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Phytoremediation

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FOREWORD

About GWRTAC

The Ground-Water Remediation Technologies Analysis Center (GWRTAC) is a national environmental technology transfer center that provides information on the use of innovative technologies to clean-up contaminated groundwater.

Established in 1995, GWRTAC is operated by Concurrent Technologies Corporation (*CTC*) in association with the University of Pittsburgh's Environmental Engineering Program through a Cooperative Agreement with the U.S. Environmental Protection Agency's (EPA) Technology Innovation Office (TIO). *CTC*, an independent nonprofit organization, is committed to assisting industry and government achieve world-class competitiveness. Through a unique concurrent engineering framework, *CTC* provides comprehensive solutions that improve product quality, productivity, and cost effectiveness.

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About "E" Series Reports

This report is one of the GWRTAC "E" Series of reports, which are developed for GWRTAC to provide a state-of-the-art review of a selected groundwater remediation technology or groundwater topic. These technology evaluation reports contain information gathered primarily from peer reviewed papers and publications and, in some instances, from personal communication with involved parties. These reports are peer-reviewed prior to being released.

Disclaimer

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1.0 SUMMARY

Phytoremediation is the use of vegetation for *in situ* treatment of contaminated soils, sediments, and water. It is best applied at sites with shallow contamination of organic, nutrient, or metal pollutants that are amenable to one of five applications: Phytotransformation, Rhizosphere Bioremediation, Phytostabilization, Phytoextraction, or Rhizofiltration. In this Technology Evaluation report, it is shown that phytoremediation has been utilized at a number of pilot and full-scale field demonstration tests. It is an emerging technology that should be considered for remediation of contaminated sites because of its cost effectiveness, aesthetic advantages, and long-term applicability. Phytoremediation is well-suited for use at very large field sites where other methods of remediation are not cost-effective or practicable; at sites with low concentrations of contaminants where only "polishing treatment" is required over long periods of time; and in conjunction with other technologies where vegetation is used as a final cap and closure of the site. There are limitations to the technology that need to be considered carefully before it is selected for site remediation. These include limited regulatory acceptance, long duration of time sometimes required for clean-up to below action levels, potential contamination of the vegetation and food chain, and difficulty establishing and maintaining vegetation at some toxic waste sites.

Plants have shown the capacity to withstand relatively high concentrations of organic chemicals without toxic effects, and they can uptake and convert chemicals quickly to less toxic metabolites in some cases. In addition, they stimulate the degradation of organic chemicals in the rhizosphere by the release of root exudates, enzymes, and the build-up of organic carbon in the soil. For metal contaminants, plants show the potential for phytoextraction (uptake and recovery of contaminants into above-ground biomass), filtering metals from water onto root systems (rhizofiltration), or stabilizing waste sites by erosion control and evapotranspiration of large quantities of water (phytostabilization).

In this technology evaluation, recent field tests of phytoremediation are reported on wastes containing petroleum hydrocarbons such as benzene, toluene, ethylbenzene, and xylenes (BTEX) and polycyclic aromatic hydrocarbons (PAHs), pentachlorophenol, polychlorinated biphenyls (PCBs), chlorinated aliphatics (trichloroethylene, tetrachloroethylene, and 1,1,2,2-tetrachloroethane), ammunition wastes (2,4,6-trinitrotoluene or TNT, and RDX), metals (lead, cadmium, zinc, arsenic, chromium, selenium), pesticide wastes and runoff (atrazine, cyanazine, alachlor), radionuclides (cesium-137, strontium-90, and uranium), and nutrient wastes (ammonia, phosphate, and nitrate). Different species of plants have been used in various applications including: Salix spp. (hybrid poplars, cottonwoods, and willow), grasses (rye, Bermuda grass, sorghum, fescue, bullrush), legumes (clover, alfalfa, and cowpeas), aquatic plants (parrot feather, duckweed, arrowroot, cattail, pondweed), and hyperaccumulators for metals (sunflowers, Indian mustard, and *Thlaspi* spp.).

Key findings of this technology evaluation show that phytoremediation has successfully been applied at a brownfields site for remediation of soil contaminated with lead; a small pond at Chernobyl with uranium contamination; a riparian zone buffer strip at Amana, Iowa for nitrate and atrazine removal from agricultural runoff; and at an engineered wetland at Milan, Tennessee for TNT removal. In addition, many successful applications have involved remediation actions at small sites, such as agricultural cooperatives with pesticide and ammonia spills where state agencies have jurisdiction. At these sites, few funds are available for long-term compliance monitoring, and it is not to the advantage of the owners to pay for monitoring voluntarily. Therefore, long-term monitoring and evaluation of phytoremediation technology is still needed to demonstrate efficacy, to further define suitable plants and applications, and to gain acceptance from regulatory agencies.

2.0 TECHNOLOGY DESCRIPTION

2.1 PHYTOTRANSFORMATION

Phytotransformation refers to the uptake of organic and nutrient contaminants from soil and groundwater and the subsequent transformation by plants. Phytotransformation depends on the direct uptake of contaminants from soil water and the accumulation of metabolites in plant tissue. For environmental application, it is important that the metabolites which accumulate in vegetation be non-toxic or at least significantly less toxic than the parent compound.

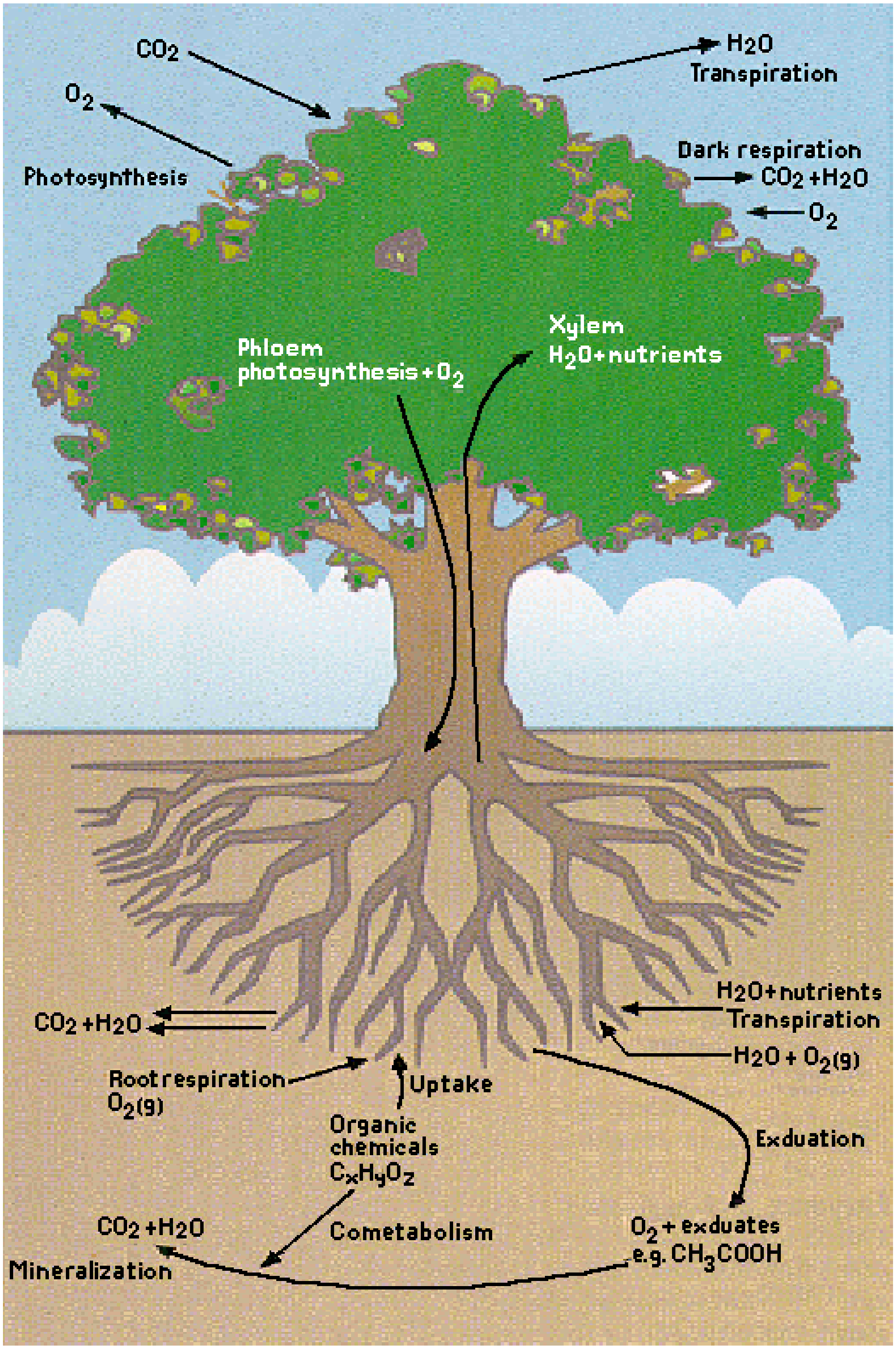
Potential applications include phytotransformation of petrochemical sites and storage areas, ammunition wastes, fuel spills, chlorinated solvents, landfill leachates (including biochemical oxygen demand (BOD) and chemical oxygen demand (COD)), and agricultural chemicals (pesticides and fertilizers). Many times, phytoremediation is not the sole treatment option, but rather it is used in conjunction with other approaches such as removal actions or ex situ treatment of highly contaminated wastes, or as a polishing treatment.

Figure 1 is a schematic of mass flows through a woody, flood-tolerant tree species (Schnoor et al., 1995). Oxygen, water and carbon transport mechanisms can vary among plant species. Plants supply oxygen to the soil rhizosphere, but roots also demand oxygen for respiration. Root turnover is a key mechanism that adds organic carbon to the soil profile. Seedlings in the laboratory can transport considerable quantities of oxygen to roots in the rhizosphere (0.5 mol O_2 per m^2 of soil surface per day) (Shimp et al., 1993). Plants are able to take-up contaminants directly from the soil water or release exudates that help to degrade organic pollutants via cometabolism in the rhizosphere (see Rhizosphere Bioremediation).

Direct uptake of organics by plants is a surprisingly efficient removal mechanism from sites contaminated at a shallow depth with moderately hydrophobic organic chemicals (octanol-water partition coefficients, log K_{ow} = 1 to 3.5). This includes most BTEX chemicals, chlorinated solvents, and short-chain aliphatic chemicals. Hydrophobic chemicals (log $K_{ow} > 3.5$) are bound so strongly to the surface of roots and soils that they cannot be easily translocated within the plant; and chemicals which are quite water soluble (log $K_{ow} < 1.0$) are not sufficiently sorbed to roots nor actively transported through plant membranes (Briggs et al., 1982). Very hydrophobic chemicals (log $K_{ow} > 3.5$) are candidates for phytostabilization and/or rhizosphere bioremediation.

Figure 1

Schematic of oxygen, water, and chemical flows through a woody tree



The direct uptake of chemical into the plant through roots depends on the uptake efficiency, transpiration rate, and the concentration of chemical in soil water (Burken and Schnoor, 1996). Uptake efficiency, in turn, depends on physical-chemical properties, chemical speciation, and the plant itself. Transpiration is a key variable that determines the rate of chemical uptake for a given phytoremediation design; it depends on the plant type, leaf area, nutrients, soil moisture, temperature, wind conditions, and relative humidity.

