concentrations. This, again, is an example of the continuum between basic research and applied development needs in this area.

Equally critical is the need to develop hyperaccumulation plants that exhibit high productivity. Conventional agronomic methods for improving plants are applicable here, and may be combined with modern gene transfer methods, such as *in vitro* tissue culture and somaclonal variation.

PHYTOREMEDIATION OF HAZARDOUS ORGANIC COMPOUNDS

Plants can take up and metabolize many toxic organic compounds. The main driving factor for plant uptake is contaminant hydrophobicity, commonly measured as the octanol:water partitioning coefficient. The partitioning coefficient also determines mobility through soil, and, thus availability to the plants. Once taken up by the plant, many xenobiotics can be metabolized by plant enzymes, such as those that degrade fungal toxins.

Mass transfer limitations of organic compounds through soil, due to low solubility and high soil adsorption, can limit plant uptake of many toxic organic compounds. Highly lipophilic compounds are generally so strongly absorbed to soil surfaces that they do not become available for bioremediation (plant or microbial). Hydrophilic compounds move quickly beyond the root zone in non-contained systems and thus are not available for plant remediation. Moderately lipophilic contaminants that can move (or be moved with surfactants) in the soil solution to the plant root zone, are the most likely candidates for phytoremediation of toxic organic compounds.

Most of the literature on plant uptake and metabolism of toxic organic compounds is for agricultural chemicals such as pesticides. There are relatively few data on other hazardous organic compounds. For chlorinated hydrocarbons, it has been established that plant tissue cultures and axenic (bacteria-free) plants can produce trichloroethanol (and other, unidentified metabolites) from trichloroethylene (TCE). However, most of the TCE loss is due to binding to a non-extractable fraction, a phenomenon also observed with some other toxic organic compounds.

The rhizosphere of soil harbors extensive microbial populations, which participate actively, and are generally the primary agents, in the *in situ* remediation of toxic organic compounds. This has been demonstrated for TCE remediation in experimental soils, where multiple microbial species acting in concert with plants were responsible for the observed degradation. Thus, in the phytoremediation of toxic organic compounds, the focus must be on the soil microflora and its interaction with the plants and soil.

The influence of plants extends well below the root zone: vegetated soils can supply the deeper subsurface microbial populations with organic substrates, stimulating degradative microbial activities ("intrinsic bioremediation").

In plant-microbial systems, the plant can be thought of as furnishing the equivalent of a "bioreactor", controlling environmental conditions and providing the substrates required by the microbes for growth and metabolism of the contaminants. The amount of fixed carbon provided by plants, either directly (plant exudates, root hair turnover) or indirectly (leaf litter decomposition), is dependent on many factors, not the least being the nutritional status of the plants.

Unbalanced growth can release a larger fraction of photosynthetic productivity into the soil through excretion below ground. Alternatively, the crop could be plowed under at the end of the growing season. Plant organic matter would greatly enhance the level of microbial activity in the rhizosphere, even extending to the deeper subsurface. This could potentially increase microbial destruction of toxic organic compounds.

Considering the costs and difficulties in providing reagents for *in situ* microbial bioremediation (e.g. nutrients; electron acceptors such O_2, H_2O_2, and NO_3; methane or phenol for co-metabolism of TCE; etc.); the low cost of plant cultivation is the major factor in potentially making phytoremediation of toxic organic compounds much less expensive than even other bioremediation processes.

At present, phytoremediation of toxic organic compounds is more a phenomenon than a process: it is observed to happen, but process control and optimization has not been well studied. It is likely that empirical research including the effects of various irrigation and fertilization schedules, the use of alternative crop plants, or the availability of easily decomposed plant biomass will significantly improve the rate of soil contaminant degradation. Only a deeper mechanistic understanding of the complex rhizosphere processes resulting in toxic organic compounds mineralization will assure the optimization of this technology. The goal is to establish some control over the soil microbial populations and their activities.

However, controlling microbial populations in any such "open" system, with an existing, highly adapted and diverse microbial population, and subject to invasion and contamination, has been a very difficult problem in applied microbiology. The problem boils down to one of selectively encouraging specific microbial subpopulations, either native or introduced, able to degrade the contaminants. One potential strategy is to change the soil environment through moisture control, pH alteration, fertilization, deep tilling, and the addition of soil amendments. These could increase mobility of the soil contaminant(s) and improve the kinetics of microbial mineralization. However, such techniques, on their own, would not likely be specific enough to enhance the desired microbial population and their metabolic activities.

