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, and radioactive compounds in soil or water. 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 µg/g [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 lysimeter 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 µg/g (ppm) Zn in the dry root tissue. Above-ground organs contained less metal. A similar experiment evaluating zinc uptake in Eastern gamagrass (*Tripsacum dactyloides*), a large, robust native grass was conducted in 1996. This study found similar patterns of partitioning and sequestration as the poplar experiments, but growth and transpiration were more suppressed at the highest levels of accumulation in gamagrass. These levels of sequestered zinc observed in both hybrid poplar and Eastern gamagrass 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. Currently ongoing studies include heavy metal uptake and fate from soil by willow trees as influenced by natural and chemical chelating agents, rooting patterns in hybrid poplar, and the uptake and fate of halogenated organics in hybrid poplar. Other research includes the development and 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.

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 (Ross 1994, Salt et al. 1995). For soils contaminated with toxic organics, the approach is similar, but the plant may take up or assist in the degradation of the organic (Schnoor et al. 1995). 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 (Hinchman and Negri, 1994a).

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 current project objectives are:

- To identify the plant species best adapted for the uptake, sequestration, or degradation of specific contaminants and evaluate these species in controlled greenhouse and field experiments.
- To elucidate the physical, chemical, physiological and metabolic mechanisms of contaminant uptake, translocation, sequestration/detoxification, partitioning and bioaccumulation in phytoremediation plants. This research is focusing on heavy metal, radionuclide, and organic contaminants that are problems at DOE and other sites.
- To demonstrate that plant-based cleanup systems for heavy metals, radionuclides and organics are low-cost, low tech, environmentally friendly, and will operate economically at actual contaminated sites.

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 12m [40 feet]) soil horizons in a trademarked process called *TreeMediation*® (Nyer and Gatliff, 1996).

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 dehalogenases, and laccases. 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 (19,000 liters or 5,000 gal), and one hectare of a herbaceous plant like saltwater cordgrass evapotranspires up to 80 m^3 of water (80,000 liters or 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. It is well known that hyperaccumulator plants can accumulate metals in large amounts. Demonstrating that large plants that are not currently classified as hyperaccumulators can compensate for somewhat lower accumulation factors in above-ground organs with greater biomass production and high transpiration rates is critical in proving the viability of field expolitability of phytoremediation. This research is also increasing the number of potential phytoremediation plants by expanding the type, range, and adaptability of species considered to be hyperaccumulators.

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.

The evaluation of plant potential for metal accumulation by several selected plant species is being investigated by growing plants in an inert substrate (quartz sand) in lysimeter pots. The inert substrate is used to eliminate any possible interferences with soil components (e.g., clay micelles, organic matter) that might affect the contaminant availability to plants. Replicated treatments in a random scheme are used. Plants are irrigated with nutrient solution, and contaminants are supplied either at low but constant doses in the nutrient, or at higher doses, at specific application times. Leachate is drained from the pots each time nutrient is added. This is done at regular intervals, frequent enough so that measurable leachate volumes are always present. Volume, pH, and conductivity are measured each time leachate is drained, and a sample is taken for metal analysis. Measuring leachate volume permits us to determine evapotranspiration rates throughout the experiments. Plant leaves and branches are collected at regular intervals for metal analysis, and at the end of the experiments (about 4 months each), the pots are disassembled, and samples are collected of the sand substrate, leachate, roots, and above-ground biomass.

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 via food chains by wildlife, domestic animals, or birds.

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 (Hinchman and Negri, 1994b). A field demonstration of a plant bioreactor for processing produced water is under way at a natural gas well site in Oklahoma. This system uses salt-tolerant marsh species that simultaneously reduce the wastewater volume through high rates of evapotranspiration, and remove contaminants through uptake, degradation, metabolism and/or complexation. 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 of 75% in less than eight days (Negri and Hinchman, 1996). 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. Our current research is expanding the plant hyperaccumulator concept to include large, robust species with high evapotranspiration rates. The plants investigated to date in our greenhouse experiments process more soil water (in which contaminants are dissolved) and sequester in their tissues as much, or more, heavy metal than the "traditional" hyperaccumulators, many of which are small, shallow-rooted plants in the Brassicaceae (mustard) family.
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.

