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# PHYTOREMEDIATION: USING GREEN PLANTS TO CLEAN UP CONTAMINATED SOIL, GROUNDWATER, AND WASTEWATER

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## ABSTRACT

Phytoremediation, an emerging cleanup technology for contaminated soils, groundwater, and wastewater that is both low-tech and low-cost, is defined as the engineered use of green plants (including grasses, forbs, and woody species) to remove, contain, or render harmless such environmental contaminants as heavy metals, trace elements, organic compounds ("organics"), and radioactive compounds in soil or water. Current research at Argonne National Laboratory includes a successful field demonstration of a plant bioreactor for processing the salty wastewater from petroleum wells; the demonstration is currently under way at a natural gas well site in Oklahoma, in cooperation with Devon Energy Corporation. A greenhouse experiment on zinc uptake in hybrid poplar (*Populus sp.*) was initiated in 1995. These experiments are being conducted to confirm and extend field data from Applied Natural Sciences, Inc. (our CRADA partner), indicating high levels of zinc (4,200 ppm) in leaves of hybrid poplar growing as a cleanup system at a site with zinc contamination in the root zone of some of the trees. Analyses of soil water from experimental pots that had received several doses of zinc indicated that the zinc was totally sequestered by the plants in about 4 hours during a single pass through the root system. The data also showed concentrations of sequestered metal of >38,000 ppm Zn in the dry root tissue. These levels of sequestered zinc exceed the levels found in either roots or tops of many of the known "hyperaccumulator" species. Because the roots sequester most of the contaminant taken up in most plants, a major objective of this program is to determine the feasibility of root harvesting as a method to maximize the removal of contaminants from soils. Available techniques and equipment for harvesting plant roots, including young tree roots, are being evaluated and modified as necessary for use with phytoremediation plants.

## INTRODUCTION AND BACKGROUND

At many hazardous waste sites requiring cleanup, the contaminated soil, groundwater, and/or wastewater contain a mixture of contaminant types, often at widely varying concentrations. These may include salts, organics, heavy metals, trace elements, and radioactive compounds. The simultaneous cleanup of multiple, mixed contaminants using conventional chemical and thermal methods is both technically difficult and expensive; these methods also destroy the biotic component of soils.

Phytoremediation, an emerging cleanup technology for contaminated soils, groundwater, and wastewater, is both low-tech and low-cost. We define phytoremediation as the engineered use of green plants, including grasses, forbs, and woody species, to remove, contain, or render harmless such environmental contaminants as heavy metals, trace elements, organic compounds, and radioactive compounds in soil or water. This definition includes all plant-influenced biological, chemical, and physical processes that aid in the uptake, sequestration, degradation, and metabolism of contaminants, either by plants or by the free-living organisms that constitute the plant's rhizosphere. Phytoremediation takes advantage of the unique and selective uptake capabilities of plant root systems, together with the translocation, bioaccumulation, and contaminant storage/degradation abilities of the entire plant body. Plant-based soil remediation systems can be viewed as biological, solar-driven, pump-and-treat systems with an extensive, self-extending uptake network (the root system) that enhances the below-ground ecosystem for subsequent productive use.

Examples of simpler phytoremediation systems that have been used for years are constructed or engineered wetlands, often using cattails to treat acid mine drainage or municipal sewage. Our work extends to more complicated remediation cases: the phytoremediation of a

site contaminated with heavy metals and/or radionuclides involves "farming" the soil with selected plants to "biomine" the inorganic contaminants, which are concentrated in the plant biomass. For soils contaminated with toxic organics, the approach is similar, but the plant may take up or assist in the degradation of the organic. Several sequential crops of hyperaccumulating plants could possibly reduce soil concentrations of toxic inorganics or organics to the extent that residual concentrations would be environmentally acceptable and no longer considered hazardous. The potential also exists for degrading the hazardous organic component of mixed contamination, thus reducing the waste (which may be sequestered in plant biomass) to a more manageable radioactive one.

For treating contaminated wastewater, the phytoremediation plants are grown in a bed of inert granular substrate, such as sand or pea gravel, using hydroponic or aeroponic techniques. The wastewater, supplemented with nutrients if necessary, trickles through this bed, which is ramified with plant roots that function as a biological filter and a contaminant uptake system. An added advantage of phytoremediation of wastewater is the considerable volume reduction attained through evapotranspiration.

