USING PHYTOREMEDIATION TO CLEAN UP CONTAMINATION AT MILITARY INSTALLATIONS

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ABSTRACT

During and following World War II, wastes from the production of munitions and other military materials were disposed of using the best available practices acceptable at that time. However, these disposal methods often contaminated soil and groundwater with organic compounds and metals that require cleanup under current regulations. An emerging technology for cleaning contaminated soils and shallow groundwater is phytoremediation, an environmentally friendly, low-cost, and low-tech process. Phytoremediation encompasses all plant-influenced biological, chemical, and physical processes that aid in the uptake, degradation, and metabolism of contaminants by either plants or free-living organisms in the plant's rhizosphere. A phytoremediation system can be viewed as a biological, solar-driven, pump-and-treat system with an extensive, self-extending uptake network (the root system) that enhances the soil and below-ground ecosystem for subsequent productive use. Argonne National Laboratory (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" from petroleum wells. Results of these studies were used to design a bioreactor for processing produced water that is being demonstrated at a natural gas well in Oklahoma; this system can reduce produced water volume by about 75% in less than eight days, representing substantial savings in waste disposal cost. During 1994, ANL conducted a TNT plant uptake and *in situ* remediation study in a ridge-and-furrow area used for the disposal of pink water at the Joliet Army Ammunition Plant. Replicated plots with three levels of TNT-contaminated soil were treated with three levels of chopped hay incorporated into the surface soil and planted with two common crops to enhance *in situ* remediation. Analytical results showed no TNT uptake by crops, and TNT concentrations in the soil of all treatments were substantially reduced, indicating these low-cost treatments can be used to clean up TNT-contaminated surface soil. During 1995, greenhouse studies were initiated on zinc uptake by plants. These experiments were conducted to confirm field data from Applied Natural Sciences, Inc., indicating high levels of zinc in leaves of hybrid poplar trees growing on a zinc-contaminated site. Analytical data from the greenhouse studies show zinc was totally sequestered by the plants with concentrations of >38,000 µg/g (3.8%) zinc in the dry root tissue. Results from recent greenhouse studies show that the pattern of zinc uptake by Eastern gamagrass is similar to that of hybrid poplars. Additional studies are now being conducted to document the uptake and sequestration of other metals by plants. Because the roots sequester most of the metal taken up in many plants, the feasibility of root harvesting is being

investigated as a method to maximize metal removal from soils. Currently, studies also are being conducted to develop analytical methods for determining concentrations of these compounds and their degradation products in plant materials and to document the uptake and detoxification of organohalogens by plants. Phytoremediation systems preserve and often improve soil productivity, minimize environmental disruption, produce few or no wastes, use a natural energy source, require minimal maintenance, and cost about one-tenth as much as traditional cleanup methods.

1. INTRODUCTION

Wastes from the production and use of munitions and other military materials in the past were disposed of using the best available and acceptable practices for the time. Wastes were frequently discharged into lagoons or land application areas and solid waste was commonly burned. These disposal practices often resulted in an accumulation of organics and/or metals in the soil and shallow groundwater of the disposal site. Because of current concerns for the environment and human health, many of these sites are classified as hazardous waste sites that now require cleanup. The cleanup of organic and metal contaminants by means of conventional methods (thermal treatment, stabilization, soil washing) is expensive and often destroys or alters the soil, limiting the future use of the site.

Phytoremediation is an emerging cleanup technology. It is defined as the engineered use of selected green plant species to degrade, accumulate, or remove such contaminants as metals, organics, and radionuclides from soil, water, or waste streams. This definition includes all the biological, chemical, and physical processes caused by the plant, by its enzymes, or by free-living organisms in the rhizosphere. Phytoremediation takes advantage of the unique and selective uptake capabilities of plant root systems, together with the metabolism, accumulation, and translocation ability of the entire plant. Plant-based remediation systems can be viewed as biological, solar-driven, pump-and-treat systems with an extensive and self-extending uptake network, the root system.

Phytoremediation has a number of advantages when compared with conventional clean-up technologies, such as incineration or soil washing. Phytoremediation is environmentally friendly because the soil is not damaged or destroyed and secondary wastes are small in volume or can be recycled. Installation and maintenance costs of a phytoremediation system are usually a fraction of the cost of most conventional clean-up methods because it is an *in situ* process that does not require soil excavation or transport. Phytoremediation can be used for a wide range of contaminants, including organics and metals and can often be used to treat several contaminants at the same time. It is a low-tech technology that requires little or no hardware. Phytoremediation is socially acceptable because the public does not object to plants growing in its backyards.

Phytoremediation systems have been used for a number of years. An example of a simple phytoremediation system is a constructed wetland with cattails to increase pH and remove iron from acid mine drainage or remove nitrates from livestock waste lagoons and municipal sewage water[1]. Research at ANL is designed to extend phytoremediation to more complex situations. For example, phytoremediation of a site contaminated with metals and/or radionuclides involves "farming" with selected plant species or crops to "biomine" the soil and concentrate contaminants in the plant biomass[2,3]. The volume of the contaminant(s), sequestered in plant biomass, is reduced for easier disposal. For soil contaminated with organics, the approach is similar, but the plant may metabolize or assist in the degradation of the organic[4]. The potential exists for degrading hazardous organics mixed with radionuclides, thus reducing the waste to a more manageable radioactive one. Sequential cropping with phytoremediation species can reduce contaminant levels to concentrations that are environmentally acceptable, so that the site would no longer considered hazardous. The objectives of

the phytoremediation research program at ANL are to (i) identify plant species best adapted for the metabolism and accumulation of metals and organic contaminants, (ii) evaluate the most promising species under controlled greenhouse conditions, (iii) investigate the use of various soil amendments to enhance phytoremediation, and (iv) demonstrate low-cost, low-tech, environmentally friendly phytoremediation systems under field conditions.