Zinc Uptake in Hybrid Poplar

Recent 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 (Negri, Hinchman, and Gatliff, 1996). These experiments (Fig. 1), 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 4.6m (15 ft) 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. The transpiration rate of potential phytoremediation plants is considered to be a critical factor, because the transpiration rate determines the rate at which contaminated soil solution is drawn into the plant to be processed.

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 µg/g (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 µg/g (ppm) Zn, respectively, while a control group (0 µg/g [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 many times the available levels in "normal" soils with the same amount of zinc, 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 µg/g (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 (Fig. 2). At levels of zinc above 1,000 µg/g (ppm) in nutrient added to the pots, leachate levels were always below 100 µg/g (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 µg/g (ppm) for the 2,000 µg/g (ppm) zinc addition, and then decreased sharply the second day after the zinc addition, to concentrations less than 100 µg/g (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 (Fig. 2).

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.

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 µg/g (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 (ppm) Zn in the dry leaf tissue, or 900 µg/g (ppm) Zn in the woody branches, on a dry-weight basis.

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 µg/g (ppm) Zn, the evapotranspiration rate decreased significantly as zinc concentration in the roots increased (Fig. 3); this physiological effect could have complex causes.

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 µg/g [ppm]), the D roots contained a mean concentration of 38,055 µg/g (ppm) 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 (ppm) Zn, respectively. The pots receiving the lower zinc doses (maximum of 1,500 µg/g [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 (ppm) Zn. The control pots also showed the same zinc uptake pattern among root types, the mean for the D roots being 232 µg/g (ppm) Zn. The small amount of zinc in the controls is from the zinc present in the nutrient solution as an essential nutrient. Figure 4 summarizes biomass zinc content.

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, approximately 18 systems are in place in the field, with additional sites being established during 1997. Soil and groundwater as deep as 9m (30 ft) 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.

Zinc Uptake in Eastern Gamagrass

In a recent experiment, Eastern gamagrass transplants were grown in inert quartz sand in lysimeter pots (Fig. 5). In April 1996, when the gamagrass was growing well and had developed a normal root system, a series of treatments was started in which three groups of plants, each group consisting of five replicates, were continuously given zinc ion in nutrient solution at concentrations of 160 µg/g Zn, 600 µg/g Zn, and 0 µg/g Zn (control), respectively, over a period of about two months. Detailed data were collected on zinc uptake, translocation, and partitioning in the gamagrass roots and shoots, as well as on evapotranspiration rates, nutrient use, and biomass increase of the rapidly growing plants. Methods were essentially identical to those described for the hybrid poplar experiment.

Leachate analyses for zinc by atomic absorption spectrophotometry indicate that initially plants subjected to both levels of zinc were removing up to 70% of the zinc from the leachate. After two months, the plants receiving 160 µg/g Zn had grown considerably and were almost the same size as the controls (no zinc), but some of the mature leaf blades were rolled; the mean zinc removal rate for these plants was 50% of the zinc in the leachate. The plants receiving 600 µg/g Zn were smaller than the controls after two months, their color was a darker green, most of the mature leaf blades were rolled, and the mean zinc removal rate was about 30% of the zinc in the soil solution (leachate).

In mid-June 1996 three replicates of each Eastern gamagrass treatment were harvested. 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. The plants were divided into tops (leaves and crowns) and roots. The root systems of all the plants had the typical structure of large primary roots and smaller, lateral,

Figure 1. Hybrid Poplar (*Populus* sp.) Cuttings Growing in Lysimeter Pots with Connecting Tubes and Leachate Collection Bottles Below