Once an organic chemical is translocated, the plant may store the chemical and its fragments into new plant structures via lignification (covalent bonding of chemical or its fragments into lignin of the plant); or it can volatilize, metabolize, or mineralize the chemical completely to carbon dioxide and water. Chlorinated aliphatic compounds such as trichloroethylene (TCE) have been reported to be mineralized to CO_2 and less toxic aerobic metabolites (trichloroethanol, trichloroacetic acid, and dichloroacetic acid by Newman et al., 1997). These products are consistent with those found in the human liver for TCE destruction by cytochrome P450, which is an abundant enzyme in plants as well as humans. Thus, plants are sometimes viewed as “green livers” in terms of their enzyme biochemistry.

Another form of phytotransformation is *phytovolatilization*, whereby volatile chemicals or their metabolic products are released to the atmosphere through plant transpiration. Many organic chemicals that are recalcitrant in the subsurface environment react rapidly in the atmosphere with hydroxyl radicals, an oxidant formed in the photochemical cycle. The transfer of contaminants from the soil or groundwater to the atmosphere is not as desirable as *in situ* degradation, but it may be preferable to prolonged exposure in the soil environment and the risk of ground-water contamination.

Nitroreductase and laccase enzymes in plants can break down ammunition wastes such as TNT (2,4,6-trinitrotoluene), and they may incorporate the broken ring structures into new plant material or organic detritus that becomes a part of sediment organic matter. Detoxification mechanisms may transform the parent chemical to non-phytotoxic metabolites that are stored in plant tissues (Schnoor et al., 1995). A thorough understanding of pathways and end-products of enzymatic processes will simplify toxicity investigations of *in situ* phytoremediation.

A summary of typical plants used in various applications of phytoremediation is presented in Table 1.

2.2 RHIZOSPHERE BIOREMEDIATION

Phytoremediation of the rhizosphere increases soil organic carbon, bacteria, and mycorrhizal fungi, all factors that encourage degradation of organic chemicals in soil. Rhizosphere bioremediation is also known as *phytostimulation* or *plant-assisted bioremediation*. Jordahl et al. (1997) showed that the numbers of beneficial bacteria increased in the root zone of hybrid poplar trees relative to an unplanted reference site. Denitrifiers, *Pseudonomad* spp., BTEX degrading organisms, and general heterotrophs were enhanced. Also, plants may release exudates to the soil environment that help to stimulate the degradation of organic chemicals by inducing enzyme systems of existing bacterial populations, stimulating growth of new species that are able to degrade the wastes, and/or increasing soluble substrate concentrations for all microorganisms. Leakage of sugars, alcohols, and acids from the plant and root turnover can amount to 10 to 20% of plant photosynthesis on an annual basis (Foth, 1990). Researchers have characterized the molecular weight distribution of organic

Table 1. Typical Plants Used in Various Phytoremediation Applications

Application	Media	Contaminants	Typical Plants
1. Phytotransformation	Soil, Groundwater, Landfill leachate, Land application of wastewater	· Herbicides (atrazine, alachlor) · Aromatics (BTEX) · Chlorinated aliphatics (TCE) · Nutrients (NO_3^-, NH_4^+, PO_4^{3-}) · Ammunition wastes (TNT, RDX)	· Phreatophyte trees (poplar, willow, cottonwood, aspen); · Grasses (rye, Bermuda, sorghum, fescue); · Legumes (clover, alfalfa, cowpeas)
2. Rhizosphere Bioremediation	Soil, Sediments, Land application of wastewater	· Organic contaminants (pesticides, aromatics, and polynuclear aromatic hydrocarbons [PAHs])	· Phenolics releasers (mulberry, apple, osage orange); · Grasses with fibrous roots (rye, fescue, Bermuda) for contaminants 0-3 ft deep; · Phreatophyte trees for 0-10 ft; · Aquatic plants for sediments
3. Phytostabilization	Soil, Sediments	· Metals (Pb, Cd, Zn, As, Cu, Cr, Se, U) · Hydrophobic Organics (PAHs, PCBs, dioxins, furans, pentachlorophenol, DDT, dieldrin)	· Phreatophyte trees to transpire large amounts of water for hydraulic control; · Grasses with fibrous roots to stabilize soil erosion; · Dense root systems are needed to sorb/ bind contaminants
4. Phytoextraction	Soil, Brownfields, Sediments	· Metals (Pb, Cd, Zn, Ni, Cu) with EDTA addition for Pb Selenium (volatilization)	· Sunflowers · Indian mustard · Rape seed plants · Barley, Hops · Crucifers · Serpentine plants · Nettles, Dandelions
5. Rhizofiltration	Groundwater, Water and Wastewater in Lagoons or Created Wetlands	· Metals (Pb, Cd, Zn, Ni, Cu) · Radionuclides (^{137}Cs, ^{90}Sr, U) · Hydrophobic organics	· Aquatic Plants: - Emergents (bullrush, cattail, coontail, pondweed, arrowroot, duckweed); - Submergents (algae, stonewort, parrot feather, Eurasian water milfoil, Hydrilla)

exudates from root systems of hybrid poplar trees. Exudates include short chain organic acids, phenolics, and small concentrations of high molecular weight compounds (enzymes and proteins).

Research at the U.S. Environmental Protection (EPA) Laboratory in Athens, Georgia, has examined five plant enzyme systems in sediments and soils (dehalogenase, nitroreductase, peroxidase, laccase, and nitrilase). Dehalogenase enzymes are important in dechlorination reactions of chlorinated hydrocarbons. Nitroreductase is needed in the first step for degradation of nitroaromatics, while laccase enzyme serves to break aromatic ring structures in organic contaminants. Peroxidase and nitrilase are important in oxidation reactions. Enzymes are active in rhizosphere soils in close proximity to the root (1 mm) for transformation of organic contaminants that would not occur in the absence of the plant. The addition of plant root systems creates an ecology in soils that is suitable for bioremediation. When plants are grown in soil or sediment slurries, pH is buffered, metals are biosorbed or chelated, and enzymes remain protected inside the plant or sorbed to plant surfaces. In EPA studies of TNT breakdown, plants like hornwort increase soil water pH from 3 to 7 and sorb high concentrations of metals that would usually inhibit bacteria, while the plants remain healthy and viable. Overall, plants and their root systems can accommodate mixed wastes (organic and metals) and other harsh conditions (Schnoor et al., 1995).

Anderson et al. (1993) have demonstrated the importance of biodegradation in the rhizosphere. Plants help with microbial transformations in many ways.

- Mycorrhizae fungi associated with plant roots metabolize the organic pollutants
- Plant exudates stimulate bacterial transformations (enzyme induction)
- Build-up of organic carbon increases microbial mineralization rates (substrate enhancement)
- Plants provide habitat for increased microbial populations and activity
- Oxygen is pumped to roots ensuring aerobic transformations

Fletcher et al. (1995) have reported that flavonoids and coumarin are released by root turnover from trees like mulberry, osage orange, and apple which select and stimulate PCB and PAH degrading organisms.

Fungi, growing in symbiotic association with the plant, have unique enzymatic pathways that help to degrade organics that could not be transformed solely by bacteria. In addition to soluble exudates, the rapid decay of fine root biomass can become an important addition of organic carbon to soils which serves to retard organic chemical transport. Microbial mineralization of atrazine is directly related to the fraction of organic carbon in the soil (Nair and Schnoor, 1993). Microbial assemblages are abundant in the rhizosphere, and typical communities may comprise 5 x 10^6 bacteria, 9 x 10^5 actinomycetes, and 2 x 10^3 fungi per gram of air dried soil; bacteria live in colonies that cover as much as 4 to 10% of the root surface area (Foth, 1990).

2.3 PHYTOSTABILIZATION

Phytostabilization refers to the holding of contaminated soils and sediments in place by vegetation, and to immobilizing toxic contaminants in soils. Establishment of rooted vegetation prevents windblown dust, an important pathway for human exposure at hazardous waste sites. Hydraulic control is possible, in some cases, due to the large volume of water that is transpired through plants which prevents migration of leachate towards groundwater or receiving waters. Phytostabilization is especially applicable for metal contaminants at waste sites where the best alternative is often to hold contaminants in place. Metals do not ultimately degrade, so capturing them *in situ* is sometimes the best alternative at sites with low contamination levels (below risk thresholds) or vast contaminated areas where a large-scale removal action or other *in situ* remediation is not feasible. Vigorously growing plants are necessary to exert hydraulic control and immobilization at the site; plants cannot die or be removed during the phytostabilization design period. Low-level radionuclide contaminants can also be held in place by phytostabilization, and this alternative can result in significant risk reduction if their half-lives are not too long. Soil amendments such as phosphate, lime, and organic matter are sometimes needed to immobilize toxic metals such as lead, cadmium, zinc, and arsenic. Cadmium is readily translocated to leaves in many plants, which represents a risk to the food chain, and this pathway may be the limiting consideration in applying phytostabilization at some metals contaminated sites.

2.4 PHYTOEXTRACTION

Phytoextraction refers to the use of metal-accumulating plants that translocate and concentrate metals from the soil in roots and above ground shoots or leaves. It has been used effectively by Phytotech® at brownfields sites with relatively low level lead and cadmium contamination for soil remediation to below action levels (McGinty, 1996). It has also been proposed for extraction of radionuclides from sites with mixed wastes. Phytoextraction offers significant cost advantages over alternative schemes of soil excavation and treatment or disposal. An important issue in phytoextraction is whether the metals can be economically recovered from the plant tissue or whether disposal of the waste is required. Design considerations include the accumulation factor (ratio of metal in the plant tissue to that in the soil) and the plant productivity (kg of dry matter that is harvestable each season). In order to have a practicable treatment alternative, one needs a vigorously growing plant (>3 tons dry matter/ha-yr) that is easily harvested and which accumulates large concentrations of metal in the harvestable portion (>1000 mg/kg metal).

As a general rule, readily bioavailable metals for plant uptake include cadmium, nickel, zinc, arsenic, selenium, and copper. Moderately bioavailable metals are cobalt, manganese, and iron; while lead, chromium, and uranium are not very bioavailable. Lead can be made greatly more bioavailable by the addition of EDTA to soils. Lead, chromium and uranium can be removed by binding to soils and root mass via rhizofiltration.

2.5 RHIZOFILTRATION

Rhizofiltration refers to the use of plant roots to sorb, concentrate, and precipitate metal contaminants from surface or groundwater. Roots of plants are capable of sorbing large quantities of lead and chromium from soil water or from water that is passed through the root zone of densely growing vegetation. The potential for treatment of radionuclide contaminants has received a great deal of attention in the press. Rhizofiltration has been employed by Phytotech® using sunflowers at a U.S. Department of Energy (DOE) pilot project with uranium wastes at Ashtabula, Ohio, and on water from a pond near the Chernobyl nuclear plant in the Ukraine.