A Cooperative Research and Development Agreement (CRADA) has been established between Argonne National Laboratory (ANL) and Applied Natural Sciences, Inc. (ANS), which uses trees as phytoremediation plants to explore deeper (down to 40 feet) soil horizons in a trademarked process called Treemediation®.

Though it is not a panacea, phytoremediation is well suited for applications in low-permeability soils, where most currently used technologies have a low degree of feasibility or success, as well as in combination with more conventional cleanup technologies (electromigration, foam migration, etc.). In appropriate situations, phytoremediation can be an alternative to the much harsher remediation technologies of incineration, thermal vaporization, solvent washing, or other soil washing techniques, which essentially destroy the biological component of the soil and can drastically alter its chemical and physical characteristics as well, creating a relatively nonviable solid waste. Phytoremediation actually benefits the soil, leaving an improved, functional, soil ecosystem at costs estimated at approximately one-tenth of those currently adopted technologies.

## RESEARCH APPROACH

The phytoremediation concept is based on the well-known ability of plants and their associated rhizospheres to concentrate and/or degrade highly dilute contaminants. Critical components of the rhizosphere, in addition to a variety of free-living microorganisms, include root exudates. These complex root secretions, which "feed" the microorganisms by providing carbohydrates, also contain natural chelating agents (citric, acetic, and other organic acids) that make the ions of both nutrients and contaminants more mobile in the soil. Root exudates may also include enzymes, such as nitroreductase and dehalogenases. These enzymes have important natural functions, but they may also degrade organic contaminants that contain nitro groups (e.g., TNT, other explosives) or halogenated compounds (e.g., chlorinated hydrocarbons, many pesticides).

Plant roots and rhizosphere microorganisms "sense" the immediate soil environment in which they are growing and have complex feedback mechanisms that permit them to adapt to changing conditions as they grow. In some plants growing in phosphorus-deficient soil, the root exudates contain large amounts of citric acid, in an attempt to mobilize and make available for uptake any phosphorus compounds present. Some rhizosphere microorganisms secrete plant hormones that increase root growth, and thereby the secretion of root exudates that contain metabolites they use as an energy source.

Large green plants have the capability to move large amounts of soil solution into the plant body through the roots and evaporate this water out of the leaves as pure water vapor in a process called transpiration. Plants transpire water to move nutrients from the soil solution to leaves and stems, where photosynthesis occurs, and to cool the plant. During this process, contaminants present in the soil water are also taken up and sequestered, metabolized, or vaporized out of the leaves along with the transpired water. Low water use is a trait considered desirable in most economically important plants. However, some plants are notoriously poor at water conservation, usually because they normally grow in moist environments (e.g., hybrid poplars, willows, bulrush, marsh grasses). Such species are good candidates for phytoremediation plants because they take up and "process" large volumes of soil water. For example, data show that a single willow tree can, on a hot summer day, transpire more than 19 $m^3$ of water (5,000 gal), and one hectare of a herbaceous plant like saltwater cordgrass evapotranspires up to 80 $m^3$ of water (21,000 gal) daily.

When we grow selected, adapted plants in contaminated substrates, the root system functions as a highly dispersed, fibrous uptake system. Contaminants over a large range of concentrations are taken up along with the water and degraded, metabolized, and/or sequestered in the plant body, while evapotranspiration from aerial parts maximizes the movement of soil solution or wastewater through the plant. Through the process of bioaccumulation, contaminants can be concentrated thousands of times higher in the plant than in the soil or wastewater. The contaminated plant biomass can be digested or ashed to reduce its volume (≈95%), and the resulting small volume of material can be processed as an "ore" to recover the contaminant (e.g., valuable heavy metals, radionuclides). If recycling the metal is not economically feasible, the relatively small amount of ash (compared to the original biomass or the extremely large volume of contaminated soil) can be disposed of in an appropriate manner.

Our studies are screening both hyperaccumulator and nonaccumulator plant species to identify candidate plants for phytoremediation of heavy metals, radionuclides, and organics. Understanding contaminant uptake mechanisms includes the study of root physiology and morphology, uptake kinetics, translocation in roots, stems, and leaves, and bioaccumulation in specific organs, as well as the role of mycorrhizae and the rhizosphere. Many known hyperaccumulator species are small and have special growth requirements, such as species in the family Brassicaceae (mustard family). Selected nonaccumulator plants, though their specific toxic metal accumulation ratios may be lower than those of hyperaccumulators, may actually sequester more total metal because of their greater biomass and water uptake rates. Total contaminant removal and binding capability are determined by many plant attributes and factors being investigated by this project. These include tolerance to the contaminant; selectivity; accumulation capability; transpiration rate; plant size, biomass, and growth form; aerial surface area; root type, fibrosity, rooting depth, and harvestability; duration (annual, biennial, or perennial); dormancy; and resistance to pests and disease.