Figure 2

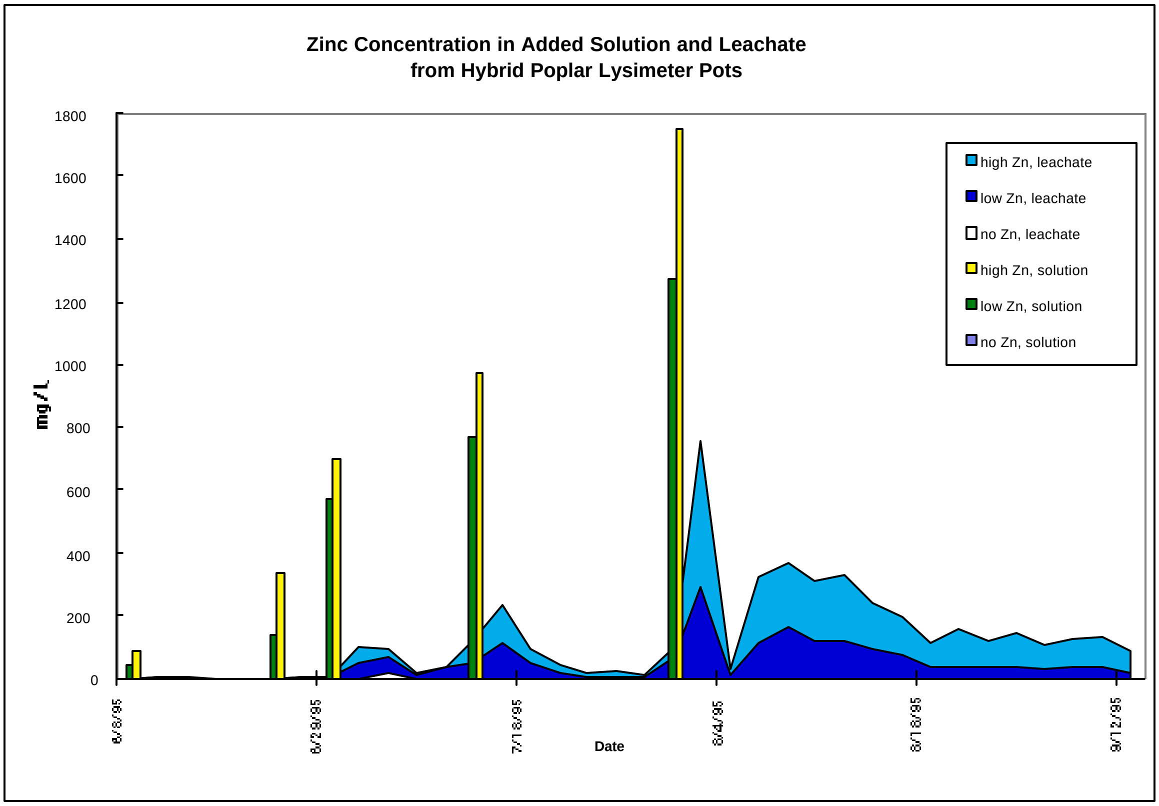


Figure 3