Table 2. Phytoremediation Applications and Demonstrations in the Field

Location	**Application**	**Plants**	**Contaminants**	**Performance**	**Contacts**
Chernobyl, Ukraine	Rhizofiltration demonstration pond near nuclear disaster	Sunflowers *Helianthus annuus*	^{137}Cs, ^{90}Sr	90% Reduction in 2 weeks. Roots concentrated 8,000 fold	I. Raskin, Rutgers U.
Ashtabula, OH	Rhizofiltration demonstration DOE energy wastes	Sunflowers *Helianthus annuus*	U	95% removal in 24 hours from 350 ppb to < 5 ppb	B. Ensley, Phytotech
Trenton, NJ	Phytoextraction demonstration 200 ft x 300 ft plot brownfield location	Indian mustard *Brassica juncea*	Pb	Pb cleaned-up to below action level in one season SITE program	B. Ensley, Phytotech
Rocky Flats, CO	Rhizofiltration from landfill leachate	Sunflowers and mustard	U and nitrate	Just beginning SITE program	Rock, 1997
Dearing, KS	Phytostabilization demonstration one acre test plot abandoned smelter, barren land	Poplars *Populus* spp.	Pb, Zn, Cd Concs. > 20,000 ppm for Pb and Zn	50% survival after 3 years. Site was successfully revegetated.	G. Pierzynski, Kansas St.
Whitewood Cr., SD	Phytostabilization demonstration one acre test plot mine wastes	Poplars *Populus* spp.	As, Cd	95% of trees died. Inclement weather, deer browse, toxicity caused die-off.	J. Shnoor, U. of Iowa
Pennsylvania	Phytoextraction pilot mine wastes	*Thlaspi caerulescens*	Zn, Cd	Uptake is rapid but difficult to decontaminate soil	R. Chaney, USDA Beltsville, MD Brown 1995
San Francisco, CA	Phytovolatilization refinery wastes and agricultural soils	*Brassica sp.*	Se	Selenium is partly taken-up and volatilized, but difficult to decontaminate soil	G. Banuelos, USDA Salinity Lab, Riverside, CA
Aberdeen, MD J-field site	Phytotransformation groundwater capture on 1 acre plot	Hybrid poplars *Populus* spp.	TCE, PCA (1,1,2,2-tetrachloroethane)	Only in second year Demonstration Project	H. Compton, EPA/ERT, Edison, NJ
Carswell AFB Ft. Worth, TX	Phytotransformation groundwater capture on 4 acre plot	Hybrid poplars *Populus* spp.	TCE	Only in second year SITE Project	G. Harvey, Ohio Wright-Patterson AFB

Table 2. Phytoremediation Applications and Demonstrations in the Field (cont.)

Location	**Application**	**Plants**	**Contaminants**	**Performance**	**Contacts**
Milan, TN	Phytotransformation engineered wetland at army ammunition plant	Elodeia Bullrush Canary Grass	TNT, RDX	> 90% removal	D. Bader, U.S. Army Aberdeen Proving Ground, MD
Middletown, IA	Phytotransformation created wetland and surrounding soil	Pondweed Coontail Arrowroot Hybrid poplars	TNT, RDX	Just beginning	J. Schnoor, U. of Iowa K. Howe, Army COE Omaha
Ogden, UT	Phytotransformation (groundwater and soil) petrochemical wastes 4 acre site	Hybrid Poplar	BTEX, TPH	Only in second year SITE Program	A. Ferro, Phytokinetics
Portland, OR	Phytotransformation on wastes of wood preservative	Hybrid Poplar	PCP, PAH	Only in second year SITE Program	A. Ferro, Phytokinetics
Martell, IA Clarence, IA Amana, IA	Phytotransformation agricultural runoff and agricultural co-op sites	Hybrid Poplar	atrazine, nitrates	90% reduction in groundwater of NO_3^- atrazine reductions	Licht, Ecolotree Paterson and Schnoor (1992)

Shallow lagoons have been engineered as wetlands and maintained as facultative microbial systems with low dissolved oxygen in the sediment. Groundwater or wastewater is pumped through the system for the removal of contaminants by rhizofiltration. Usually this technology is intended for metals or mixed wastes, but it is suitable for ammunition wastes as well. 2,4,6-Trinitrotoluene (TNT) is an organic contaminant that sorbs strongly to roots and is not translocated to any appreciable degree. An engineered wetland technology has been used at the Milan, Tennessee, and Volunteer Army Ammunition Plants with bullrush (Table 2). In addition, an engineered wetland has been approved for full scale treatment of a CERCLA site at the Iowa Army Ammunition Plant at Middletown, Iowa, for TNT and RDX polishing of soil and groundwater after removal actions.

Wetlands have been used with great success in treating nutrients, metals, and organic contaminants for many years (Young, 1996). Long-term utilization of wetland plants and sulfate-reducing conditions result in an increase in pH and a decrease in toxic metals concentrations for treatment of acid mine drainage (Wieder, 1993; Walski, 1993). Root systems and sediments in wetlands are facultative (aerobic and anaerobic zones) which facilitates sorption and precipitation of toxic metals.

3.0 APPLICATIONS AND PERFORMANCE

3.1 LIMITATIONS

Limitations of phytoremediation include the difficulty with treating wastes greater than three meters deep, possible uptake of contaminants into leaves and release during litter fall, inability to assure clean-up below action levels in a short period of time, difficulty in establishing the vegetation due to toxicity at the site, and possible migration of contaminants off-site by macropore flow or by binding with soluble plant exudates. Regulatory restrictions sometimes will not allow contaminants to be left in place, even when a vegetative cover prevents erosional or hydrological pathways of exposure. Phytoremediation is most effective at sites with shallow contaminated soils where contaminants can be treated in the rhizosphere and by root uptake. Sites where contamination is relatively deep and those with pools of nonaqueous phase liquids (NAPL) would not be good applications. However, deep ground-water contaminants or leachate pond effluent can be treated by pumping and irrigation on plantations of trees. Degradation of organics may be limited by mass transfer, i.e., desorption and mass transport of chemicals from soil particles to the aqueous phase may become the rate determining step. Therefore, phytoremediation may require more time to achieve clean-up standards than other more costly alternatives such as excavation and treatment or disposal, especially for hydrophobic pollutants that are tightly bound to soil particles. In many cases, phytoremediation may serve as a final “polishing step” to close sites after other clean-up technologies have been used to treat the hot spots.

Winter operations may pose problems for phytoremediation when deciduous vegetation loses its leaves, transformation and uptake cease, and soil water is no longer transpired. Mathematical modeling of the hydrology and contaminant transport is recommended in order to ensure that migration of contaminants and/or leaching to groundwater during seasonal periods of vegetation dormancy does not preclude the phytoremediation option.

3.2 PERFORMANCE

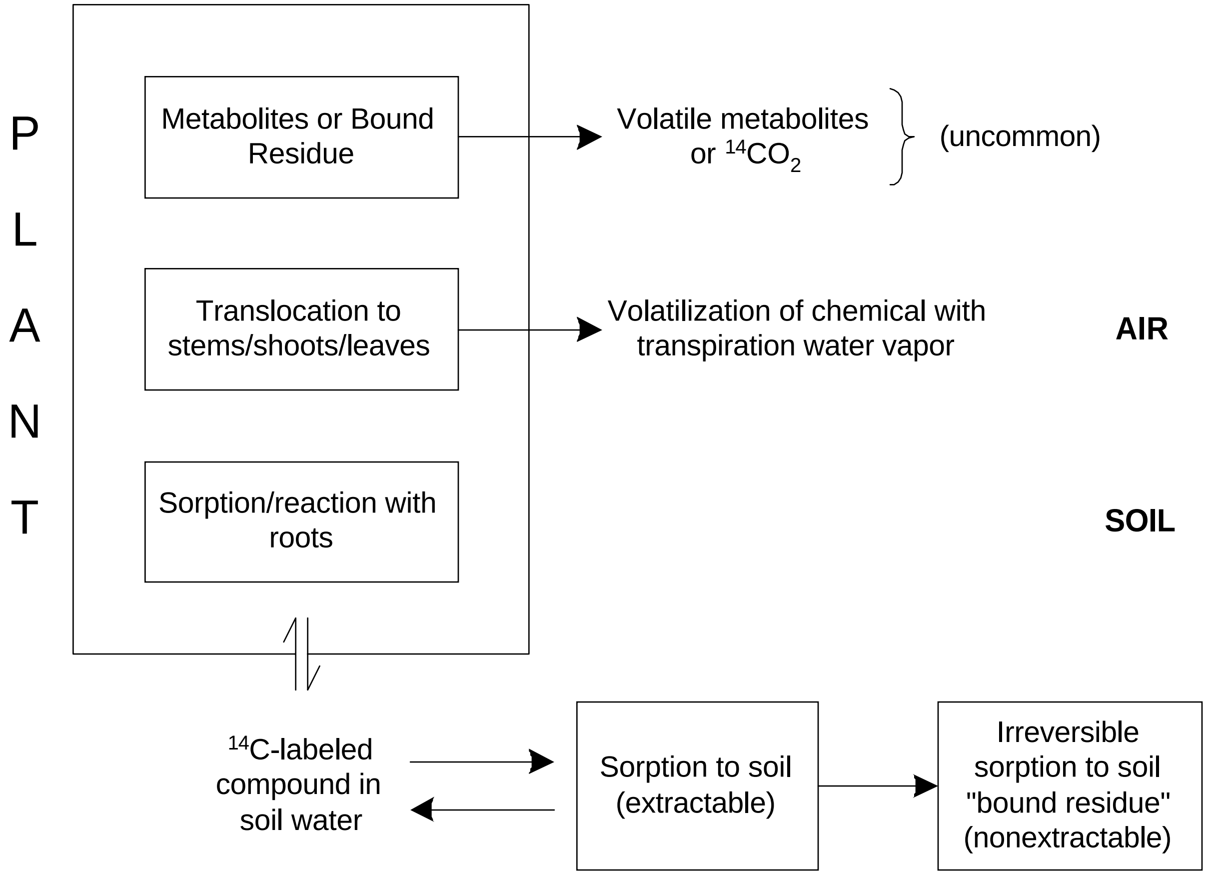
Phytotransformation

The concept of phytotransformation for organic compounds has been verified in the laboratory, greenhouse, and small plots. Contaminants are either immobilized as bound residues in the soil or plant, metabolized, or volatilized as shown in Figure 2.

Mass balance studies have been completed using ^{14}C-labeled compounds, and the fate and transport of the chemicals through plants has been documented (Newman et al., 1997; Burken and Schnoor, 1997; Dushenkov et al., 1995; Ferro et al., 1994).

Newman et al. (1997) have demonstrated that TCE is transformed to trichloroethanol, trichloroacetic acid, and dichloroacetic acid by hybrid poplar trees. This observation is indicative of an aerobic transformation, such as that in the human liver by cytochrome P-450 enzymes (mono-oxygenase). Trace quantities of $^{14}CO_2$ were released by the plant demonstrating partial mineralization of TCE. Mineralization is fairly unusual, however, and there are no reports in the literature of aromatic compounds being completely mineralized by plants.

Figure 2
Fate and Transport of Organic Chemicals in Phytoremediation Laboratory Experiments with Radiolabeled ^{14}C-isotopes



Burken and Schnoor (1997) showed that the aromatic pesticide atrazine was uptaken and transformed to ammeline, a fully hydroxylated and dealkylated metabolite, but there was no indication of mineralization to $^{14}CO_2$. A fraction (~ 15%) of the ring-labeled atrazine was incorporated into biomass as bound residue.