2. BIOTREATMENT OF PRODUCED WATERS

Initial research in the area of phytoremediation at ANL began in 1990 with the investigation of the biotreatment of produced waters. Water that is brought to the surface from wells during oil and natural gas production is referred to as "produced water" and includes the water stripped from the oil or gas during cleanup before transport by pipeline. Produced water originates from the same geologic formation as the gas, and its most common characteristic is the elevated concentration of dissolved salt, often greater than that of sea water. Other constituents can include bicarbonates, carbonates, sulfates, hydrocarbons and other organics, and metals. Produced waters are a waste product; their constituents are considered contaminants and are subject to EPA regulations[5]. The current accepted method of disposal is by injection into deep wells, with disposal costs based on volume.

The goal of the this investigation is to develop and evaluate a biological approach to reduce the volume of produced water by using a cost-effective engineered ecosystem, called a contained plant bioreactor system. This plant bioreactor would use natural saltmarsh ecosystems as a model and incorporate salt-tolerant wetland plant species with innovative hydroponic plant growth techniques. Specific objectives to accomplish this goal include (i) identification of candidate halophyte species having attributes appropriate for use in a plant bioreactor system, (ii) conducting greenhouse evaluations to determine salt tolerance and evapotranspiration rates of the most promising species, (iii) use of the data generated in the greenhouse evaluation to develop a plant bioreactor model, and (iv) demonstration of the plant bioreactor under typical field conditions.

To develop biological treatment systems for produced waters, it was logical to look at the halophytes that have already developed a natural ability to grow and be highly productive at high external salinity levels. The plants that occupy the highly saline habitats along sea coasts, in salt and brackish marshes, in estuaries, and in dry, saline interior habitats have already undergone strong selection for their ability to tolerate salt. This large pool of plants provided candidates for evaluation and potential use in an engineered, plant-based bioreactor designed to reduce the volume of produced waters.

Because of the large number of halophytes that potentially could be evaluated, additional selection criteria were used to reduce the number of candidate halophyte species to be evaluated. Besides being tolerant to saline waters, the species should be able to tolerate metals, organics, and low fertility. Species should be easily propagated or available at low cost from commercial sources. Perennials were preferred over annuals for quick startup, a generally longer growing season, and reduced re-establishment time and cost. Because a high transpiration rate was a major consideration, fast-growing plants with extensive root systems were needed. A data base containing the attributes over 65 candidate species was assembled and the five species, one with two varieties, with the highest rating were selected for greenhouse evaluation. The plants tested were saltwater cordgrass (*Spartina alterniflora)*, dropseed (*Sporobolus virginicus*), perennial glasswort (*Salicornia virginica*), sawgrass (*Cladium jamaicense*), Vermillion saltwater cordgrass (*Spartina alterniflora* var. Vermillion), and great bulrush (*Scirpus validus*).

After a period of one to several weeks of acclimatization and growth in the nursery trays, a set of plants was selected for a greenhouse experiment to determine salt tolerance and evapotranspiration rates. Candidate plant species were tested at three salt concentrations (0%, 1.5% and 3%) in

modified Hoagland's solution[6]. Each treatment was replicated three or four times, with replicates consisting of several plants growing in a plastic bucket with the root system submerged in the appropriate salt solution. The plants in each bucket were supported by a styrofoam float, with each plant placed in a hole in the float that was just large enough to support the plant at the transition between stem and roots. The float fit snugly inside the bucket and maintained root immersion in the nutrient solution regardless of the depth. The styrofoam float also inhibited direct evaporation of water from the surface of the solution in the buckets. Thus, most water loss from the buckets with plants was from plant transpiration. In the experiment with bulrush plants, styrofoam pellets floating on the surface of the nutrient solution were used instead of the floats and had the same effect. The stems of the bulrush are rather soft and subject to bending and collapse if manipulated to any extent, as when threading the stems through the holes in the float. Another set of replicated buckets containing test solutions, but without floats or plants, was used to measure direct evaporation from the open liquid surface.

Evapotranspiration is the total amount of water evaporated from an area of plants and substrate. In the greenhouse experiments, direct evaporation from the test solution surface in the buckets containing plants was minimized by the styrofoam floats or pellets, and the principal route of water loss was by plant transpiration. Direct evaporation from the buckets was determined from the open water buckets with no plants. The water depth was measured in each bucket, using a float gauge. The observed depth, subtracted from the previous depth measurement and converted to a volume, represented the amount of water evapotranspired or evaporated during the time interval between measurements. Observed volume changes were mathematically adjusted to standardize all volumes to 24-hour intervals.

Salt concentrations in the buckets were determined by measuring the electrical conductivity of the solution at 24- or 48-hour intervals. The conductivity meter was calibrated against a set of NaCl solutions in nutrient of known concentration. Conductivity readings were converted to a value for salt concentration. When a bucket reached a predetermined minimum solution depth, nutrient solution at the same salt concentration as the starting concentration was added to the bucket to bring it to the original volume. The new salt concentration was higher than the original because all the salt added to the bucket remained and only water was lost, but the concentration was not as high as before the addition. Thus, the experimental plants experienced a fluctuating, but generally increasing, salt concentration over the course of the experiment. Such fluctuations are similar to those found in natural systems caused by tidal fluctuations. This procedure was used to measure evapotranspiration over an essentially continuous range of salt concentrations, up to the salt tolerance limit of the species. As the salt tolerance limit was approached, the plants usually went through stages characterized by a slowing or cessation of growth, severe wilting, drying up of the leaves, and eventually death of the plant.