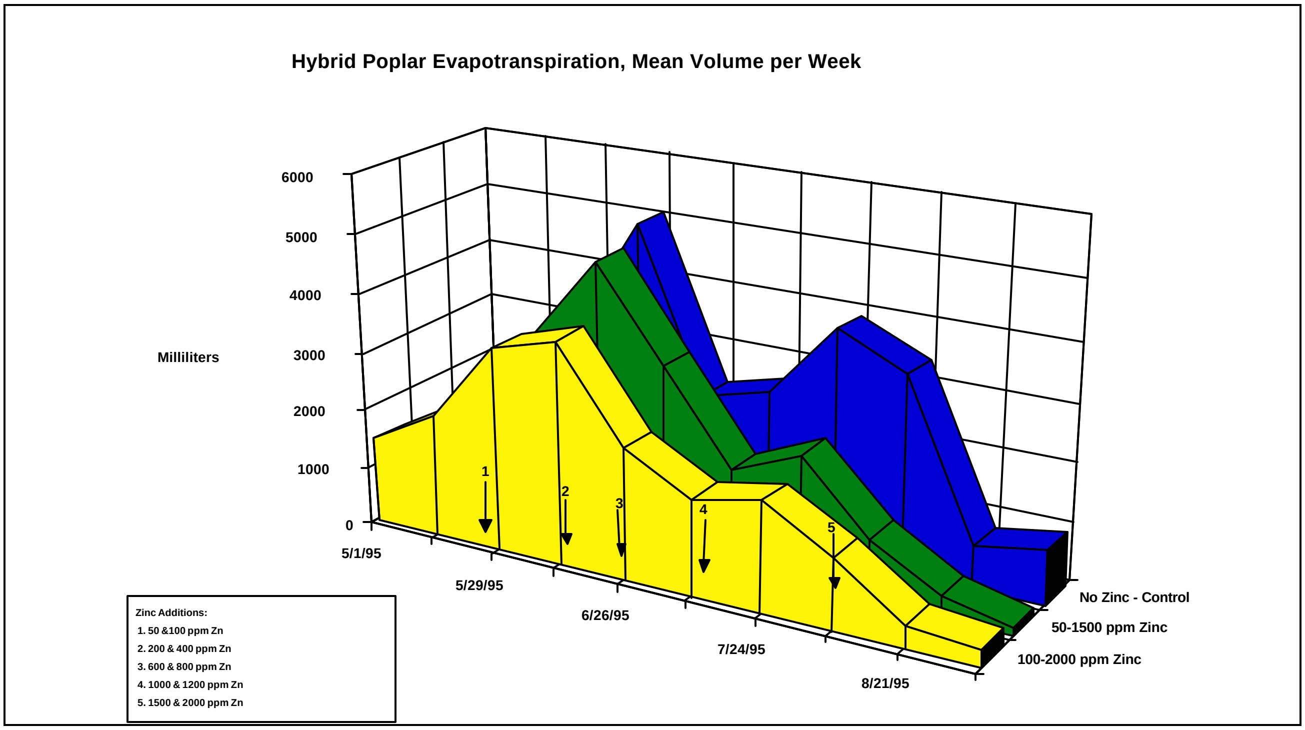
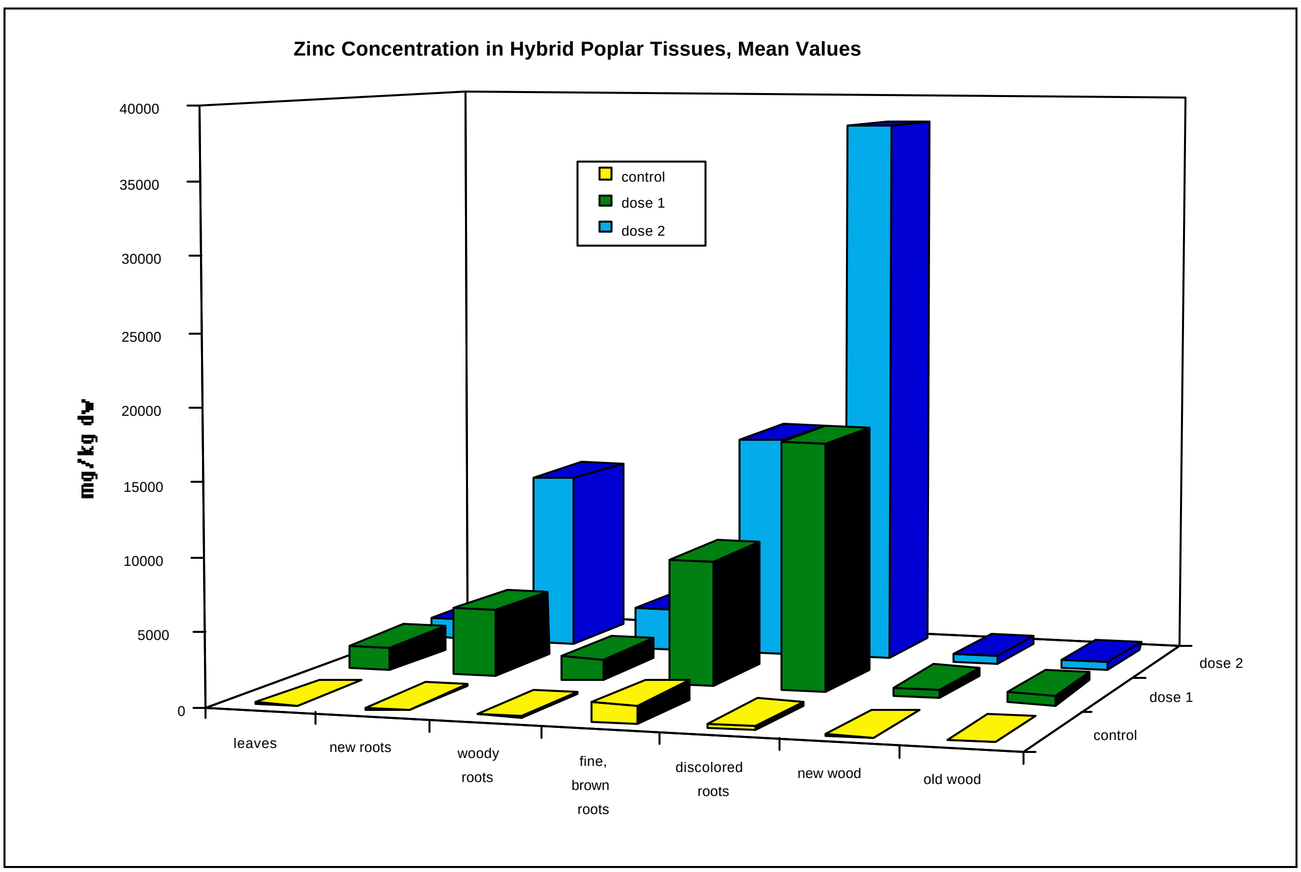