Hydrophobic chemicals (log $K_{ow} > 3.5$) are expected to be sorbed strongly to soils and not bioavailable to plants for translocation. Phytoremediation of hydrophobic compounds such as PCBs and PAHs may be possible by enhancement of rhizosphere microbial degradation processes and sorption to roots. Moderately hydrophobic chemicals (log K_{ow} = 1 to 3.5) are expected to be taken-up by plants and metabolized, volatilized, or incorporated into plant tissues as nonextractable bound residue. Bound residues are generally viewed as much less toxic to animals (non-bioavailable) in the food chain, but further research may be necessary to confirm this for some compounds. Volatile organic chemicals (VOCs) may be transpired by the plant, and simple air toxics models can be used to determine if they pose an unacceptable risk to the atmosphere. In the case of TCE, the half-life in aerobic soil and groundwater is on the order of years; in the atmosphere, it reacts with the hydroxyl radical yielding a half-life of hours to days.

Hydrophilic chemicals (log $K_{ow} < 1$) are not expected to be taken up or sorbed by plants. However, exceptions do exist and treatability studies are recommended. Phytoremediation may be a viable option for some hydrophilic chemicals that are quite mobile in the subsurface environment and not amenable to microbial degradation.

Table 2 lists information on field demonstrations of phytoremediation. Despite a number of investigations in the lab and greenhouse, very few documented cases of full scale clean-up by phytotransformation exist. There are no Superfund Innovative Technology Evaluations (SITE Program by EPA) that have been completed, however five are currently in progress (Steve Rock, EPA Cincinnati, personal communication). These include demonstrations or evaluations of phytoremediation for lead, uranium, nitrate, TCE, BTEX, total petroleum hydrocarbons (TPH), pentachlorophenol, and PAHs (Table 2). Many other demonstration projects are underway, but they do not have detailed reporting requirements. A dozen small sites (such as pesticide dealerships) have utilized phytoremediation where states held the lead in hazardous waste programs. But these small projects generally do not have the funds necessary to document the extent of remediation in the field. Usually, ground-water monitoring is the only requirement placed upon the principal responsible party in these cases, and it is not in their self interest to conduct detailed monitoring at the site.

Rhizosphere Bioremediation

Rhizosphere bioremediation has been demonstrated in the field at Oak Ridge National Laboratory by Anderson (1992) and Anderson et al. (1993) for TCE contaminated soils. It was not possible to perform mass balance studies, but the project did show disappearance of TCE over time and differences among five different plant species. Aerobic rhizosphere bioremediation is thought to be effective for aromatic hydrophobic chemicals such as PAHs, BTEX, and phenolics (Hedge and Fletcher, 1996) at sites with shallow contamination (Hsu et al., 1992).

Phytostabilization

Phytostabilization is a potentially effective remediation strategy for hydrophobic chemicals and metals at contaminated sites where removal or treatment is not practical or as a polishing step where contaminant concentrations are below regulatory action levels. Hse (1996) reports on phytoremediation of metals at two locations: a mine tailings Superfund site in South Dakota with up to 1,000 mg/kg of arsenic (and lesser amounts of cadmium); and an abandoned smelter in Kansas with up to 200,000 mg/kg of zinc and 20,000 mg/kg of lead. The goal at each site was to stabilize soils and to decrease vertical migration of leachate to groundwater using deep-rooted hybrid poplar trees. At the South Dakota site, the trees died due to harsh climatic conditions, deer browse, and possible toxic stress. At the Kansas site, survival of the trees has been greater than 50% in the third year, and the effort is successful to date. Sites with high concentrations of metals are difficult to phytostabilize due to soil toxicity, but it is inexpensive relative to excavation and treatment or disposal. Soil amendments such as phosphate, lime, N/P/K, and organic matter (sewage sludge, compost, aged manure, straw, leaves, etc.) are usually required. Treatability and toxicity studies in small pots in a greenhouse are recommended.

Phytoextraction

Phytoextraction has been proven effective at a brownfields site in Trenton, New Jersey for remediation of lead-contaminated shallow soils (Blaylock et al., 1996). Approximately 50% of the lead was removed from the surface soil (~ 700 mg/kg) in order to achieve clean-up standards (400 mg/kg) in one year using *Brassica juncea*, a relative of the mustard family. For phytoextraction to be effective, one needs vigorously growing plants (> 3 tons dry matter/acre-yr), an easily harvestable above-ground portion, and a plant that accumulates large amounts of metals (~ 1000 mg/kg) in above-ground biomass. To achieve clean-up within three to five years, the plant must accumulate about ten times the level in soil (for example, if the level in soil is 500 mg/kg, then the concentration in the plant must be almost 5000 mg/kg to clean-up the soil in a few years). Some sites have metals that are bioavailable while others do not. Generally, cadmium, nickel, zinc, arsenic, and copper are relatively bioavailable while lead, chromium, and uranium are not taken-up and translocated to the harvestable biomass. Plants which accumulate nickel, cobalt, copper, manganese, lead, zinc, and selenium have been reported in the literature (Kumar et al., 1995). Zinc and boron are phytotoxic to some plants at levels above 200 mg/kg in soil. Addition of EDTA (0.5 to 10 µg EDTA/kg soil) has greatly enhanced the bioavailability of lead, but the enhancement must be weighed against the increased probability of lead migration to groundwater. Mathematical modeling of water movement and metals transport may be required to further understand the fate of lead under these conditions.

Rhizofiltration

Rhizofiltration has been pioneered by Ilya Raskin and the group at Rutgers University (Dushkenov, et al. 1995). It is effective in cases where wetlands can be created and all of the contaminated water allowed to come into contact with roots. Contaminants should be those that sorb strongly to roots such as hydrophobic organics, lead, chromium (III), uranium, and arsenic (V).

4.0 DESIGN

Design of a phytoremediation system varies according to the contaminant(s), the conditions at the site, the level of clean-up required, and the plant(s) that are used. Clearly, phytoextraction has different design requirements than phytostabilization or rhizosphere bioremediation. Nevertheless, it is possible to specify a few design considerations that are a part of most phytoremediation efforts. These include:

- Plant selection;
- Treatability;
- Planting density and pattern;
- Irrigation, agronomic inputs, and maintenance;
- Ground-water capture zone and transpiration rate;
- Contaminant uptake rate and clean-up time required; and,
- Analysis of failure modes.

4.1 PLANT SELECTION

Plants are selected according to the needs of the application and the contaminants of concern. For phytotransformation of organics, the design requirements are that vegetation is fast growing and hardy, easy to plant and maintain, utilizes a large quantity of water by evapotranspiration (if groundwater is an issue), and transforms the contaminants of concern to non-toxic or less toxic products. In temperate climates, phreatophytes (e.g., hybrid poplar, willow, cottonwood, aspen) are often selected because of fast growth, a deep rooting ability down to the surface of groundwater, large transpiration rates, and the fact that they are native throughout most of the country. At the Iowa Army Ammunition Plant CERCLA site (Table 2), design requirements included the use of native plants (to avoid introduction of nuisance species) and species which showed nitroreductase activity. In pre-screening ELISA immunoassays for transformation of TNT. Hybrid poplar was selected for the terrestrial species and pondweed, arrowroot, and coontail were selected for the aquatic species. At petrochemical sites, other trees (mulberry, apple, and osage orange) have been selected for their ability to release flavonoids and phenolics (via fine root turnover), compounds that are known to induce enzymes in PCB and PAH-degrading organisms (Fletcher, 1995). Hybrid poplars have been shown to uptake and transform TCE. A screening test or knowledge from the literature of plant attributes will aid the design engineer in selection of plants. Engineers should work in interdisciplinary teams which includes a botanist and/or agricultural specialist to identify and select plants that will grow well at the site.

Grasses are often planted in tandem with trees at sites with organic contaminants or as the primary remediation method. They provide a tremendous amount of fine roots in the surface soil which is effective at binding and transforming hydrophobic contaminants such as TPH, BTEX, and PAHs. Grasses are often planted between rows of trees to provide for soil stabilization and protection against wind-blown dust that can move contaminants off-site. Legumes such as alfalfa, alsike clover, and peas can be used to restore nitrogen to poor soils. Fescue, rye, and reed canary grass have been used successfully at several sites, especially those contaminated with petrochemical wastes. The grasses are harvested periodically and disposed to compost or burned. Hydrophobic contaminants do not translocate appreciably, so the top portion of grasses are not contaminated. The system achieves phytoremediation via rhizosphere processes and sorption to roots.

Selection of plants for phytoremediation of metals depends on the application: phytostabilization, rhizofiltration, or phytoextraction. In phytoextraction, one is seeking to concentrate the metal(s) in the above-ground portion of the biomass, and to harvest and recover metals from the biomass, if practicable. Plants used to date in phytoextraction remedies include sunflowers and Indian mustard plants for lead; *Thlaspi* spp. for zinc, cadmium, and nickel; and sunflowers and aquatic plants for radionuclides (Table 2). Screening tests for hyperaccumulators around the world have been led by Alan J.M. Baker, University of Sheffield, UK. Ilya Raskin, Rutgers University, has led a development effort for screening plants for phytoextraction capabilities in the laboratory. Recovery of metals from vegetation has centered on incineration and recovery from ash, or wet extraction techniques. Even if it is not practicable to recover the metals from plant biomass or ash, they will have been concentrated into a much smaller volume for ultimate disposal.

Aquatic plants are used in created wetlands applications. They fall into two categories: emergent and submerged species. Emergent vegetation transpires water, and it is easier to harvest the vegetation if desired. Submerged species do not transpire water, but they provide more biomass within the aquatic portion of the system for uptake and sorption of contaminants. Aquatic species in created wetlands have included bullrush, cattail, coontail, duckweed, arrowroot, pondweed, parrot feather, Eurasian water milfoil, stonewort, and *Potamogeton* spp.

4.2 TREATABILITY

It is necessary to utilize treatability studies prior to design in order to assure that the phytoremediation system will achieve desired results. Toxicity and transformation data are obtained in treatability studies. There is a large amount of variation in toxicity and transformation rates that can be expected from one plant species to another, and even from one variety or cultivar to another. Boron, zinc, ammonium, some metals and salts are especially toxic to plants. Thus, it is critical to obtain treatability information in the laboratory or greenhouse, if prior knowledge has not been reported for the waste with that plant. The sequence of design information that is required typically ranges from hydroponic studies, to small pot studies with soils from the site in a greenhouse, to plot studies (up to 15 x 15 m). Different concentrations of contaminant can be analyzed for toxicity, and plant tissues can be harvested for metabolite or parent compound analysis. Regulators may require total mass balance information which necessitates use of radiolabeled compounds in the laboratory.

Treatability laboratory studies may be needed to assess the fate of the contaminant(s) in the plant system. For example, the potential for volatile compounds such as benzene and trichloroethylene to move through the plant and become transpired to the atmosphere as air toxics must be examined. Volatiles are often transpired to the atmosphere by plants, in which case, air toxics calculations would be needed to estimate the atmospheric concentrations and whether these emissions would be considered acceptable. Similarly, moderately hydrophobic organics (log K_{ow} = 1 to 3.5) are often translocated to the leaves of the plant and metabolized. Measurement of leaf concentrations of parent compound and metabolites would be needed in this case to determine if acceptable levels are exceeded.