Data from the greenhouse studies were used to design a plant bioreactor model and select the species used in the bioreactor. Because volume reduction of produced water was the primary objective, it was decided that a batch system would be the most effective. The bioreactor also would be designed in modular units so it could be adapted to the different volumes of produced water from individual wells or groups of gas wells. The bioreactor consisted of two compartments or tanks filled with an inert material to support the plants, with the second compartment about one-half the size of the first compartment. Bulrush was selected as the species for the first compartment and the more salt-tolerant saltwater cordgrass for the second compartment. Pipes connect the compartments and valves are installed at the inlet and outlet and between the compartments. A final or second storage tank is required at the well head for storage of the concentrated produced water for disposal.

The bioreactor is operated using the following procedure. The bioreactor (with all valves closed) is filled with produced water from the well head storage tank with low levels of nutrients added. When

the level of produced water in the compartments reaches about one-half the original volume, the valve on the outlet of the second compartment is opened, the concentrated produced water is drained into the second or final storage tank, and the outlet valve is closed. The valve between the compartments is opened, produced water from the first compartment is drained into the second compartment, and the valve is closed. The last step in the operation is to refill the first compartment with produced water from the well head storage and add nutrients. On the bases of data generated by the greenhouse studies, the total time required for one cycle of the bioreactor would be about 7.5 days, and the volume of produced water would be reduced by about 75%.

In 1995, a full-scale field demonstration of the plant bioreactor was installed at an active gas well in Beaver County, Oklahoma. The plant bioreactor installed is a low-cost, low-tech system. Cattle watering troughs, used as the compartments, are filled with pea gravel to support the bulrush and saltwater cordgrass. The piping, valves, and second storage tank are plastic. The system is gravity-operated and no external power is required. Produced water levels in the cattle troughs are checked daily when the well operation is checked. The only maintenance cost is the small amount of soluble fertilizer added to the produced water to maintain the plants. During operation in the summer, the bioreactor reduces the volume of produced water to about one quarter the original volume in about eight days.

The field demonstration of the plant bioreactor shows that a low-cost, low-tech system can be used to reduce the volume of produced water. The 75% reduction in volume in about eight days results in a similar reduction in produced water disposal cost. The major disadvantage of the system is the reduction in efficiency during cold weather and plant dormancy in the winter. The well operator has constructed a plastic shelter over the unit to keep it in operation during cold weather. For a more detailed description of the development and demonstration of the plant bioreactor, see Negri and Hinchman[7].

3. FATE OF TNT IN CROPS AND SOILS

In the past, wastes from the production of 2,4,6-trinitrotoluene (TNT) were disposed of by using the best available and acceptable practices for the time. Waste streams were frequently discharged into lagoons or land application areas and solid waste was often burned, resulting in an accumulation of TNT residues in the soil. A major environmental and human health concern is that TNT, its by-products, and degradation products could enter animal and human food chains through plant uptake by crops growing on explosives-contaminated soils.

Published information on the uptake of TNT by plants is limited to results from hydroponic and greenhouse investigations. Palazzo and Leggett[8] reported uptake of TNT by yellow nutsedge (*Cyperus esculentus* L.) from hydroponic solutions and also reported that TNT and its metabolites, 4-amino-2,6-dinitrotoluene (4A-DNT) and 2-amino-4,6-dinitrotoluene (2A-DNT) were found throughout the plants. Folsom et al.[9] and Pennington[10] also studied TNT uptake by yellow nutsedge from soil amended with TNT, and they reported minimal uptake of TNT by plants. Banwart and Hassett[11] studied the effect of organic residue additions on plant tolerance and extractable TNT from TNT-contaminated soil. Initial TNT levels in the soil were 1,000 and 2,000 mg kg^{-1}, and species tested were perennial ryegrass (*Lolium multiflorum* Lam.), sorghum x sudangrass (*Sorghum bicolor* x *S. sudanense* (L.) Moench.), and alfalfa (*Medicago sativa* L.). Little or no growth occurred on unamended soil, but growth and dry matter production were dramatically improved with an addition of 5% (by weight) ground wheat straw (*Triticum aestivum* L.). TNT in soil originally containing 2,000 mg kg^{-1} TNT amended with wheat straw was less than 2% of the initial value after 90 days of crop growth. Cataldo et al.[12] also studied the fate and degradation products of explosives in soils and three plant species: wheat, "Blando" brome (*Bromus mollis* L.), and bush beans. Plants were grown for 60 days in explosives-amended soil. They reported about 70% of the TNT underwent

transformation to A-DNT isomers and unknown chemical species. Explosive uptake by plants appeared to be dependent on soil properties and plant species. TNT uptake in its original form was low, with TNT residues primarily accumulated in plant roots. The majority of contaminants found in plants grown in TNT-amended soils was in the form of 2A- and 4A-DNT. Both of these compounds were also found primarily in root tissues. The highest TNT uptake was observed in plants grown in the soil with the lowest organic matter content. The nonextractable fraction of TNT increased more rapidly and to higher levels in soil with higher organic content compared with a soil with lower organic content. Results from these investigations indicate uptake of TNT and/or its degradation products by plants grown under greenhouse and hydroponic conditions, but data are not available on plants grown under field conditions. Objectives of this field investigation were to determine: (i) explosive uptake by crops grown on TNT-contaminated soils, (ii) the influence of organic material additions to TNT-contaminated soils on TNT uptake by crops, and (iii) the effect of organic material additions on levels of TNT in contaminated soils.