Figure 4



secondary roots. In the controls, most of the roots were light colored, with the newest roots being white. However, in the plants receiving the 160 µg/g Zn treatment, although the root systems were almost as large as the controls, some of the secondary roots were dark or black, while in the 600 µg/g Zn treatment the root systems were small with many black roots. All tissue samples were dried at 80 C and analyzed for zinc by digestion, extraction, and atomic absorption spectrophotometry.

As was observed in the hybrid poplar zinc uptake experiment, the Eastern gamagrass root tissues harvested in June 1996 showed much higher concentrations of accumulated and sequestered metal than did the aboveground parts (Fig. 6). In the pots receiving the highest level of zinc in the nutrient solution (600 µg/g Zn), the roots contained a mean concentration of about 10,000 µg/g Zn in the dry root tissue, while the tops contained about 1000 µg/g Zn. In the plants receiving 160 µg/g Zn, the roots contained about 4000 µg/g Zn, and the tops about 400 µg/g Zn. The roots of the control plants contained about 75 µg/g Zn, and the tops about 50 µg/g Zn. The small amount of zinc in the controls is from the zinc present in the nutrient solution as an essential nutrient.

The two remaining replicates in each treatment were allowed to continue growing under the same treatment regime after the tops were pruned back to a length of 10 cm above the substrate surface, to observe regrowth characteristics and any changes in zinc uptake.

The levels of sequestered zinc observed in both the gamagrass and 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. In hybrid poplar the evapotranspiration rate decreased significantly as zinc concentration in the roots increased; while in gamagrass both top growth and evapotranspiration rate decreased at the higher zinc level (600 µg/g Zn) as a constant dose.

In any case, a reduction of zinc from the soil solution of between 90-50% (Eastern gamagrass), and 99+% (hybrid poplar) when the concentration in the soil solution is several hundred ppm has major implications for the development of effective plant-based cleanup systems for contaminated soils, groundwater, and wastewater that is both low-tech and low-cost. These data demonstrate a very effective uptake, bioaccumulation, and sequestration system for zinc in both hybrid poplar and Eastern gamagrass, as well as high evapotranspiration rates and a wide range of adaptability for both of these versatile plants.

Eastern gamagrass is ideally suited to plant-based, hydroponic wastewater treatment systems consisting of above-ground open top tanks in which the plants are densely grown in a bed of pea gravel, through which the wastewater is trickled for purification. Gamagrass can attain a height of over 2 meters, and develops an extensive, fibrous root system when grown hydroponically. This very large root biomass provides numerous sites for contaminant sequestration and/or degradation. Because both tops and roots of gamagrass are uniform in development, the harvest of either of these organs will be relatively easy.

Bioremediation of Contaminated Soils by Enhanced Plant Accumulation: Chelation-Assisted Metal Accumulation in Plants

Key factors that control the rate at which the contaminants are removed from the soil by plant-based cleanup systems are the ability of the plant to accumulate, without suffering toxic effects, large concentrations of contaminant, and the rate and extent at which the contaminants are made available for plant uptake. Phytoremediation is the result of matching the growth of selected, adapted plants with the gradual but complete release of the contaminants from the soil's binding sites into the soil solution in plant-available forms.

Heavy metals are bound to soil components in varying degrees, depending on soil conditions such as pH, clay content, organic matter, redox potential. Plant-available metals include species that are readily soluble or exchangeable, while metals that are more strongly adsorbed/bound to organic matter or co-precipitated with oxides are generally not available for plant uptake. One way to increase metals availability is to selectively “leach” them into the soil solution using chelating agents.