4.3 PLANT DENSITY AND PATTERN

Planting density depends on the application. Louis Licht, Ecolotree, has pioneered the use of hybrid poplar trees as riparian zone buffer strips, landfill caps, and at hazardous waste sites. For hybrid poplar trees, 1000 to 2000 trees per acre are typically planted with a conventional tree planter at 12 to 18 inches depth or in trenched rows one to six feet deep. Poplars have the ability to root along the entire buried depth. If a row conformation is used, the trees may be spaced with two feet between trees and ten feet between rows. The poplars are planted simply as "sticks", long cuttings that will root and grow rapidly in the first season. Several phreatophytes in the Salix family, such as willow and cottonwood, can be planted in a similar manner. Hardwood trees and evergreens may require a lower planting density initially. A high initial planting density assures a significant amount of evapotranspiration in the first year which is normally desirable, but the trees will naturally thin themselves by competition to 600 to 800 trees per acre over the first six years. If desirable, hybrid poplars can be harvested on a six-year rotation and sold for fuelwood or pulp and paper, and the trees will grow back from the cut-stump (coppicing trait). The dense, deep root system stays in place to sustain growth for the next year. The lifetime of hybrid poplars such as *Populus deltoides x nigra* DN-34 (Imperial Carolina) is on the order of 30 years which is usually sufficient as the design life of the project.

Grasses are usually drilled or broadcast for planting at waste sites. Biomass densities (above ground) of 200 to 600 g/m^2 are achieved by the second crop, with 1 to 3 crops per year depending on climate and water availability.

The initial planting density of aquatic species in a created or natural wetland is normally three plants to a pod, located on three foot centers. Replanting and maintenance should be estimated in the cost of the project. One should consider that at least 30 percent of the plants may need to be replanted in the second or third year, as a contingency. At Milan, Tennessee, the final plant density in four created wetlands cells ranges from 2400 to 4000 g/m^2 with addition of 350 to 700 mg/L of fertilizer addition (N = 3.6%, P = 0.7%, K = 2.4%, O.C. = 43.7%, trace elements = Mg, Na, Si, S, Fe, Zn, Mn). The application of large amounts of organic fertilizer at the Milan site ensured that most of the TNT treatment was due to anaerobic microbiological processes rather than by plant uptake and phytotransformation.

4.4 IRRIGATION, AGRONOMIC INPUTS AND MAINTENANCE

For terrestrial phytoremediation applications, it is often desirable to include irrigation costs in the design, on the order of 10 to 20 inches of water per year. Irrigation of the plants ensures a vigorous start to the system even in a drought. On the other hand, hydrologic modeling may be required to estimate the rate of percolation to groundwater under irrigation conditions. Over time, irrigation should be withdrawn from the site, provided the area receives sufficient rainfall to sustain the plants. Operation and maintenance (O&M) costs should be considered in the design of phytoremediation systems. Costs for mowing, replanting, pruning, harvesting, monitoring vegetation for contaminants, fertilizer costs, and performance monitoring should all be included in the initial estimated costs if they are needed.

Agronomic inputs include the nutrients necessary for vigorous growth of vegetation and rhizosphere bacteria. These include N/P/K from commercial fertilizer mixes, and carbon addition and soil conditioners such as aged manure, sewage sludge, compost, straw, or mulch. Typical application rates of fertilizer include 50 lbs P/acre and 100 lbs N/acre each year, especially for production of grasses and fine roots at petrochemical sites. It is critical that the site soils have sufficient water holding capacity to sustain vegetation. This is often not the case at mine tailings sites, abandoned smelters, and rocky terrains. In these cases, soil amendments are necessary to improve soil tilth and allow water to be absorbed. Sometimes it is desirable to neutralize pH by lime addition; a standard agronomic analysis of site soils will allow assessment of the necessity for pH adjustment.

Biomass production can be estimated at 7 tons dry matter/acre-yr for fast growing trees. The amount of nitrogen stored in woody tissue is typically 0.5 to 1.0%, so nitrogen uptake can be calculated. Stoichiometries of woody tissue and leaf tissue are available in the literature to estimate major nutrient uptake requirements.

In some cases, chemical inputs are a part of the total phytoremediation design. For phytostabilization, it is necessary to bind metals to soil particles so that they are not available for plant uptake or leaching. Phosphate rock or phosphate fertilizers are effective in binding lead and zinc. They can be added to trenches or disked into the soil prior to planting. For phytoextraction, the opposite effect is desired: metals must be bioavailable for plant uptake. In this case, chelates such as EDTA (0.5 to 10 µg EDTA/kg soil) have been added to soils in irrigation water to assure plant uptake and concentration from the soil to biomass (Raskin, 1996).

4.5 GROUND-WATER CAPTURE AND TRANSPIRATION

One must understand where the water is moving at a site in order to estimate contaminant fate and transport. For applications involving ground-water remediation, a simple capture zone calculation (Domenico and Schwartz, 1997) can be used to estimate whether the phytoremediation “pump” can be effective at entraining the plume of contaminants. Trees can be grouped for consideration as average withdrawal points. The goal of such a phytoremediation effort is to create a water table depression where contaminants will flow to the vegetation for uptake and treatment. It is important to realize that organic contaminants are not taken-up at the same concentration as in the soil or groundwater, rather there is a transpiration stream concentration factor (a fractional efficiency of uptake) that accounts for the partial uptake of contaminant (due to membrane barriers at the root surface). The uptake rate is given by the following equation.

$$U = (TSCF)\,(T)\,(C) \qquad (1)$$

where U = uptake rate of contaminant, mg/day

$TSCF$ = transpiration stream concentration factor, dimensionless

T = transpiration rate of vegetation, L/day

C = aqueous phase concentration in soil water or groundwater, mg/L

If the contaminant plume is not taken-up by the vegetation, the plume that emerges will be evapoconcentrated, i.e., the mass of contaminant in the plume will be less due to uptake by vegetation, but the concentration remaining will actually be greater. This is a potential concern for phytoremediation of ground-water plumes or with created wetlands, where a relatively hydrophilic contaminant can be concentrated on the downstream side of the phyto system.

A method for estimating the Transpiration Stream Concentration Factor (TSCF) for equation (1) is given in Table 3. The Root Concentration Factor is also defined in Table 3 as the ratio of the contaminant in roots to the concentration dissolved in soil water (µg/kg root per µg/L). It is important in estimating the mass of contaminant sorbed to roots in phytoremediation systems.

Table 3
Estimating the Transpiration Stream Concentration Factor (TSCF) and Root Concentration Factor (RCF) for Some Typical Contaminants
(from Burken and Schnoor, 1997b)

The TSCF and RCF for metals depends on their redox state and chemical speciation in soil and groundwater.

Chemical	**+Log K_{ow}**	**+Solubility -Log C_w^{sat} @ 25°C, (mol/l)**	**+Henry's Constant $k_{H'}$, @25°C (dimensionless)**	**+Vapor Pressure -Log P_o @ 25°C (atm)**	**Transpiration Stream Conc. Factor (TSCF)***	**Root Conc. Factor, RCF† (L/kg)**
benzene	2.13	1.64	0.2250	0.90	0.71	3.6
toluene	2.69	2.25	0.2760	1.42	0.74	4.5
ethylbenzene	3.15	2.80	0.3240	1.90	0.63	6.0
m-xylene	3.20	2.77	0.2520	1.98	0.61	6.2
TCE	2.33	2.04	0.4370	1.01	0.74	3.9
aniline	0.90	0.41	2.2×10^{-5}	2.89	0.26	3.1
nitrobenzene	1.83	1.77	0.0025[a]	3.68	0.62	3.4
phenol	1.45	0.20	$>1.0 \times 10^{-5}$	3.59	0.47	3.2
pentachloropheno	5.04	4.27	1.5×10^{-4} [a]	6.75[a]	0.07	54
atrazine	2.69	3.81	1.0×10^{-7} [a]	9.40[a]	0.74	4.5
1,2,4-trichlorobenzene	4.25	3.65	0.1130	3.21	0.21	19
RDX	0.87	4.57	- - -	- - -	0.25	3.1

+ Physical chemical properties (Schwarzenbach, et al., 1993) unless otherwise noted.

* TSCF = $0.75 \exp\{-[(\log K_{ow} - 2.50)^2/2.4]\}$ Burken & Schnoor, 1997b

† RCF = $3.0 + \exp(1.497 \log K_{ow} - 3.615)$ Burken & Schnoor, 1997b

[a] Source: (Schnoor, 1996)

Mature phreatophyte trees (poplar, willow, cottonwood, aspen, ash, alder, eucalyptus, mesquite, bald cypress, birch and river cedar) typically can transpire three to five acre-ft of water per year (36 to 60 inches of water per year). This is equivalent to about 600 to 1000 gallons of water per tree per year for a mature species planted at 1500 trees per acre. Transpiration rates in the first two years would be somewhat less, about 200 gallons per tree per year, and hardwood trees would transpire about half the water of a phreatophyte. Two meters of water per year is a practical maximum for transpiration in a system with complete canopy coverage (a theoretical maximum would be 4 m/yr based on the solar energy supplied at 40^{o}N on a clear day that is required to evaporate water). If evapotranspiration of the system exceeds precipitation, it is possible to capture water that is moving vertically through soil. Areas that receive precipitation in the wintertime (dormant season for deciduous trees) must be modeled to determine if the soil will be sufficiently dry to hold water for the next spring's growth period. The Corps of Engineers HELP model (Vicksburg, Mississippi) and other codes have been used to estimate vertical water movement and percolation to groundwater.

4.6 CONTAMINANT UPTAKE RATE AND CLEAN-UP TIME

From equation (1) above, it is possible to estimate the uptake rate of the contaminant(s). First order kinetics can be assumed as an approximation for the time duration needed to achieve remediation goals. The uptake rate should be divided by the mass of contaminant remaining in the soil:

$$k = U/M_o \tag{2}$$

where k = first order rate constant for uptake, yr^{-1}
U = contaminant uptake rate, kg/yr
M_o = mass of contaminant initially, kg

Then, an estimate for mass remaining at any time is expressed by equation (3) below.

$$M = M_o e^{-kt} \tag{3}$$

where M = mass remaining, kg
t = time, yr

Solving for the time required to achieve clean-up of a known action level:

$$t = -(\ln M/M_o)/k \tag{4}$$

where t = time required for clean-up to action level, yr
M = mass allowed at action level, kg
M_o = initial mass of contaminant, kg

4.7 ANALYSIS OF FAILURE MODES

Phytoremediation systems are like any other treatment scheme; one cannot simply walk away from them and expect success. There are events that can cause failure that should be realistically assessed at the outset. These include killing frosts, wind storms, animals (voles, deer, beaver), disease or infestation (fungus, insects), and latent toxicity. A contingency fund should be provided for periodic replanting of a certain percentage of the site in order to ensure a viable vegetation system.

5.0 EXAMPLES

Equations (1 through 4) from Section 4.0 can be applied to most sites where soil clean-up regulations are known for metals or organic contaminants. Two examples follow, one for TCE treatment by phytotransformation and another for lead removal by phytoextraction, which demonstrate the use of the design equations.