This investigation of the fate of TNT in crops and soils was conducted at government-owned, contractor-operated Joliet Army Ammunition Plant (JAAP). This facility was constructed in the early 1940s for the production of munitions during World War II. The site selected at JAAP for sampling the crop uptake experiment was Group 61. Group 61 facilities were originally constructed in 1941 for crystallizing ammonium nitrate, but were expensively modified in 1945 to reclaim TNT from high-explosive shells. Shell reclamation operations involved the removal and recovery of explosives and a shell-washout operation. Process water (pink water) from the washout operation was disposed of by evaporation and infiltration in a 1.6-ha ridge-and-furrow area (R&FA). All operations at Group 61 ceased in the mid-1950s; in the 40 years following the closure of the Group 61 facilities, erosion has partially leveled the ridges and filled the furrows. After 40 years, the presence of TNT in the furrow surface soil is still evident by a reddish color and lack of vegetation.

Design of the crop uptake experiment involved three levels of soil TNT contamination (high, intermediate, and zero), three levels of organic material additions (high, intermediate, and zero), two crops (forage and small grain), and four replications of each treatment (soil TNT level, organic material addition, crop). Crop establishment and growth were observed and roots, forage, straw, and grain analyzed to determine the influence of TNT level, organic material addition, and crop type on TNT uptake. Soil samples were periodically collected and analyzed to determine the influence of hay additions on concentration of TNT in the soil.

During the fall of 1993 information from a previous investigation[13] and results from soil analyses from samples collected in the R&FA were used to select locations for three 16 m by 24 m areas in the Group 61 at JAAP. High and intermediate TNT-contaminated areas were within the R&FA, while the zero TNT area was in an uncontaminated area next to the R&FA. Arrangements were made with a local contractor to perform soil mixing in the areas during October 1993, and mixing was done by using the following method. Along one edge of the area, parallel with the original furrows, a road grader was used to excavate a trench to a depth about 15 to 20 cm below the original furrow bottom. Soil from the trench was rolled along the length of the blade to mix the surface soil with soil from the lower depths. A second trench was excavated parallel and next to the original trench, with the soil rolled along the blade into the first trench. This operation was repeated across the width of the area. The area was then disked to further mix the soil and smooth the surface. Following disking, the soil was mixed again, using the road grader, but the operation started at the opposite edge of the area. The sequence of excavating with the grader and disking was repeated several times in an attempt to uniformly distribute TNT-contaminated soil throughout the upper 15 to 20 cm of the area soil. After each session of grading and disking, five soil samples were taken from random locations near each of the corners and the center of the area. Each sample was analyzed for TNT concentration, using the field method developed by Jenkins[14]. If the results of the five samples were not within 20% of each other, the area was graded and disked again.

Following the soil mixing operation, each area was divided into 4 m by 4 m plots. Level of organic material addition, crop, and replicate number for each plot for each TNT level area were randomly assigned. Application and incorporation of ground smooth bromegrass hay, purchased from a local farmer, was the next step in plot preparation. Ground hay at rates of 1 and 2% (46 and 92 kg per plot) was weighed for each plot, spread on the soil surface, and incorporated into the soil by using the tractor-mounted rototiller. On 28 October 1994, fertilizer was applied to all plots at a rate equivalent to 224 kg ha^{-1} nitrogen and 112 kg ha^{-1} P_2O_5. Following the application of fertilizer, all plots were rototilled and seeded. The small grain crop was soft red winter wheat (*Triticum aestivum* L.) seeded at 135 kg ha^{-1}, and the forage was common perennial ryegrass seeded at 13.5 kg ha^{-1}. Both wheat and ryegrass seed were hand-broadcast and the plots lightly raked after seeding to cover the seed. A composite soil sample was collected from each plot immediately following seeding. The late planting date and below-normal rainfall after seeding resulted in a complete failure of both the ryegrass and winter wheat on all plots, including the control plots. As a result, all plots were tilled using the tractor-mounted rototiller and replanted on 22 March 1994. Procedures were the same as those used during the October planting, with one exception: oat broadcast at 125 kg ha^{-1} was planted in place of the winter wheat. Rainfall received at the site remained well below normal through June, resulting in poor stand establishment of both oat and ryegrass on all plots, including control plots. On 7 June 1994, barren areas in all the plots were raked, reseeded with oat or ryegrass, and the seed covered by lightly raking.

Oat harvest and initial ryegrass samples were collected on 19 July 1994. Plants were clipped about 2.5 cm above the soil surface within the center square meter of each plot. Care was taken not to include plant material that had been in direct contact with the soil and to prevent contact of the plants sampled with the soil. Plant tops from each plot were bagged as a single sample, but oat grain was separated from the straw in the laboratory for separate analysis. Soil in the root zone was loosened, the root mass of the sampled plants extracted, excess soil removed, and roots bagged for transport to the analytical laboratory. A second sampling from the ryegrass plots was conducted on 21 September 1994, using the procedure described above. Soil and plant analyses were performed in a U.S. Army Environmental Center (USAEC) certified laboratory at the University of Illinois at Urbana, Illinois. U.S. EPA SW-846 method 8330 was used to determine concentrations of TNT and its derivatives in soil samples. A method developed by Banwart and Hassett[11] was used to analyze plant materials for TNT and its derivatives.