Chelating agents increase metals diffusion in the soil solution and keep them in plant available forms by forming large, less reactive ions, by increasing the concentration of these larger chelated ions in solution, and by decreasing the ability of the free ions to react with the soil. Natural chelating agents (organic acids such as citric and acetic) released by plant roots make the ions of both nutrients and contaminants more mobile in the soil. Plants can usually break the chelation bond, take up the metal, and release the chelant back in the soil solution.

Current greenhouse experiments aim at investigating the feasibility of utilizing various chelating agents to induce the release of heavy metals from soil binding sites and increase the rate and extent of metal accumulation in plant tissues. These experiments are investigating the changes in concentration factors of several heavy metals (Zn, Cd, Pb, Cu) and radionuclides (U and Th) from soil/sediment from the Miami-Erie Canal, near Miamisburg, Ohio. This soil, although considered "clean", has concentrations of these contaminants sufficient to provide information on the

Figure 5. Zinc Uptake in Eastern gamagrass (*Tripsacum dactyloides*) Growing in Lysimeter Pots, Experimental Setup

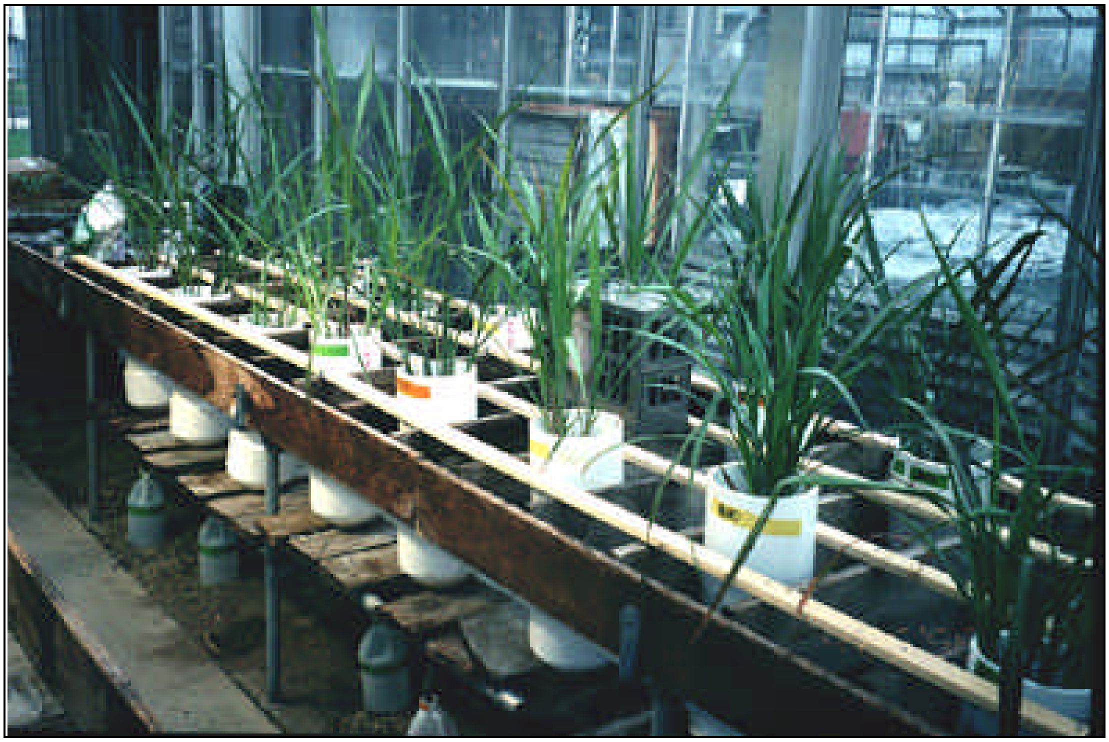
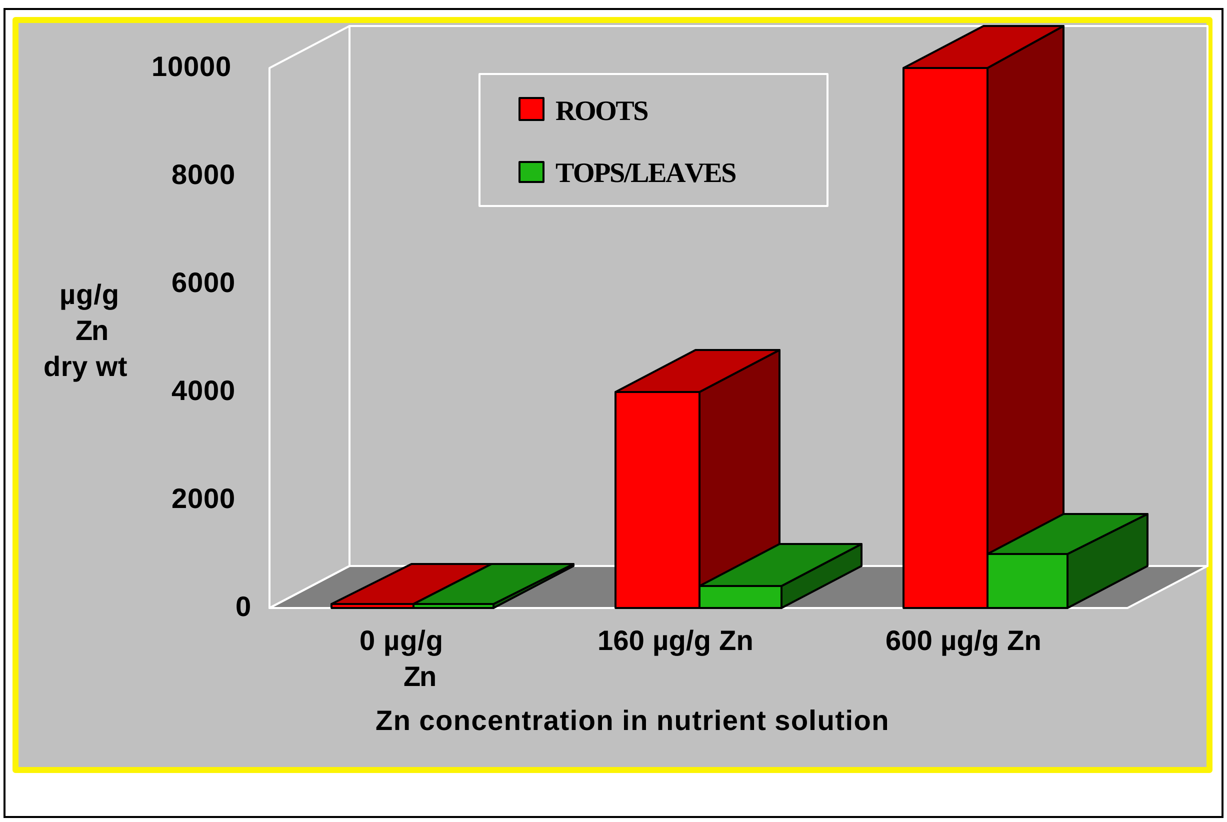