Organics - Example 1)

TCE residuals have been discovered in an unsaturated soil profile at a depth of 3 meters. From lysimeter samples, the soil water concentration is approximately 100 mg/L. Long cuttings of hybrid poplar trees will be planted through the waste at a density of 1500 trees per acre for uptake and phytotransformation of the TCE waste. By the second or third year, the trees are expected to transpire 3 acre ft/yr of water (36 in/yr) or about 600 gal/tree per year. Estimate the time required for clean-up if the mass of TCE per acre is estimated to be 1000 kg/acre, and the clean-up standard has been set at 100 kg/acre (90% clean-up).

$$U = (TSCF)\ (T)\ (C) \qquad (1)$$

where TSCF = 0.74 from Table 3
T = (600 gal/tree-yr)(1500 tree/acre)(3.89 L/gal) = $3.5\text{x}10^6$ L/acre-yr
C = 100 mg/L (given)
U = 2.59×10^8 mg/acre-yr = 259 kg/acre-yr
k = U/M_o (2)
k = (259 kg/yr)/1000 kg
k = 0.259 yr^{-1}
t = $-(\ln M/M_o)/k$ (4)
t = $-(\ln 100/1000)/k$
t = 8.9 yr

Most of the TCE that is taken-up by the poplars is expected to volatilize slowly to the atmosphere. A portion will be metabolized by the leaves and woody tissue of the trees.

Metals - Example 2)

Lead at a lightly contaminated Brownfield Site has a concentration in soil of 600 mg/kg to a depth of one foot. The clean-up standard has been set at 400 mg/kg. Indian mustard, *Brassica juncea*, will be planted, fertilized, and harvested three times each year for phytoextraction. Using small doses of EDTA, it is possible to achieve concentrations in the plant of 5000 mg/kg (dry weight basis), and harvestable densities of 3 tons dry matter per crop. Estimate the time required for clean-up.

$U =$ Uptake Rate $= (5000\ \text{mg/kg})\ (9\ \text{tons/acre-yr})\ (908\ \text{kg/ton})$

$\qquad\qquad = 4.09 \times 10^{7}\ \text{mg/acre-yr} = 40.9\ \text{kg/acre-yr}$

$M_o =$ Mass of Pb in soil at a dry bulk density of 1.5 kg/L

$$M_o = (600\ \text{mg/kg})(1.5\ \text{kg/L})(1\ \text{ft})(43{,}560\ \text{ft}^3/\text{acre-ft})(28.32\ \text{L/ft}^3)\left(10^{-6}\ \frac{\text{mg}}{\text{kg}}\right)$$

$M_o =$ 1110 kg/acre (initial mass in soil)

$M =$ 740 kg/acre (clean-up standard of 400 mg/kg)

We assume zero-order kinetics (constant rate of Pb uptake each year) because EDTA will make the lead continue to be bioavailable to the sunflowers.

$$t = \frac{M_o - M}{U} = 9.0\ \text{yr}$$

The time to clean-up may actually be somewhat less than 9 years if Pb migrates down in the soil profile with EDTA addition, or if tillage practices serve to “smooth out” the hot spots. Regulatory clean-up levels are usually based on a limit that cannot be exceeded, such as 400 mg/kg, and soil concentrations would need to be analyzed to ensure compliance at the end of each year.

6.0 COST

Phytoremediation is very competitive with other treatment alternatives. It is aesthetically pleasing and its public acceptability is high. Darlene Bader of the U.S. Army Environmental Center at Aberdeen Proving Ground, reports that two anaerobic wetlands cells followed by two aerobic cells with canary grass were successful in removing TNT at 30% of the cost of granular activated carbon treatment (Table 2). Tables 4 through 6 provide three different estimates for phytoremediation versus competing technologies. In Table 4, a five year cost comparison is made for a phytoremediation design versus a pump and treat system with reverse osmosis for nitrate contaminated groundwater. Phytoremediation is less than half the cost of the pump and treat technology. Table 5 shows the estimated cost advantage of phytoextraction for metals compared to *in situ* fixation, excavation and landfilling in a RCRA approved hazardous waste facility, and soil extraction. Phytoremediation is far less expensive, but it requires five years rather than shorter periods for the competing technologies. In Table 6, the advantage of phytoremediation on petrochemical wastes is shown relative to competing technologies. Once again, phytoremediation offers cost advantages, but the trade off is the amount of time that is required to achieve treatment to action levels.

Phytoremediation is most comparable to *in situ* bioremediation and natural attenuation. In these technologies, mathematical modeling and monitoring are necessary to demonstrate the effectiveness of the technology to regulatory agencies. The same will be true of phytoremediation.

Table 4
Five-Year Cost Comparison of Phytoremediation by Hybrid Poplar Trees versus Conventional Pump and Treat
(Gatliff, E.G., 1996)

1.	Phytotransformation	
	Design and Implementation	$ 50,000
	Monitoring Equipment	
	Capital	10,000
	Installation	10,000
	Replacement	5,000
	5-Year Monitoring	
	Travel and administration	50,000
	Data collection	50,000
	Reports (annual)	25,000
	Sample analysis	50,000
TOTAL		$ 250,000
2.	Pump and Treat (3 wells and Reverse Osmosis System)	
	Equipment	$ 100,000
	Consulting	25,000
	Installation/Construction	100,000
	5-Year Costs	
	Maintenance	105,000
	Operation (electricity)	50,000
	Waste disposal	180,000
	Waste disposal liability	100,000
TOTAL		$ 660,000

Table 5
Cost Advantage of Phytoextraction for Metals
(Phytotech Technical Summary, 1997)

Type of Treatment	Cost/m^3 ($)	Time Required (months)	Additional factors/ expense	Saftety Issues
Fixation	90-200	6-9	Transport/excavation Long-term monitoring	Leaching
Landfilling	100-400	6-9	Long-term monitoring	Leaching
Soil extraction, leaching	250-500	8-12	5,000 m^3 minimum Chemical recycle	Residue disposal
Phytoextraction	15-40	18-60	Time/land commitment	Residue disposal

Table 6
Cost Advantage of Phytoremediation (Rhizosphere Bioremediation)
of Soils Using Fine-Rooted Grasses Compared to Other Techniques
(E. Drake, Exxon, Anandale, NJ, personal communication)

Type of Treatment	Range of Costs $/Ton
Phytoremediation	$10-35
In situ Bioremediation	$50-150
Soil Venting	$20-220
Indirect Thermal	$120-300
Soil Washing	$80-200
Solidification/Stabilization	$240-340
Solvent Extraction	$360-440
Incineration	$200-1,500

7.0 REGULATORY ISSUES

Phytoremediation is too new to be approved by regulatory agencies in *pro forma* reviews. The design team needs to work with regulatory personnel early and often to obtain a satisfactory solution for all parties at the site. Experience dictates that EPA and state agency personnel appreciate being involved at the conceptualization stage because they are interested in testing this emerging technology also. The main question that regulators must answer is whether phytoremediation can remediate the site to standards and reduce risk to human health and the environment.

The answer to this question requires pilot studies and demonstrations on a variety of wastes. This process is beginning to occur with a number of demonstrations listed in Table 2. The questions that remain for most of these projects are the same as those for bioremediation or natural attenuation:

- Can it clean-up the site to below action levels? On what time scale?
- Does it create any toxic intermediates or products?
- Is it as cost-effective as alternative methods?
- Does the public accept the technology?

The answer to the latter two questions appears to be positive because phytoremediation has a large impetus at the present time. The answer to the first two questions will determine whether phytoremediation will become a major new technology in the future.

8.0 CONCLUSIONS

Phytoremediation is an emerging technology for contaminated sites that is attractive due to its low cost and versatility. It is not a panacea for hazardous waste problems, but it shows tremendous potential in several applications for treatment of metals and organics at sites where contamination is shallow. The role of enzymes, metabolites, and the selection of plant systems for various wastes must be better understood. Plants have the ability to withstand relatively high concentrations of pollutants; they can sometimes take-up the chemicals and convert them to less toxic products, and they are known to stimulate degradation of organics in the rhizosphere. The technology has not been demonstrated conclusively at many sites to date, and it remains to be seen if it is effective at full scale. Table 7 is a summary of some of the key factors required for the success of phytoremediation.

Table 7. Summary of Phytoremediation Critical Success Factors and Conditions

Phytoremediation Process	Critical Success Factors/Design Considerations	Conditions for Optimum Likelihood of Success	Basis	Data Needs*	Vegetation
Phytotransformation	uptake by plant; bound residue or metabolism/ volatilization required	log K_{ow} = 1-3.5; nontoxic concentrations	moderately hydrophobic organics taken-up	toxicity, fate	trees, grasses
Rhizosphere Bioremediation	degradation by microbes; dense root system needed	compounds amenable to aerobic biodegradation	dense roots sorb chemicals and enhance microbial degradation	toxicity, fate	trees, grasses, legumes
Phytostabilization	hydraulic control, soil stabilization, immobilization	vigorously growing roots; hydrophobic or immobile chemicals	roots hold soil and water, immobilize metals	toxicity, fate	trees, grasses, legumes
Phytoextraction	plant productivity accumulation in harvestable portion of plant	>3 tons dry matter/ acre-yr; >1,000 mg/kg metals lightly contaminated soil near to clean-up standard	vigorous plant growth provides acceptable uptake rate high ability to accumulate contaminants desirable	toxicity, fate	terrestrial plants or aquatic emergent plants for sediments
Rhizofiltration	sorption/filtration by roots; water in contact with roots; hydraulic detention time	plant densities 200-1000 grams/m^2; hydraulic detention time of several days	roots sorb and immobilize contaminants	toxicity, fate	aquatic emergent or submergent plants

9.0 GLOSSARY

Bound residues - chemical contaminants that are not extractable from plant tissues by conventional methods (covalent bonding, polymerization, or lignification within the plant)

Exudates - release of soluble organic matter from the roots of plants to enhance availability of nutrients or as a by-product of fine root degradation

Lignification - the synthesis of lignin and woody tissue by plants which may incorporate chemical contaminants and immobilize them from the environment

Macropores - openings in the soil matrix caused by worms, burrowing animals, old root channels or soil properties that allow the relatively free flow of water and contaminants through soil

Phytoextraction - the use of plants at waste sites to accumulate metals into the harvestable, above-ground portion of the plant and, thus, to decontaminate soils

Phytostabilization - the use of plants to immobilize contaminants *in situ* by decreasing soil erosion and curtailing vertical migration of contaminants to groundwater by transpiration (hydraulic control)

Phytotransformation - the uptake and transformation (metabolism) or volatilization of organic chemical contaminants by plants as an *in situ* treatment technology

Rhizofiltration - the use of plant roots and rhizosphere to sorb, concentrate, transform, and precipitate organic and metal contaminants from surface water, groundwater, or wastewater

Rhizosphere - the soil profile in close contact with roots of plants, usually taken to be the soil within 1 mm of roots and fine roots

Rhizosphere bioremediation - the microbial transformations of organic contaminants by bacteria, fungi, and protozoans within the biologically-rich zone of the immediate vicinity around plant roots

Root turnover - the rapid decay of fine roots in the soil profile by endogenous respiration

10.0 REFERENCES AND BIBLIOGRAPHY

Adhya, T.K. et al. (1995) Leaching of Hexachlorocyclohexane Isomers in a Flooded Field Unplanted or Planted to Rice. *Water, Air, and Soil Pollut.* 81:305-313.

Ahuja, L.R. et al. (1996) A Field-Test of Root-Zone Water-Quality Model: Pesticide and Bromide Behavior. *Pest. Sci.* 48:101-108.

Anderson, T.A. (1992) Comparative Plant Uptake and Microbial Degradation of Trichloroethylene in the Rhizosphere of Five Plant Species - Implications for Bioremediation of Contaminated Surface Soils. University of Tennessee, Ph.D Dissertation.