Inoculation would appear to be the most straightforward strategy. The literature is replete with descriptions of laboratory selected microbial strains able to mineralize toxic organic compounds at high rates. However, in field trials (or even pot tests) laboratory bred strains do not generally persist long enough to effectuate the desired result. Grazing by protozoa and competition by indigenous microbes are the general causes for this lack of persistence.

The major issue is, thus, how desired microbial activities, or even specific microbes, can be controlled and maintained in the rhizosphere. Most participants at the workshop considered this something beyond our current capabilities, deserving attention by both basic and applied R&D programs. At the basic level, there is a need to study mechanisms used by plants to structure microbial communities in the rhizosphere. At an applied level, a number of different approaches were suggested by the participants on how such "biased rhizosphere" or "phyto-bacto-remediation" processes could be developed.

One approach is to provide a unique carbon-energy source, which only the desired bacterial strain is able to metabolize. Indeed, this approach has already been anticipated by some leguminous plants whose roots excrete specific carbon compounds (rhizopine and other opines) that can only be used by specific symbiotic soil microbes. It may be possible to exploit this system by adapting microbes dependent on such unique substrates to be able to degrade specific toxic organic compounds. Genetic engineering could be used to develop specific plant-microbe associations for phytoremediation of toxic organic compounds.

The feasibility of actually achieving successful inoculation, even with a competitive advantage, is far from assured. The view expressed at the workshop was that "one of the biggest problems arises when one tries to introduce the (modified) bacteria into the rhizosphere." At present only the enhancement of pre-existing microbial populations has been successfully applied in bioremediation.

Two themes recurred during these discussions:

- integrated rhizosphere systems outperform bacteria or plants by themselves; and
- our understanding of soil factors, both natural and managed, which affect the mineralization of organic compounds, is rudimentary.

The research and development needs for phytoremediation of toxic organic compounds identified at the workshop included better understanding of microbial vs. plant roles in the degradation of organic compounds, soil-root-microbe interfaces and rhizospheric chemistry generally, how plant roots might structure-bias rhizosphere microbial populations-films, and the development of techniques for controlling the microbial populations in the rhizosphere.

PHYTOREMEDIATION: FROM LABORATORY INTO THE FIELD

There was a strong consensus expressed at the workshop that research in phytoremediation must include early field trials. Field experiments can generate the data and feedback needed to direct applied, and even basic, research efforts. For example, field trials can justify further laboratory work in probing specific biochemical and genetic systems. Combining field trials with laboratory work will result in early identification of potential problems, allowing for correction of research and development plans. Another argument for early field trials is the need to develop and validate test protocols that would allow improving the, currently rather poor, extrapolation of laboratory results to the field.

Field trials force the question of site selection and characterization as well as the choice of plants suitable for the soil type. Site variability and complexity make "generic" tests of phytoremediation (e.g. with artificially contaminated soils) of questionable relevance, in the absence of experiments with actual contaminated soils. Site selection must be guided by the available data on the potential effectiveness of the plants to be tested and site-specific characteristics, including the solution chemistry of the contaminants to be removed or degraded.

Initially, the plants must be tested using standardized screening protocols such as *in vitro*, sand, hydroponic, and other culture methods that allow handling of large numbers of plants. After selection and matching of plants and test sites, small-scale laboratory and greenhouse (pot) tests must confirm the suitability of the plants for the remediation process. These are the minimum preliminary steps required before small field-tests are started.

It will be important to develop standard protocols for hydroponic and other laboratory culture methods, soil and plant analysis, pot tests, field tests, and other aspects of such applied R&D. Standardization of techniques is a major requirement in such research to permit comparative evaluation of results from different research groups and field trials. Comparative data are important for directing the next steps in technology development: from the improvement of plant cultivars to field management techniques.

Reiterative and parallel field tests and laboratory studies would likely achieve near-term applications of phytoremediation at favorable locations. More difficult sites and contaminants will require the application of advanced plant breeding, selection, and/or genetic techniques and a deeper understanding of underlying mechanisms of contaminant uptake and transport in vascular plants.