For many contaminants, passive uptake via micropores in the root cell walls (the apoplastic pathway) may be a major route into the root, where sequestration or degradation can take place. The apoplast is a hydrated free space continuum between the external soil solution and the cell membranes of the root cortex and vascular tissue. The cell wall micropores exist within a network of cellulose, hemicellulose, pectins, and glucoprotein containing many negative charges (generated by carboxylic groups) that act as cation binding sites and exchangers and as anion repellers. Di- and polyvalent cations (the form of many heavy metal and radionuclide contaminants) are preferentially attracted to, and bound on, these cation exchange sites within the root cortex cell walls. For metal ions to be metabolized or translocated to the aboveground parts of the plant, they must pass through the plasma membrane of a living cell, and this can only occur by active transport processes. The inner limit of the root cell wall free space, and hence of the passive apoplastic radial transport of ions, is the endodermis, which forms the outer limit of the root vascular system, or stele.

A major objective of this program is to determine the feasibility of root harvesting as a method to maximize the removal of contaminants from soils, since in most plants the roots sequester most of the contaminant that is taken up. Available techniques and equipment for harvesting plant roots, including young tree roots, are being evaluated and modified as necessary for use with phytoremediation plants. Sequestration of heavy metals in roots, as opposed to translocation to aboveground plant parts, reduces the likelihood of environmental dispersion of the contaminants into food chains by wildlife or domestic animals.

We are also evaluating the use of large, robust plants with high transpiration rates for the treatment of wastewater, using a variety of hydroponic and aeroponic growth systems. The mobility of heavy metals and other contaminants in soils by means of chelating agents, root exudates, and other compounds is also being studied.

## RESEARCH STATUS AND PLANS

ANL has been conducting basic and applied research in phytoremediation since 1990. Initial greenhouse studies evaluated salt-tolerant wetland plants to clean up and reduce the volume of salty "produced water," the wastewater brought to the surface from wells producing natural gas and crude oil. A field demonstration of a plant bioreactor for processing produced water is under way at a natural gas well site in Oklahoma. In this small-scale, two-compartment bioreactor, each compartment processes wastewater of increasing salinity and contains a different plant species or a combination of species. The effluent water is considerably reduced in both volume and contaminants, while its salt concentration is increased. The usual disposal method for produced water is deep well injection, the cost of which depends on the volume of wastewater injected. The bioreactor is based on a model that uses conservative greenhouse data and predicts a produced-water volume reduction 75% in less than eight days. This represents a major potential cost saving for the petroleum industry.

Currently, we are extending these studies and the phytoremediation concept to a wider variety of hazardous contaminants and other waste site problems, through greenhouse studies of plant uptake of heavy metals in both woody and herbaceous species, and through partitioning of the metal in specific plant organs. In addition, we are developing a database of phytoremediation literature. We have also identified commercial sources for seed and transplants of many of the promising phytoremediation species.

Current greenhouse experiments on heavy metal uptake and sequestration by green plants include an experiment studying zinc uptake in hybrid poplar (*Populus* sp.) cuttings growing in inert quartz sand in lysimeter pots. These experiments, initiated in late March 1995, seek to confirm and extend field data from ANS indicating high levels of zinc in leaf tissue of hybrid poplar growing at a cleanup site that had zinc contamination approximately 15 feet below the surface. Detailed data were collected on contaminant uptake, translocation, and partitioning in plant organs, as well as on evapotranspiration rates, nutrient use, and biomass increase of the rapidly growing poplar shoots. To obtain these data, leachate volume, conductivity, and pH were measured each day. To replace water lost via evapotranspiration, fresh nutrient or water was added to the leachate each day to bring the total volume to either 1 or 1.5 liters.

In June 1995, when the poplars were growing well and had developed a normal root system, a series of treatments was started in which three groups of plants were given increasing doses of zinc ion in nutrient solution over a period of about two months. These doses were given in five zinc additions, with concentrations ranging from 50 to 2,000 ppm Zn. Each group consisted of three replicates. On each zinc addition date, two groups received increasing doses up to 1,500 and 2,000 ppm Zn, respectively, while a control group (0 ppm) received nutrient only.