Figure 6. Zinc Concentrations in Eastern gamagrass (*Tripsacum dactyloides*) Tissues, Mean Values



relative changes in plant accumulation induced by the addition of chelating agents. Willow plants growing in this soil in lysimeter pots are periodically irrigated with constant doses of dilute chelating agents in aqueous solution. At predefined time intervals, samples of soil and plant tissues are taken and analyzed for metals and radionuclides. The soil samples are analyzed via sequential extraction techniques, which provide the empirical information on the probable species in which the contaminants are present, and the relative percentage of the total content. Plant concentration is considered as an additional sequential extraction step, which represents the sum of metal species that are effectively taken up by the plant.

The Uptake of Lead and Arsenic by Phreatophytic Trees

In this recent greenhouse experiment we evaluated the uptake, translocation, partitioning, and sequestration of lead and arsenic in the organs of hybrid willow (*Salix* sp.) and hybrid poplar (*Populus* sp.) trees grown in contaminated soil from a field site. Another group of both trees was grown in quartz sand and watered with solutions containing soluble compounds of lead and arsenic. The contaminated soil contained about 1000 µg/g lead and up to 200 µg/g arsenic on a total basis, but the plant-available concentrations in the soil water were below 2 µg/mL for both metals. The goal of this project, conducted in collaboration with our CRADA partner, Applied Natural Sciences, Inc., was to determine the role of deep rooting phreatophytic trees in the phytoremediation of sites contaminated with heavy metals, and to develop optimal tree-based cleanup methodologies to be tested in the field.