Anderson, T.A., Guthrie, E.A. and Walton, B.T. (1993) Bioremediation. *Environ. Sci. Technol.* 27:2630-2636.

Anderson, T.A. and Walton, B.T. (1995) Comparative Fate of ^{14}C Trichloroethylene in the Root Zone of Plants from a Former Solvent Disposal Site. *Environ. Toxicol. Chem.* 14:2041-2047.

Aprill, W.S., Sims, R.C. (1990) Evaluation of the Use of Prairie Grass for Stimulating PAH Treatment in Soil. *Chemosphere*, 20:253-265.

Banuelos, G.S. et al. (1996) Accumulation of Selenium by Different Plant-Species Grown under Increasing Sodium and Calcium-Chloride Salinity. *Plant and Soil* 183:49-59.

Barkovskii, A.L., Boullant, M.L. and Balanbreau, J. (1994) Polyphenolic Compounds Respired by Microorganisms *in*: Bioremediation Through Rhizosphere Technology. American Chemical Society.

Barona, A. and Romero F. (1996) Study of Metal Accumulation in Wild Plants using Principal Component Analysis and Ionic Impulsions. *Toxicol. and Environ. Chem.* 54:75-86.

Bedell, G.W. (1992) Phytoremediation. *The World & I* 12, 260-263.

Berti, W.R., and Jacobs, L.W. (1996) Chemistry and Phytotoxicity of Soil Trace Elements from Repeated Sewage Sludge Applications. *J. Environ. Qual.* 25:1025-1032.

Blaylock, M.J., Muhr, E., Page, D., Montes, G., Vasudev, D., Kapulnik, Y. (1996) Phytoremediation of Lead Contaminated Soil at a Brownfield Site in New Jersey. *Proceedings of Am. Chem. Soc.*, Birmingham, AL, Sept. 9-11.

Blum, U. et al. (1991) Phenolic Acid Content of Soils From Wheat-No Till, Wheat-Conventional Till, and Fallow Conventional Till Soybean Cropping Systems. *J. Chem. Ecology,* 17:1045-1068.

Bowen, G.B. and Rovira, A.D. (1976) Microbial Colonization of Plant Roots. *Ann. Rev Phytopathology*, 14:121-144.

Briggs, G.G., Bromilow, R.H. and Evans, A.A. (1982) Relationships Between Lipophicity and Root Uptake and Translocation of Non-Ionized Chemicals by Barley. *Pestic. Sci.*, 13:495-504.

Brock, T.D. and Madigan, M.T. (1991) *Biology of Microorganisms*. Prentice-Hall, Inc., Englewood Cliffs, NJ.

Brown, K.S. (1995) The Green Clean: The Emerging Field of Phytoremediation Takes Root. *BioScience*, 45:579-582.

Burken, J.G. (1993) Vegetative Uptake by *Populus* spp and Mineralization of Atrazine in Variable Soil Types. University of Iowa, Masters Thesis.

Burken, J.G. and Schnoor, J.L. (1996) Phytoremediation: Plant Uptake of Atrazine and Role of Root Exudates. *J. Environ. Engrg.*, ASCE, 122(11), 958-963.

Burken, J.G. and Schnoor, J.L. (1997a) Uptake and Metabolism of Atrazine by Poplar Trees. *Environ. Sci. Technol*., 31, 1399-1406.

Burken, J.G. and Schnoor, J.L. (1997b) Estimating the Uptake of Organic Contaminants by Hybrid Poplar Trees. *Environ. Sci. Technol.* (submitted).

Chaineau, C.H., Morel, J.L. and Oudot, J. (1996) Biodegradation of Fuel Oil Hydrocarbons in the Rhizosphere of Maize. *Phytoremediation*, IBC Conference, Arlington, VA.

Chen, J., Huang, J.W. and Caspar, T.C. (1996) Using Arabidopsis to Study Lead Accumulation and Tolerance in Plants. American Chemical Society, Orlando, FL.

Clarkson, D.T. (1991) Root Structure and Sites of Ion Uptake *in:* Plant Roots the Hidden Half. Marcel-Dekker, Inc., New York.

Cunningham, S.D. and Ow, D.W. (1996) Promises and Prospects of Phytoremediation. *Plant Physiol.* 110:715-719.

Curl, E.A. and Truelove, B. (1986) *The Rhizosphere*. Springer-Verlag.

Domenico, P.A. and Schwartz, F.W. (1997) *Physical and Chemical Hydrogeology*, John Wiley, New York.

Donnelly, P.K. and Fletcher, J.S. (1994) Potential Use of Mycorrhizal Fungi as Bioremediation Agents *In:* Bioremediation Through Rhizosphere Technology. (Eds: T.A. Anderson and J.R. Coats) American Chemical Society, Washington, D.C.

Dudka, S. et al. (1996) Forms of Cu, Ni, and Zn in Soils of Sudbury, Ontario and the Metal Concentrations in Plants. *Water, Air, and Soil Pollut.* 90:531-542.

Dunn, C.E. et al. (1996) Biogeochemical Studies of Metal-Tolerant Plants from Southern Morocco. *J. Geochemical Exploration* 56:13-22.

Dushenkov, V., Kumar, P.B.A.N., Motto, H., and Raskin, I. (1995) Rhizofiltration: The Use of Plants to Remove Heavy Metals from Aqueous Streams. *Environ. Sci. Technol.* 29:1239-1245.

Ensley, B.D. (1991) Biochemical Diversity in Trichloroethylene Metabolism. *Annu. Rev. Microbiol.*, 45:283-299.

Entry, J.A. et al. (1996) Phytoremediation of Soil Contaminated with Low Concentrations of Radionuclides. *Water, Air, and Soil Pollut.* 88:167-176.

Ferro, A.M. et al. (1996) Uptake and Biodegradation of Volatile Petroleum Hydrocarbons in Planted Systems. *Phytoremediation*, IBC Conference, Arlington, VA.

Ferro, A.M., Sims, R.C. and Bugbee, B. (1994) Hycrest Crested Wheatgrass Accelerates the Degradation of Pentachlorophenol. *J. Environ. Qual.*, 23:272-279.

Fletcher, J. et al. (1995) *Chemosphere* 28:881, 31:3009.

Floyd, R.A. and Ohlrogge, A.J. (1970) Gel Formation on Nodal Root Surfaces of *Zea Mays*. I. Investigation of the Gel's Composition. *Plant and Soil*, 33:331-343.

Foth, H.D. (1990) *Fundamentals of Soil Science*, 8th edition. Wiley, New York.

Gardea-Torresday, J.L. et al. (1996) Ability of Silica-Immobilized *Medicago sativa* (Alfalfa) to Remove Copper Ions from Solution. *J. Hazardous Materials* 48:181-190.

Gardea-Torresday, J.L. et al. (1996) Removal of Nickel Ions from Aqueous Solution by Biomass and Silica-Immobilized Biomass of *Medico sativa* (Alfalfa). *J. Hazardous Materials* 49:205-216.

Gardiner, D.T. et al. (1995) Effects of Repeated Sewage-Sludge Applications on Plant Accumulation of Heavy Metals. *Agriculture Ecosystems and Environment* 55:1-6.

Gatliff, E.G. (1996) Phytoremediation. Ground Water Monitoring Review, Winter/1996.

Gatliff, E.G. (1994) Vegetative Remediation Process Offers Advantages over Pump-and-Treat. *Remediation*, Summer/1994, 343-352.

Goel, A. et al. (1997) Plant Cell Biodegradation of a Xenobiotic Nitrate Ester, Nitroglycerin. *Nature Biotech.* 15:174-177.

Gouin, F.R.; Flamino, P., "Sludge Tree Farm", University of Maryland, College of Agriculture, College Park, Maryland, 1988.

Gravrilova, E.A., Kruglov, Y.V., and Garankina, N.G. (1983) Effects of Plants and Microflora of the Rhizosphere on the Degradation of Basudin in the Soil. *Trudy Vsesoyuznyi Nauchno-Issledovatelskii Institut Selskokhozyaistevennoi Mikrbiologii*, 52:67-70.

Harms, H.H. (1997) Bioaccumulation and Metabolic Fate of Sewage-Sludge Derived Organic Xenobiotics in Plants. *Sci. Tot. Environ.* 185:83-92.

Hartwig, U.A., Joseph, C.M. and Phillips, D.A. (1991) Flavonoids Released Naturally from Alfalfa Seeds Enhance the Growth Rate of *Rhizobium meliloti*. *Plant Physiol.*, 95:797-803.

Hedge, R.S. and Fletcher, J.S. (1996) Influence of Plant-Growth Stage and Season on the Release of Root Phenolics by Mulberry as Related to Development of Phytoremediation Technology. *Chemosphere* 32:2471-2479.

Hopkins, W.G. (1995) *Introduction to Plant Physiology*. John Wiley & Sons, New York.

Hse, W. (1996) Metals Soil Pollution and Treatment by Hybrid Poplar Trees, University of Iowa, M.S. Thesis, Iowa City, IA.

Hsu, M.S.; Schnoor, J.L.; Licht, L.A.; St.Clair, M.A.; Fannin, S.A. *Compost Science & Utilization* 1993, 1(4), 36-48.

Huang, J.W. and Cunningham, S.D. (1996) Lead Phytoextraction: Species Variation in Lead Uptake and Translocation. *New Phytol.* 134:75-84.

Jordahl, J., Foster, L., Alvarez, P.J., and Schnoor, J. (1997) Effect of Hybrid Poplar Trees on Microbial Populations Important to Hazardous Waste Bioremediation. *Environ. Toxicol. Chem.* 16:1318-1381.

Jungk, A.O. (1991) Nutrient Movement at the Soil- *in:* Plant Roots the Hidden Half. Marcel-Dekker, Inc., New York.

Kramer, U. et al. (1996) Free Histidine as a Metal Chelator in Plants that Accumulate Nickel. *Nature* 379:635-638.

Kubiak, R. et al. (1995) Volatilization of Pesticides from Plant and Soil Surfaces: Field Versus Laboratory Experiments. *Internat. J. Environ. Analytical Chemistry* 58:349-358.

Kull, D. (1995) Landfill Leachate and Poplar Tree Perimeter Buffer, M.S. Thesis, University of Iowa, Iowa City, IA.

Kumar, P.B.A.N., Dushenkov, V., Motto, H., and Raskin, I. (1995) Phytoextraction: The Use of Plants to Remove Heavy Metals from Soils. *Environ. Sci. Technol.* 29:1232-1238.

Licht, L.A. (1990) Poplar Trees for Nonpoint Source Pollution Control and Biomass Production. University of Iowa, Ph.D. Dissertation, Iowa City, IA.

Licht, L.A.; Marinko, A.; Macarol, B. (1994) Ecolotree™ Cap at the Barje Landfill in Ljubljana, Slovenia. Proceedings of the Second International Conference on Energy and Environment: Transitions in East Central Europe, Prague, Czech Republic, November.

Madison, M.; Licht, L.A.; Wilson, D. (1994) Ecolotree™ Buffer for Landfill Leachate Management Installation and Operational Summary, 94-WA86.03, Proceedings of the 87th Meeting of the Air & Waste Management Assoc., Cincinnati, Ohio, June.

Madison, M.F., Licht, L.A. (1991) Landfill Cap Closure Utilizing a Tree Ecosystem. Proceedings of the American Society of Agricultural Engineers ASAE, Albuquerque, New Mexico, December.