The zinc was added to three replicate pots, in 500 mL of nutrient solution spiked with $ZnSO_4$. These levels of zinc, totally dissolved in nutrient solution, are equivalent to several times the levels in "normal" soils, as revealed by available zinc analyses (DTPA extraction). In our experiments, there are essentially no binding sites in the sand growth substrate, as internal controls showed, in which the zinc concentration of nutrient solution remained unchanged when passed through lysimeter pots with quartz sand substrate but no plants. Prior to each zinc addition, sand substrate samples and plant tissues (roots, leaves, and branches) were collected to determine how much zinc remained in the substrate, and to track zinc translocation and partitioning of bioaccumulation in poplar aboveground tissues.

Leachate analyses for zinc by atomic absorption spectrophotometry indicate that in all cases, up to 800 ppm Zn added in nutrient solution, the zinc was totally and selectively absorbed and sequestered by the plants in about 4 hours during a *single* pass through the root system contained in the lysimeter pot. At levels of zinc above 1,000 ppm in nutrient added to the pots, leachate levels were always below 100 ppm in samples making one pass through the lysimeter pot and taken the same day as the zinc addition; these levels increased the following day, to concentrations up to 548 ppm for the 2,000 ppm zinc addition. and then decreased sharply the second day after the zinc addition, to concentrations less than 100 ppm. Thereafter. the zinc concentration steadily decreased as the plants apparently reabsorbed the zinc as the nutrient was cycled through the pots on subsequent days. During the experiment. this pattern of zinc concentration changes in leachate following a zinc addition was observed twice (Figure 1).

The hybrid poplar plants in the lysimeter pots were harvested in early September 1995. All of the aboveground tissue was harvested and divided into four categories (mature leaves, expanding leaves. old wood, and new wood). The sand substrate was removed from each pot as a "cylinder," with the undisturbed roots remaining where they had grown. After the sand cylinder was photographed with the roots in place. sand samples were collected, and the sand was washed from the root system with deionized water. Four types of roots (A. B. C, and D) were identified. on the basis of appearance. morphology, and color, and collected as separate samples. These types were clumps or wefts of white or light-colored, new roots (A) originating at the perimeter or the ends of large, woody roots (B). Very fine, hairlike. dark-colored roots (C) densely covered the surfaces of the woody roots. Large-diameter, non-woody roots and fine roots that were grayish to brownish in color and occurred primarily in the bottom of the lysimeter pots (which were saturated most of the time) were grouped into a single category (D). All tissue samples were dried at 80°C and analyzed for zinc by digestion. extraction, and atomic absorption spectrophotometry. Results from the biomass analysis are summarized in Figure 2.

Several leaf harvests were made during the course of the experiment. Initial leaf analyses showed 528 µg/g (ppm) Zn in mature (large) leaves, 300 µg/g Zn in medium-sized leaves, and 140 µg/g Zn in small leaves of plants that received a single dose of 50 ppm Zn in nutrient solution. In aboveground plant parts (leaves and branches) harvested at the end of the experiment, zinc concentrations did not exceed 2,250 µg/g Zn in the dry leaf tissue. or 900 µg/g Zn in the woody branches, on a dry-weight basis.

The root tissues harvested at the end of the experiment showed much higher concentrations of accumulated and sequestered metal than did the aboveground parts. In the pots receiving the highest dose of zinc (2,000 ppm), the D roots contained a mean concentration of 38,055 µg/g Zn in the dry root tissue, while the C roots had 15,470, the A roots had 12,225, and the B roots had 2,814 µg/g Zn, respectively. The pots receiving the lower zinc doses (maximum of 1,500 ppm) had a similar pattern of distribution of zinc among root types; in this case, the D roots contained a mean concentration of 17,053 µg/g Zn. The control pots also showed the same zinc uptake pattern among root types, the mean for the D roots being 232 µg/g Zn. The small amount of zinc in the controls is from the zinc present in the nutrient solution as an essential nutrient. Figure 2 summarizes biomass zinc content.