The practical objective of phytoremediation is to achieve major cost reductions in the clean-up of remediate sites. One objective of the field trials is to adopt agricultural equipment and supplies for phytoremediation technology, a major reason for the potentially low cost of this process. Thus, in addition to their remediation qualities, the agronomic characteristics of the plants must be evaluated (e.g. planting densities, pest control, fertilization schedules, harvesting, etc.)

Plant growth and biomass production are only a part of the overall phytoremediation costs, which include site characterization, security, monitoring, management, regulatory and legal, and eventual site-closure costs. The processing and ultimate disposal of the biomass generated is likely to be a major factor of overall costs, particularly when dealing with highly toxic elements and radionuclides. The leaching of toxic metals and organic compounds into the groundwater as a consequence of phytoremediation activities is another area of concern.

Many alternative biomass processing and volume reduction options are available (gasification, combustion, chemical leaching, fermentations, etc.). Testing and development for specific applications will be required. However, even though processing and disposal will be a major cost component of phytoremediation processes, R&D on such technologies is not a high priority, as it is not considered a major impediment to this technology. Nevertheless, a better definition of the applicability and costs of the various alternatives, and perhaps some small-scale tests, would be useful.

It is difficult to predict or generalize the actual costs of phytoremediation in comparison of overall site clean-up costs. In many cases phytoremediation may be used as a follow-up technique after "hot-spots" are mitigated, or in conjunction with other remediation technologies, making cost-analysis even more difficult. However, it would be useful to better define the costs of phytoremediation. Site complexity argues against generic, and in favor of site-specific, cost analysis. Such analysis must include the entire cycle of the process from the growing and harvesting of the plants to the final processing and disposal of the biomass.

As with any technology, the potential for negative environmental consequences must be considered. Some examples of negative impacts discussed at the workshop are the potential for the phytoremediating plants to support food chains (earthworms, insects, small mammals, birds, and other herbivores) and biomagnify the contaminants into higher trophic levels. Another is the potential for release of foreign plant types which could become established beyond the site or after clean-up is completed. Side-effects from agricultural practices, biomass volume reduction processes, and the ultimate disposal of the toxic elements must also be considered. While these possible environmental effects need to be considered, methods exist to control or mitigate their impact. Also, the seriousness of such impacts must be weighed against the alternatives of either no action or much more expensive physical-chemical clean-up technologies and their potential environmental consequences.

CONCLUSIONS

Soil contamination is a national and global problem. A major challenge is the remediation of large sites contaminated with radionuclides and toxic metals, often present in relatively small amounts but above regulatory action levels. Despite the function of phytoremediation processes in nature for millenia, the technology of phytoremediation is, for the most part, still a concept. There are many different pollutants, plant uptake mechanisms, soil matrices, and plant species that need to be investigated, without overlooking the microbial participation in this technology. Developing actual practical applications will require a significant and coordinated research and development effort, due to the complexity of both biological systems and the soil contamination problems.

Research and development in this area must involve scientists and engineers in Federal and state agencies, foreign organizations and industry. The representation at the workshop of researchers from many disciplines, organizations and countries, augurs well for a cooperative and interdisciplinary research effort and the rapid application of this technology. The urgent needs for effective, low-cost technologies to clean-up contaminated soils, both in the U.S. and around the world, suggests phytoremediation as a high national and international research priority. The availability of scientists trained in the interdisciplinary topics relating to phytoremediation will be a major factor in expediting development of this technology.

Basic and applied research in phytoremediation could have applications to other urgent problems, beyond the clean-up of contaminated sites. For example, in a number of regions soils exhibit high levels, from natural sources, of aluminum, other toxic elements, or salts, reducing crop productivity. Research in phytoremediation could benefit mankind by helping to increase land productivity by making more land arable to feed a rapidly increasing population.

Current information on the mechanisms by which plants take up trace and toxic elements are fragmentary and incomplete. A deeper understanding of basic mechanisms is required. The techniques of plant molecular biology and biochemistry developed by basic plant and microbiological research have significantly shortened the time required for advancing this area of research and developing novel biotechnologies. However, practical applications are possible even with current knowledge: hyperaccumulator plants offer significant promise for near-term implementation of this technology, using conventional agronomic tools of plant management and improvement. Many toxic organic compounds in soils can be degraded by synergistic or interacting microbial and plant communities, with neither the plant nor microbial populations alone being effective. Both fundamental and empirical studies will advance such applications.

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