McCrady, J.K., McFarlane, C. and Gander, L.K. (1990) Transport and Fate of 2,3,7,8-TCDD in Soybean and Corn. *Chemosphere*, 21:359-376.

McGinty, T. (1996) Plants Weed Out Lead Contamination. *Princeton Metro*, Princeton, New Jersey, April 24, 1996, page 1.

McFarlane, J.C. et al. (1987) The Uptake, Distribution and Metabolism of Four Organic Chemicals by Soybean Plants and Barley Roots. *J. Environ. Toxicol. Chem.*, 6:847-856.

McFarlane, J.C., Pfleeger, T. and Fletcher, J.S. (1987) Transpiration Effect on the Uptake and Distribution of Bromacil, Nitrobenzene, and Phenol in Soybean Plants. *J. Environ. Qual.*, 16:372-376.

McFarlane, J.C., Pfleeger, T. and Fletcher, J.S. (1990) Effect, Uptake and Disposition of Nitrobenzene in Several Terrestrial Plants. *J. Environ. Toxicol. Chem.*, 9:513-520.

McKone, T.E. (1993) The Precision of QSAR Methods for Estimating Intermedia Transfer Factors in Exposure Assessments. *SAR and QSAR in Environmental Research*, 1:41-53.

Moser, M. and Haselwandter, K. (1983) Ecophysiology of Mycorrhizal Symbiosis *in:* Physiological Plant Ecology III: Responses to the Chemical and Plant Environment. Lange, O.L., Nobel, P.S., Osmond, C.B., Ziegler, H., Eds., Springer-Verlag, Berlin, pp. 391-411.

Nair, D.R. and Schnoor, J.L. (1992) Effect of Two Electron Acceptors on Atrazine Mineralization Rates in Soil. *Environ. Sci. Technol.*, 26:2298-2300.

Nair, D.R. and Schnoor, J.L. (1993) Effect of Soil Conditions on Model Parameters and Atrazine Mineralization Rates. *Water Res*, 28:1199-1205.

Nair, D.R., Burken, J.G., Licht, L.A., and Schnoor, J.L. (1993) Mineralization and Uptake of Triazine Pesticide in Soil-Plant Systems. *ASCE J. Environ. Eng.*, 119:842-854.

Nellesen, J.E. and Fletcher, J.S. (1993) Assessment of Published Literature Pertaining to the Uptake/Accumulation, Translocation, Adhesion, and Biotransformation of Organic Chemicals by Vascular Plants. *Environ. Toxicol. Chem.*, 12:2045-2052.

Newman, L.A.; Strand, S.E.; Choe, N.; Duffy, J.; Ekuan, G.; Ruszaj, M.; Shurtleff, B.B.; Wilmoth, J.; Heilman, P.; Gordon, M.P. (1997) Uptake and Biotransformation of Trichloroethylene by Hybrid Poplars, *Environ. Sci. Technol.* 31(4):1062-1067.

O'Connor, G.A. (1996) Organic Compounds in Sludge-Amended Soils and Their Potential for Uptake by Crop Plants. *Sci. Tot. Environ.* 185:71-81.

Oborn, I., Jansson, G. and Johnsson, L. (1995) A Field-Study on the Influence of Soil-pH on Trace-Element Levels in Spring Wheat (*Triticum aestivam*), Potatoes (*Solanum tuberosum*) and Carrots (*Daucus carota*). *Water, Air, and Soil Pollut.* 85:835-840.

Onken, B.M. and Hossner, L.R. (1995) Plant Uptake and Determination of Arsenic Species in Soil Solution Under Flooded Conditions. *J. Environ. Qual.* 24:373-381.

Painter, D. (1996) Phytoremediation of TNT. *Military Engineer* 88:45-46.

Paterson, K.G. and Schnoor, J.L. (1992) Fate of Alachlor and Atrazine in a Riparian Zone Field Site. *Water Environment Research*, 64:274-283.

Paterson, K.G. and Schnoor, J.L. (1993) Vegetative Alteration of Nitrate Fate in the Unsaturated Zone. *J. Environmental Engineering,* ASCE, 119:986-993.

Perchovich, B.S. et al. (1996) Enhanced Mineralization of (C-14) Atrazine in a *Kochia scoparia* Rhizospheric Soil from a Pesticide-Contaminated Site. *Pesticide Science* 46:391-396.

Raskin, I. (1996) Making Phytoremediation Work. Presented at Conference on Phytoremediation - Cost-Effective Clean-Up, International Business Communications, Arlington, Virginia, May 8-10, 1996.

Rock, S. (1997) Personal Communication, March 17, 1997.

Rogers, H.B. et al. (1996) Selection of Cold-Tolerant Plants for Growth in Soils Contaminated with Organics. *J. Soil. Contam.* 5:171-186.

Roper, J.C., Dec, J. and Bollag, J.M. (1996) Using Minced Horseradish Roots for the Treatment of Polluted Waters. *J. Environ. Qual.* 25:1242-1247.

Rovira, A.D., Foster, R.C. and Martin, J.R. (1979) Note on Terminology: Origin, Nature, and Nomenclature of Organic Materials in the Rhizosphere *in:* The Soil-Root Interface. Academic Press, New York.

Ryan, J.A., Bell, R.M., Davidson, J.M. and O'Connor, G.A. (1988) Plant Uptake of Non-Ionic Organic Chemicals from Soils. *Chemosphere*, 17:2299-2323.

Sajwan, K.S., et al. (1996) Uptake of Soil Applied Cadmium, Nickel, and Selenium by Bush Beans. *Water, Air, and Soil Pollut.* 91:209-217.

Salim, R., Alsubu, M.M. and Ismail Y.S. (1995) Effects of Several Factors on the Growth and on the Metal Uptake and Uptake Distribution of Okra Plant Treated with Cadmium. *J. Environ. Sci. & Health*, Part A 30:2027-2040.

Salim, R., Alsubu, M.M. and Ismail Y.S. (1995) Effects of Several Factors on the Growth and on the Metal Uptake Distribution of Pepper Plants Treated with Cadmium. *J. Environ. Sci. & Health*, Part A 30:1659-1684.

Salim, R., Alsubu, M.M. and Ismail Y.S. (1995) Growth, Metal Uptake, and Uptake Distribution of Spinach and Parsley Plants Irrigated with Copper Solutions. *J. Environ. Sci. & Health,* Part A 30:2057-2069.

Salt, D.E. et al. (1995) Phytoremediation: A Novel Strategy for the Removal of Toxic Metals from the Environment using Plants. *Biotechnology*, 13:468-474.

Samtsesvich, S.A. (1965) Active Excretions of Plant Roots and their Significance. *Sov. Plant Phys.*, 12:731-739.

Scheunert, I. (1992) Fate of Pesticides in Plants and in Soil Fauna *In: Chemistry of Plant Protection* 8.

Schnoor, J.L. (1996) *Environmental Modeling - Fate and Transport of Pollutants in Water, Air, and Soil*, John Wiley and Sons, New York, 682 pp.

Schnoor, J.L., Licht, L.A., McCutcheon, S.C., Wolfe, N.L. and Carriera, L.H. (1995) Phytoremediation: An Emerging Technology for Contaminated Soils. *Environ. Sci. Technol.*, 29:318-323A.

Schwartz, P. (1991) Effect of Poplar Trees on Fate of Atrazine in a Model Tree System Through Metabolism, Degradation and Accumulation. University of Iowa, Iowa City, Masters Thesis.

Schwarzenbach, R.P., Gschwend, P.M. and Imboden, D.M. (1993) *Environmental Organic Chemistry*. John Wiley & Sons, Inc., New York.

Shimp, J.F.; Tracy, J.C.; Davis, L.C.; Lee, E.; Huang, W.; Erickson, L.E.; Schnoor, J.L. (1993) Beneficial Effects of Plants in the Remediation of Soil and Groundwater Contaminated with Organic Materials. *Crit Rev in Env Sci & Tech*, 23:41-77.

Shurtliff, M.M., Parkin, G.F., Weathers, L.J., and Gibson, D.T. (1996) Biotransformation of Trichloroethylene by a Phenol-Induced Mixed Culture. *ASCE Environmental Journal*, 122:581-589.

Strand, S.E. et al. (1995) Removal of TCE from Aquifers Using Trees. ASCE Conf. Proceedings, Pittsburgh, PA.

Tashiro, W. at al. (1995) Metabolic Fate of Insecticide Pyraclofos in Cabbage Plants. *J. Pesticide Sci.* 20:463-470.

Thompson, P.L. and Schnoor, J.L. (1996) Phytoremediation of Ammunition Wastes (TNT, RDX) by *Populus* spp. Emerging Technologies in Hazardous Waste Management VIII, Birmingham, AL, American Chemical Society, Washington, D.C.

Trapp, S. McFarlane, C. and Matthies, M. (1994) Model for Uptake of Xenobiotics into Plants: Validation with Bromacil Experiments. *Environ. Toxicol. Chem.*, 13:413-422.

Truksa, M. et al. (1996) Transgenic Plants Expressing Metal-Binding Proteins in Phytoremediation. *Chemicke Listy* 90:707-707.

Tsai, S.M. and Phillips, D.A. (1991) Flavonoids Released Naturally from Alfalfa Promote Development of Symbiotic *Glomus* Spores *in vitro*. *Appl. Environ, Microbiol.*, 57:1485-1488.

Vanderkrol, D. et al. (1995) Metabolism of (ring-2,6-C-14) Parathion in Plant Cell Suspension Cultures of Carrot (*Daucus carota*), Purple Foxglove (*Digitalis purpurea*), Soybean, Thorn-Apple (*Datura stramonium*) and wheat (*Triticum aestivum*). *Pesticide Science* 45:143-152.

Voudrias, E.A. and Assaf, K.S. (1996) Theoretical Evaluation of Dissolution and Biochemical Reduction of TNT for Phytoremediation of Contaminated Sediments. *J. Contam. Hydrol.* 23:245-261.

Walker, A. (1972) Availability of Atrazine to Plants in Different Soils. *Pestic. Sci.*, 3:139-148.

Walker, A. and Featherstone, R.M. (1973) Uptake and Translocation of Atrazine and Linuron by Carrot and Parsnip. *Journal of Experimental Botany*, 24:450-458.

Walski, T.M. (1993) Long-Term Effects of Wetland Treatment of Mine Drainage. *J. Environmental Engineering*, ASCE, 119:1004-1005.

Wang, M.J. and Jones, K.C. (1994) Uptake of Chlorobenzenes by Carrots from Spiked and Sewage Sludge-Amended Soil. *Environ. Sci. Technol.*, 28:1260-1267.

Wichman, M. (1990) Fate and Toxicity of Volatile Organic Chemicals in a Poplar Plot. University of Iowa, M.S. Thesis, Iowa City, IA.

Wieder, R.K. (1993) Ion Input-Output Budgets for Five Wetlands Constructed for Acid Coal Mine Drainage Treatment. *Water, Air, and Soil Pollution*, 71:231-270.

Wilken, A., Bock, C., Bokern, M. and Harms, H. (1995) Metabolism of Different PCB Congeners in Plant-Cell Cultures. *Environ. Toxicol. Chem.* 14:2017-2022.

Young, P. (1996) The New Science of Wetland Restoration. *Environ. Sci. Technol.*, 30:292A-296A.