The crop uptake experiment at JAAP was compromised by a number of problems sometimes encountered in field studies. One problem was the heterogeneity of TNT in the soil within plots and areas. Regulations require that any contaminated soil that is picked up must be decontaminated before being put down. This presented a soil mixing problem, because the TNT was concentrated in the surface soil of the furrow bottoms and furrows were on about 2.1-m centers. Although soil in the areas was repeatedly mixed by using the road grader, disk, and rototiller, TNT-contaminated soil was not evenly distributed throughout the areas of plots. Composite soil samples were collected from plots after application of the ground hay and analyzed. Mean TNT concentration in the high TNT area was 1120 mg kg,$^{-1}$, but plots ranged from 72 to 2690 mg kg^{-1}, with a coefficient of variation (CV) of 72%. In the intermediate area, the mean TNT concentration was 119 mg kg^{-1} and plots ranged from 1 to 525 mg kg^{-1} with a CV of 123%. Other problems encountered in this field study were related to incorporation of the ground hay, project schedule, and weather. Amounts of ground hay incorporated into the soil had to be reduced because it was impractical to incorporate the designed amount of 5 and 10% into the soil with equipment available. The late planting date and below-normal rainfall resulted in a complete crop failure of the fall seeding. All plots were tilled and seeded again during March, resulting in dramatic change, and often increase, in soil TNT concentration on many plots. Immediately after tilling, mean TNT concentration in the high TNT area was 2021 mg kg^{-1}, and plots ranged from 76 to 5780 mg kg^{-1} with a CV of 76%. The new mean for the intermediate area was 108 mg kg^{-1}, and plots ranged from 1 to 871 mg kg^{-1}, with a CV of 179%. These data indicated that instead of replicates with about equal TNT concentrations in plots,

there was a continuum of TNT concentrations ranging from 1 to almost 5900 mg kg^{-1} of TNT in soil. Below-normal rainfall continued throughout the spring and early summer, resulting in a poor stand of the ryegrass and oats. Spot reseeding of crops during June resulted in an uneven stand of both crops. These factors affected the original design and potentially the results of the experiment.

Stands of both ryegrass and oat on most plots, including control plots, were less than normal, probably due to low germination rates caused by dry seedbed conditions following seeding on 22 March. Following spot reseeding of all plots on 7 June, it became apparent that ryegrass was not becoming established on most of the plots in the high TNT area. Only scattered plants became established on several plots in the high TNT plot. The soil surface in these plots had a reddish color, indicating the presence of TNT. Comparison of TNT concentrations from barren plots with that on plots having crop growth showed TNT levels were generally higher on barren plots. The level of hay addition did not influence the establishment of ryegrass in the high TNT plot because all but one ryegrass plot remained barren. This would indicate there is some difference in TNT tolerance between ryegrass and oat. During the summer, there also appeared to be some differences in plant growth among plots in the intermediate TNT area and control area. Establishment and growth of ryegrass and oat were better on plots with hay added, with some apparent difference between levels of hay additions.

Data on the uptake of explosives by crops are not reported because neither TNT nor any of its degradation products were found in the ryegrass tops, oat straw, or oat grain from any of the plots. TNT was not detected in any root samples, but 4A-DNT, and 2A-DNT were found in some of the root samples of both crops. There was no apparent relationship between soil TNT concentrations and presence of 4A-DNT and 2A-DNT in root samples. Ryegrass and oat have fibrous root systems, and the presence of these compounds may be due to contamination by soil not removed during root washing.

Results from greenhouse investigations[11,12] indicate levels of TNT are reduced in soils with higher organic content or by the addition of organic material to soils. This portion of the study was intended to determine if this relationship (observed under greenhouse conditions) also existed in the field. The original experimental design for this study was intended to duplicate some of the variables tested by Banwart and Hassett[11] in their greenhouse investigation. Two levels of soil TNT contamination with replicate plots of known and uniform soil contamination were required to statistically analyze data. A major complicating factor in this field study was the heterogeneity of TNT contamination in plot soils. A part of the original experimental design was also intended to duplicate the 5% and 10% (by weight) organic material additions used in the greenhouse study. However, incorporation of these large volumes of bromegrass hay into the upper 15 cm of plot soil in the field was impractical with equipment available. These factors compromised the field investigation with respect to determining changes in extractable TNT from soil due to level organic material additions.

Table 1 shows concentrations of extractable TNT in soil in plot 20 (high TNT area, 1% hay addition, ryegrass, replicate 4) during the study. Initial sample collection was on 28 October 1993 and the final sample collection was on 21 September 1994. The concentration of extractable TNT decreased by more than 82% by 10 December and continued to decrease to only about 1.6% of the original concentration by 19 July. This decrease in TNT concentration is similar to results reported by Banwart and Hassett[11]. However, the concentration increased slightly, to about 3.4% of the original concentration, on 21 September. Of the 48 plots in this study, this plot is the best example of the expected decrease of TNT in soil. Unfortunately, results from this plot are not typical of the general response observed.

TABLE 1. TNT CONCENTRATION IN PLOT 20† SOIL ON EIGHT COLLECTION DATES (mg kg^{-1})

28 Oct	10 Dec	17 Feb	22 Mar	26 Apr	7 Jun	19 Jul	21 Sep
2220	388	357	259	249	85	35	74

† High TNT area, 1% hay addition, ryegrass, replicate 4.

Table 2 shows the mean concentration of TNT in plot soils with three levels of hay additions in the intermediate and high TNT areas on eight collection dates. Each mean represents eight observations: four replications of each of the oat and ryegrass plots. Mean concentrations of TNT in the intermediate TNT area with 2% hay added generally decreased during the study, with the exception of increases on 22 March and 19 July. The increase on 22 March was about 65% more than the original mean concentration, and the increase on 19 July was twice the mean concentration on 7 June. All plots were tilled and reseeded on 22 March, and soil samples were collected after plots were tilled. Values from plots varied widely following tillage. Mean concentrations of TNT in the intermediate TNT area that did not receive hay also generally decreased during the study, again with two notable exceptions. The first, on 26 April, was probably related to the spring tilling on 22 March, and the second, on 21 September, was due to very high values from two of the eight plots. Without these two high values, the mean would have been 15.1 mg kg^{-1}. Means of TNT concentration from the intermediate TNT area plots with 1% hay added show no trend, only wide variability. Extractable TNT values of individual plots often indicate that one or more values for each sampling date were one or two orders of magnitude higher than other plot values. Mean TNT concentrations in the high TNT area for plots of all three hay additions decreased following the initial sampling, but means for later sample collections indicate this trend did not continue. Values for individual plots varied widely from one sample collection to the next.