Rooted cuttings of hybrid willow and hybrid poplar were grown in replicated sets of lysimeter pots containing either contaminated soil or quartz sand for a period of four weeks under greenhouse conditions. The pots containing contaminated soil were watered with deionized water only, while separate sets of sand pots were watered with solutions of lead nitrate and sodium arsenite, respectively. The concentrations of the metals in the watering solutions used on the sand pots were 10 and 100 µg/mL for both lead and arsenic. At these concentrations, arsenic was more toxic than lead to both species. No toxicity symptoms were observed in either tree species for both concentrations of lead.

Both willow and poplar showed uptake and partitioning of both lead and arsenic in the sand media and in the contaminated soil. In the one-month test period, the willows were able to remove approximately 9.5 percent of the available lead and about one percent of the total arsenic from the contaminated soil. In the same time period, the less mature poplars removed about one percent of the available lead and 0.1 percent of the total arsenic from the same soil. in the sand experiment, the willows took up about 40 percent of the administered lead and 30 to 40 percent of the administered arsenic in the one-month test period. Considerably more of both lead and arsenic was sequestered in the roots of both plants than in the above ground parts (branches and leaves).

Evaluation of Rooting Patterns and Biomass Production of Hybrid Poplar as a Function of Planting Method of Cuttings.

This experiment is evaluating the rate and success of root generation and aboveground biomass production in hybrid poplar cuttings planted in quartz sand in both vertical ("normal") and horizontal patterns at different depths. This experiment is aimed at developing planting techniques that may enhance or facilitate root recovery during whole-plant harvesting.

Data generated so far confirm that hybrid poplar can root extensively and produce multiple vertical shoots from horizontally planted stem cuttings. The roots produced by horizontal cuttings are formed all along the length of the buried cutting and explore the shallow soil horizons more completely than roots of vertically-planted cuttings. Thus, hybrid poplar rooting patterns can be relatively easily molded to match the existing contamination pattern and the needs of root harvesting machines.

Evaluation of TCE and PCE Uptake and Fate in Plants.

For many organic contaminants such as trichloroethylene (TCE) and tetrachloroethylene (PCE),, there is evidence that plants can degrade a portion of the organohalide that is taken up to form less volatile compounds such as trichloroacetic acid (TCAA) which are sequestered in the plant tissue, while passing the remainder out of the leaf tissue with the transpiration stream.

In hybrid poplar and several other species there is evidence for an enzyme that breaks down TCE and PCE to TCAA in the plant. We are developing new gas chromatographic analytical methods for the detection of TCAA and TCEh in woody plant tissue that is rapid and easy. A number of samples of plant tissue have been analyzed using this method, and studies are ongoing to correlate the presence and concentration of TCAA in plants and its contamination "history" in the underlying groundwater or soil.

These methods have the potential for tracking the plant-based cleanup of groundwater and soil contaminated with organohalides, as well as providing a very quick and easy method of monitoring a site for organohalide contamination using plant tissue samples from the site rather than obtaining soil and groundwater samples by coring or drilling operations.

Future Plans

Future plans include the following research and application studies on plant-based cleanup systems:

- Field demonstration of heavy metal removal by selected tree species, including root harvesting, at our CRADA partner's sites.
- Conduct high resolution microanalyses of hyperaccumulator species (scanning or transmission electron microscopy with x-ray energy dispersive spectroscopy) to determine the discrete sites of metal sequestration and bioaccumulation in specific plant organs, tissues, cells, and organelles.
- Evaluate procedures for the disposal, processing, and volume reduction of metal-contaminated plant biomass.
- Conduct studies of root and other plant biomass decomposition in soils to understand kinetics and cycling of contaminants.
- Evaluate the removal and sequestration of radionuclides from soils using plant-based cleanup systems.
- Continue the evaluation of demonstration units of an ANL-developed plant bioreactor for wastewater (produced water) treatment at natural gas well sites in Oklahoma.
- Extend investigations of the most promising lines of current research on plant-based cleanup systems:
  - phytoremediation using trees and large, robust, herbaceous plants.
  - degradation of halogenated organics.
  - bioreactors for wastewater cleanup and volume reduction.

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