These levels of sequestered zinc observed in hybrid poplar roots exceed the levels found in either roots or tops of many of the known hyperaccumulator species. We hypothesize that the greater portion of the zinc is sequestered in the cell wall tissue of the root cortex through internal complexation and detoxification, with subsequent translocation of a relatively small amount of the metal to the leaves and branches. Even at the highest zinc uptake levels, there were only subtle visual toxicity symptoms (slight leaf chlorosis and some leaf "drooping") in the aboveground parts of the experimental plants when compared to controls. However, in plants that received more than 800 ppm Zn. the evapotranspiration rate decreased significantly as zinc concentration in the roots increased; this physiological effect could have complex causes.

These data demonstrate a very effective uptake, bioaccumulation, and sequestration system for zinc in hybrid poplar, as well as high evapotranspiration rates and a wide range of adaptability for this versatile woody plant. We now need to determine if other heavy metals, such as cadmium and uranium, are sequestered in a similar manner, and if other plant species show root accumulation characteristics. To begin to answer these questions, and many others generated by the poplar data, a second lysimeter pot experiment is currently being performed to evaluate zinc uptake and sequestration in Eastern gamagrass (*Tripsacum dactyloides*), a large, robust grass with an extensive, fibrous root system and high transpiration rates. Results from this experiment may also contribute to the eventual confirmation of our hypothesis that root harvesting is an effective method to maximize heavy metal removal from contaminated soil, regardless of the type of phytoremediation plant used.

The hybrid poplar greenhouse experiments complement field studies and data collection on zinc uptake by hybrid poplar trees growing under field conditions in installed remediation systems (Treemediation®), conducted by Applied Natural Sciences, Inc. The implications for engineered soil and wastewater cleanup systems that use hybrid poplar growing either directly in the soil or in special hydroponic systems are very encouraging. Results from several Treemediation® systems that have been in place five years indicate the hydraulic control of a downgradient plume in a tight soil matrix. Currently, eight systems are in place in the field, with six to twelve additional sites likely by Spring 1996. Soil and groundwater as deep as 30 feet are being treated, and plants are under investigation for deeper conditions. With a properly engineered system, such as Treemediation®, rooting activity in the contaminated area has been realized in one to two years.


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Fig. 1. Zinc Concentration in Added Solution and Leachate

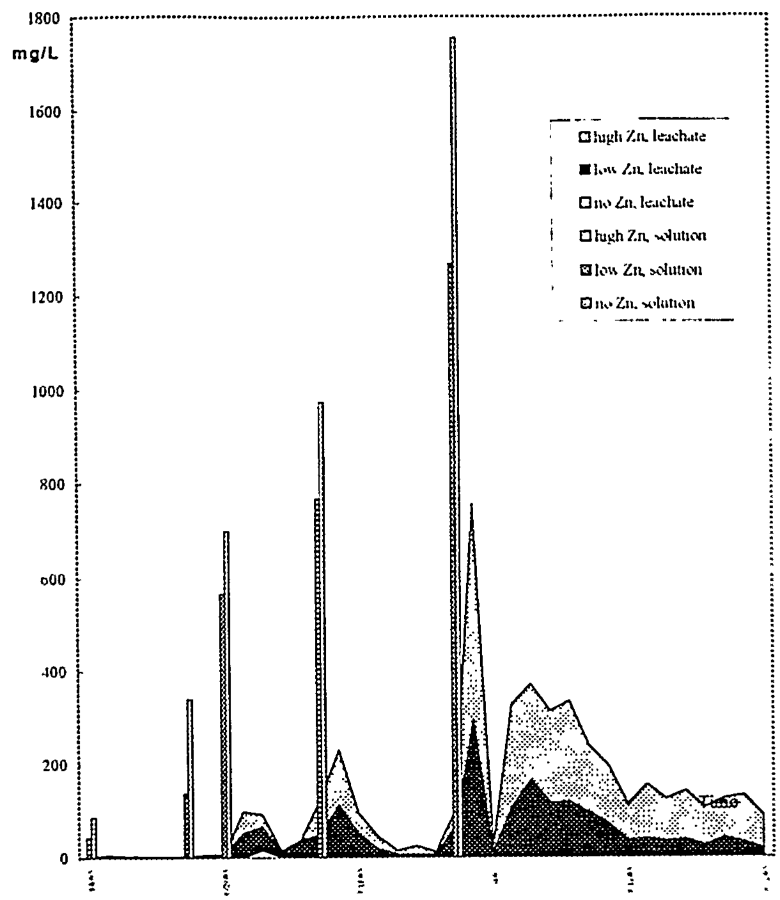


Fig. 2. Zinc Concentrations in Plant Tissue, Mean Values