TABLE 2. MEAN TNT CONCENTRATIONS IN SOIL ON EIGHT COLLECTION DATES

TNT Area	Hay Addition (%)	Concentration (mg kg^{-1}) by Collection Day and Month							
		28 Oct	10 Dec	17 Feb	22 Mar	26 Apr	7 Jun	19 Jul	21 Sep
Int†	0	170‡	151	133	111	201	87.7	47.9	203
Int	1	150	573	89.1	159	1230	244	427	447
Int	2	37.0	29.9	21.1	61.5	4.9	8.1	16.1	3.1
High	0	1260	891	1090	2590	902	1140	2700	726
High	1	1290	937	1170	2310	2090	2410	2540	2450
High	2	808	742	688	1170	922	3580	567	651

† Intermediate.

‡ Each mean represents eight observations, four replications of each for oat and ryegrass plots.

Trends of means shown in Table 1 and of 0 and 2% hay additions for the intermediate TNT area in Table 2 confirm results reported by Banwart and Hassett[11] and Cataldo et al.[12]. The most probable explanation for the decrease in TNT concentration in the soil is that TNT is degraded by microorganisms and enzymes in the soil and/or rhizosphere. Adding organic material to a soil increases nutrient levels, with corresponding increases in microorganism activities and populations. Concentrations of TNT degradation products 4A-DNT and 2A-DNT were determined for plot soil samples. A trend was noted in concentrations of these degradation products in the intermediate TNT plot. Generally, if the concentration of extractable TNT was high, concentrations of 4A-DNT and 2A-DNT were often below detection limits. As TNT levels decreased, concentrations of 4A-DNT and 2A-DNT increased to a maximum of about 15 mg kg^{-1}. As the concentration of TNT approached the detection limit, concentrations of these degradation products also decreased. The

presence of 4A-DNT and 2A-DNT indicates microorganisms and enzymes in the soil and/or rhizosphere are degrading TNT if conditions for their survival are favorable.

Results from this study indicate TNT and its degradation products are not translocated to plant tops in crops growing on explosives-contaminated soils. 4A-DNT and 2A-DNT were sometimes found to be associated with plant root samples growing in TNT-contaminated soils. Because TNT, 4A-DNT, and 2A-DNT were not detected in the aboveground portions of plants, vegetation growing on TNT-contaminated soils is not considered a health hazard. However, soil and plant roots may contain TNT degradation products that may be toxic, and their consumption is not advised. Crop uptake of TNT was not changed by the level of hay addition because neither TNT nor its degradation products were detected in any aboveground crop tissues. Data from this investigation show that phytoremediation, the addition of organic material (chopped hay) combined with the growing of green plants, is a viable option for the remediation of surface soils contaminated with low levels of TNT. For a complete description of this study, see Zellmer et al.[15] For an additional investigation of explosives uptake by plants at the Iowa Army Ammunition Plant, see Schneider et al.[16]

4. METAL UPTAKE AND ACCUMULATION

Much of the current research on the uptake and accumulation of metals by plants involves "traditional" hyperaccumulators, many of which are small, shallow-rooted plants in the Cruciferae (mustard) family. Phytoremediation research at ANL is expanding the definition of hyperaccumulators to include large, robust woody and herbaceous species that have high evapotranspiration rates. These plants process more soil solution containing dissolved metals, and this higher rate of water use draws more soil solution to the plant root system, sequestering metals in the plant.

Interest in using woody species with high evapotranspiration rates was initially generated when elevated levels of zinc were observed in the leaves of hybrid poplar (*Populus* sp.) trees at a field site. Applied Natural Sciences, Inc. (ANS), had planted and was monitoring hybrid poplars to remediate nitrate and ammonia contamination at a site. The site contained a buried galvanized piping system that is believed to be the source of zinc in the soil solution. ANS uses trees as phytoremediation plants to remove contaminants from soil and groundwater to depths as great as to 12 m (40 ft) in a trademarked process called "Treemediation"[17].

The observed elevated zinc concentration in the poplar leaves and a mutual interest in phytoremediation led to the development of a Cooperative Research and Development Agreement (CRADA) between ANS and ANL. The CRADA partnership provides an effective way to bring observations from the field into the controlled environment of a greenhouse for detailed investigation and a method to transfer laboratory research results back to the field. Objectives of CRADA partnership research with ANS are to (i) identify plant species best adapted for the uptake, accumulation, and degradation of specific contaminants; (ii) evaluate these species in controlled greenhouse experiments; (iii) explain the physical, chemical, physiological, and metabolic mechanisms of contaminant uptake, translocation, sequestration/detoxification, partitioning, and bioaccumulation in phytoremediation plants; and (iv) demonstrate low-cost, low-tech, environmentally friendly phytoremediation systems for remediating metal, radionuclide, and organic contaminants economically at contaminated sites.

During late March 1995, greenhouse experiments were initiated to confirm and extend field data from ANS observations of high levels of zinc in leaf tissue of hybrid poplars. After the poplar cutting had developed a normal root system in June, three groups of cuttings, with three replicates each, were given five increasing doses of zinc during a period of about two months. Cuttings were grown in lysimeters filled with quartz sand to prevent the zinc from binding on the growth substrate.

Analysis of leachate from control lysimeters with quartz sand, but no plants, showed zinc concentration in nutrient solution remained unchanged. The zinc was given in five doses in nutrient solution spiked with $ZnSO_4$, with concentrations ranging from 50 to 2,000 mg L^{-1} Zn. On each zinc addition date, two groups received increasing doses up to 1,500 and 2,000 mg L^{-1} Zn, respectively, while a control group received only nutrient solution containing a low concentration of zinc as an essential nutrient. Available zinc analyses of the nutrient solution showed the levels of zinc were equivalent to many times the levels of available zinc in "normal" soils. Before each zinc addition, sand samples and plant tissues (roots, leaves, and branches) were collected to determine how much zinc remained in the sand, and to track zinc translocation and partitioning of bioaccumulation in poplar aboveground tissues.

The poplars were watered daily, and leachate that passed over the poplar roots and through the lysimeters was collected after each watering. Leachate volume, conductivity, and pH were measured daily to determine the evapotranspiration rates, nutrient use, and biomass increase of the rapidly growing poplar cuttings. Fresh nutrient solution and/or water was added to the leachate before it was recycled to replace water lost by evapotranspiration. Measurement of the transpiration rate was considered to be a critical factor because it determines the rate at which contaminated soil solution is drawn into the plant to be processed.

Leachate analyses showed that in all cases with zinc additions up to 800 mg L^{-1}, the zinc was totally absorbed and sequestered by the plants in about four hours during a single pass through the lysimeters. At zinc additions above 1,000 mg L^{-1}, leachate levels were always below 100 mg L^{-1} in samples taken on the same day as the zinc addition after only one pass through the lysimeter. Zinc levels in the leachate increased the following day, to concentrations up to about 550 mg L^{-1} in the lysimeters that received 2,000 mg L^{-1} Zn, and then decreased sharply during second the day, to concentrations less than 100 mg L^{-1}. After that, zinc concentrations steadily decreased as the plants reabsorbed the zinc as the nutrient solution was cycled through the lysimeters on subsequent days.

Even at the highest zinc application, there were only subtle visual toxicity symptoms: slight leaf chlorosis and some leaf "drooping" in the poplar cuttings compared with the control plants. However, in plants that received more than 800 mg L^{-1} Zn, the evapotranspiration rate decreased significantly as zinc concentration in the roots increased, indicating this physiological effect could have complex causes.

The hybrid poplar plants in the lysimeter pots were harvested in early September 1995. Aboveground tissue was divided into four categories; mature leaves, expanding leaves, old wood, and new wood. The sand was removed from the lysimeters as a "cylinder," without disturbing the roots, sand samples were collected, and the sand was washed from the root system with deionized water. Roots were divided into four types based on appearance, morphology, and color. Type A roots were the clumps of new white or light-colored roots, and type B roots originated at the ends of large, woody roots. Type C roots were very fine, hairlike, dark-colored roots that densely covered the surfaces of the woody roots, and type D roots were the large-diameter, non-woody, fine roots, grayish to brownish, in the bottom of the lysimeter, which was saturated most of the time.

Several leaf harvests were made during the experiment (zinc concentrations in tissues are on a dry-weight basis). Initial leaf samples from plants that received a single dose of 50 mg kg^{-1} Zn showed 528 mg kg^{-1} Zn in the large mature leaves, 300 mg kg^{-1} in medium-sized leaves, and 140 mg kg^{-1} in small leaves. In the leaves and branches harvested at the end of the experiment, zinc concentrations did not exceed 2,250 mg kg^{-1} in the dry leaf tissue and 900 mg kg^{-1} in the woody branches. Roots harvested at the end of the experiment showed much higher concentrations of accumulated and sequestered metal than did any of the aboveground tissues. In the lysimeters receiving the highest dose, 2,000 mg L^{-1}, type D roots contained a mean concentration of 38,055 mg kg^{-1} (3.8%) Zn, type C had 15,470 mg kg^{-1}, type A had 12,225 mg kg^{-1}, and type B had 2,814 mg kg^{-1}. The lysimeters

receiving the lower zinc doses with a maximum of 1500 mg L^{-1} had a similar pattern of distribution of zinc among root types, with the type D roots containing a mean concentration of 17,053 mg kg^{-1} (1.7%) Zn. The control lysimeters also showed the same zinc uptake pattern among root types, with the mean concentration for the D roots being 232 mg kg^{-1}. This zinc was from the nutrient solution that contained zinc as an essential nutrient.

The hybrid poplar greenhouse experiments complement field studies and data collection on zinc uptake by hybrid poplars that are growing under field conditions, using the Treemediation® system installed by ANS. The implications for engineered soil and wastewater clean-up systems that use hybrid poplar growing either directly in the soil or in special hydroponic systems are very encouraging.

During April 1996, an experiment was started to determine the uptake and accumulation of zinc by Eastern gamagrass (*Tripsacum dactyloides*). Methods used were nearly the same as those described for the hybrid poplar experiment. Three groups of gamagrass plants, each group consisting of five replicates, were continuously given zinc in nutrient solution over a period of about two months at concentrations of 160 mg L^{-1}, 600 mg L^{-1}, and a control with only the zinc present in the nutrient solution.

Leachate analyses for zinc 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 mg L^{-1} Zn had grown considerably and were almost the same size as the control plants, but some of the mature leaf blades were rolled. The mean zinc removal rate for these plants had dropped to 50% of the zinc in the leachate. At this time, the plants receiving 600 mg L^{-1} Zn were smaller than the controls, their color was a darker green, most of the mature leaf blades were rolled, and the mean zinc removal rate was only about 30% of the zinc in the leachate.

In mid-June 1996, the gamagrass was harvested, using the same procedure described in the poplar experiment. The plants were divided into shoots (leaves and crowns) and roots. The root systems of all the plants had the typical structure of large primary roots and smaller, lateral, secondary roots. In the controls, most of the roots were light in color, with the newest roots being white. The plants receiving the 160 mg L^{-1} Zn had root systems almost as large as the controls, but some of the secondary roots were dark colored or black. The plants that received 600 mg L^{-1} Zn had small root systems with many black roots.

As observed in the hybrid poplar experiment, the Eastern gamagrass roots showed much higher concentrations of zinc than did the shoots. In the lysimeters receiving 600 mg L^{-1} Zn, the roots contained a mean concentration of about 10,000 mg kg^{-1} (1.0%) Zn on a dry-weight basis, while the tops contained about 1,000 mg kg^{-1} (0.1%) Zn. In the plants receiving 160 mg L^{-1} Zn, the roots contained about 4,000 mg kg^{-1} Zn, and the tops about 400 mg kg^{-1} Zn. The roots of the control plants contained about 75 mg kg^{-1}, and the tops about 50 mg kg^{-1} Zn. Again, the small amount of zinc in the controls is from the zinc present in the nutrient solution as an essential nutrient.

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. It is hypothesized that the greater portion of the zinc is sequestered in the cell wall tissue of the root cortex through internal complexation and detoxification, with translocation of a relatively small amount of the zinc to the leaves and branches. The evapotranspiration rate of the hybrid poplar decreased significantly as zinc concentration in the roots increased. Both top growth and evapotranspiration rate of the gamagrass decreased at the higher zinc level as a constant dose. For a more detailed description of zinc uptake and accumulation studies, see Negri et al.[18]

Results from these greenhouse studies and the field experience of ANS indicate that a number of species can be used for the uptake and accumulation of metals, in addition to the traditional hyperaccumulator species. Another major factor influencing metal uptake and accumulation is the evapotranspiration rate of the plant species. The higher the evapotranspiration rate, the larger the volume of water that is pumped through the phytoremediation system. A high rate of water consumption draws more soil solution to the plant roots, resulting in a higher exposure of soluble metals to the root system. A high evapotranspiration rate requires a large, robust plant with an extensive root system. For the phytoremediation of many sites, perennial woody plants are preferred because woody plants generally have deeper root systems and perennials have a longer growing season. Results from the greenhouse studies show the highest proportion of metals is accumulated in the roots. Plans are now being formulated to evaluate root harvesting methods to remove the highest concentration of metals from a site with metal contamination in surface soils.

Several other greenhouse investigations are being conducted to evaluate other aspects of phytoremediation. A key factor controlling the rate at which metals are removed from the soil by plant-based clean-up systems is the availability of the metal to the plant. Metals are bound to soil components in varying degrees, depending on such soil conditions as pH, clay content, organic matter, and redox potential. Only the readily soluble or exchangeable metals are available to plants. One way to increase metal availability is to selectively increase their concentration in the soil solution by using chelating agents. Chelating agents increase metal diffusion in the soil solution and keep the metals in plant-available forms. Natural chelating agents (organic acids, such as citric and acetic) are released by plant roots, making 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 into the soil solution.

Current greenhouse experiments are investigating the feasibility of utilizing various chelating agents to induce the release of metals and increase the rate and extent of metal accumulation in plant tissues. These experiments are measuring changes in concentration of metals (Zn, Cd, Pb, Cu) and radionuclides (U and Th) in 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 relative changes in plant accumulation induced by the addition of chelating agents. Willow plants are being grown in soil-filled lysimeters that are periodically irrigated with constant doses of dilute chelating agents in aqueous solution. Periodically, soil and plant tissue samples are collected and analyzed for metals and radionuclides. The soil samples have been analyzed by sequential extraction techniques to provide empirical information on the probable contaminant species present and the relative percentage of the total contaminant content. Plant concentration is considered as an additional sequential extraction step that represents the sum of metal species that are effectively taken up by the plant.

For many organic contaminants, such as trichloroethylene (TCE) and tetrachloroethylene (PCE), there is evidence that plants can degrade the organohalide to form less volatile compounds, such as trichloroacetic acid (TCAA). The less volatile compounds are sequestered in the plant tissue, while the remainder, passes 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. A study at ANL is developing rapid, easy, gas chromatographic analytical methods for the detection of TCAA in woody plant tissue. Samples of plant tissue have been analyzed using this new method, and results are being correlated with the presence and concentration of TCAA in plants and the contamination "history" of the site where the samples were collected. This method has the potential for monitoring phytoremediation systems used to cleanup groundwater and soil contaminated with organohalides. The analytical method also can provide a very quick and easy way to determine organohalide contamination by using plant tissue samples, rather than obtaining soil and groundwater samples by coring or drilling operations.

5. CONCLUSIONS

As with any remediation method, phytoremediation is not a panacea and it has limitations. Phytoremediation is only effective while the plants are actively growing; it slows down or stops during plant dormancy. Some contaminants are toxic at low concentrations to plants, and some sites may have contaminant concentrations that are too high and toxic for the phytoremediation plants to survive and grow. At other sites, the contaminant may be too deep in the soil for the root system to reach, even using special planting techniques. Phytoremediation can be a slow process and site cleanup may require years, but often the additional time required for remediation can be offset by the much lower cost.

Phytoremediation has a number of advantages compared with such remediation methods as soil incineration. It is an *in situ* process and doesn't require excavation or transport of contaminated soil. Phytoremediation is low-tech, requiring little if any hardware or operational facilities. Installation and maintenance costs are normally a fraction of those of other remediation methods. Phytoremediation does not damage the physical, chemical, or biological properties of the soil, and often the process improves soil fertility and conditions. It can be used for a number of contaminants and can often be used to remediate several contaminants at the same time. A major advantage of phytoremediation is that it generates little or no secondary waste. Finally, it is socially acceptable, because the public does not object to plants growing in its back yards, as it sometimes does to a soil incinerator or other remediation facilities.

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