Phytoremediation of TCE in Groundwater using *Populus*

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FOREWORD

The potential use of plants to remediate contaminated soil and groundwater has recently received a great deal of interest. EPA's Technology Innovation Office (TIO) provided a grant through the National Network for Environmental Management Studies (NNEMS) to assess the status of phytoremediation technologies to clean up shallow groundwater. This report was prepared by a graduate student from Duke University during the summer of 1997. It has been reproduced to help provide federal and state project managers responsible for hazardous waste sites with information on the current status of this technology.

About the National Network for Environmental Management Studies (NNEMS)

NNEMS is a comprehensive fellowship program managed by the Environmental Education Division of EPA. The purpose of the NNEMS Program is to provide students with practical research opportunities and experiences.

Each participating headquarters or regional office develops and sponsors projects for student research. The projects are narrow in scope to allow the student to complete the research by working full-time during the summer or part-time during the school year. Research fellowships are available in Environmental Policy, Regulations, and Law; Environmental Management and Administration; Environmental Science; Public Relations and Communications; and Computer Programming and Development.

NNEMS fellows receive a stipend determined by the student's level of education and the duration of the research project. Fellowships are offered to undergraduate and graduate students. Students must meet certain eligibility criteria.

About this Report

This report is intended to provide a basic orientation and current status of phytoremediation for shallow groundwater. It contains information gathered from a range of currently available sources, including project documents, reports, periodicals, Internet searches, and personal communication with involved parties. No attempts were made to independently confirm the resources used.

Jonathan Chappell, NNEMS fellow, would like to acknowledge the support and encouragement received for the completion of this report from the EPA's Technology Innovation Office and from the contributors to this report for the invaluable information and comments they provided for the completion of this paper.

While the original report included color images, this copy is printed in one color. Readers are directed to the electronic version of this report to view the color images; it is located at **http://clu-in.com/phytoTCE.htm**

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The Technology Innovation Office (TIO) would like to acknowledge and thank the individuals who reviewed and provided comments on draft documents. The reviewers included representatives of business, community and grassroots organizations, EPA Headquarters and regional offices, local government and city planning offices, and professional associations representing local and state government officials.

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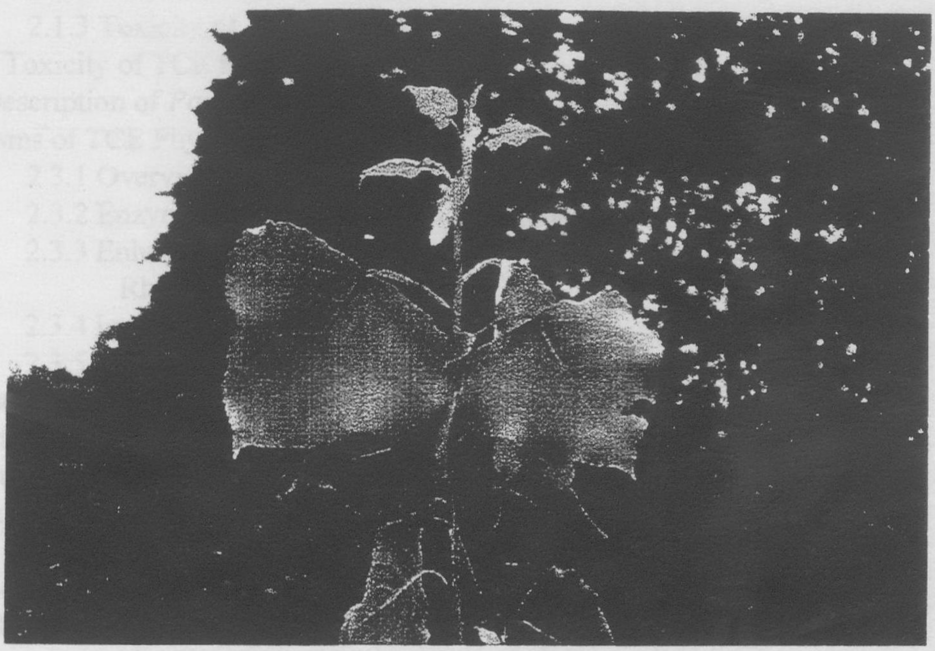

Hybrid poplar (*Populus charkowiiensis x incrassata*, NE 308) at Edward Sears Property

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Purpose

The purpose of this report is to briefly summarize the current state of phytoremediation technology, and then focus on the use of poplar trees (*Populus* sp.) to degrade trichloroethylene (TCE) in groundwater. The summary of phytoremediation will serve as an introduction to this technology. It will address some common concerns such as the cost and performance of phytoremediation. The analyzation of TCE phytoremediation will begin with separate discussions of TCE and poplars, followed by a detailed section on the use of poplars to treat TCE contamination. The final section will present three case studies detailing Department of Defense and Superfund sites where poplars have been planted in order to treat TCE contamination in groundwater.

1. Overview of Phytoremediation

Phytoremediation is an emerging technology which uses plants and their associated rhizospheric microorganisms to remove, degrade, or contain chemical contaminants located in the soil, sediments, groundwater, surface water, and even the atmosphere. Researchers have found that plants can be used to treat most classes of contaminants, including petroleum hydrocarbons, chlorinated solvents, pesticides, metals, radionuclides, explosives, and excess nutrients. Plant species are selected for phytoremediation based on their potential to evapotranspirate groundwater, the degradative enzymes they produce, their growth rates and yield, the depth of their root zone, and their ability to bioaccumulate contaminants.

Table 1 lists the various applications of phytoremediation technologies. This list indicates that phytoremediation is actually a broad class of remediation techniques which include many treatment strategies. Obviously, the common thread through all of these techniques is the use of plants to treat a contaminant problem. However, due to the diverse nature of chemical contamination and the diversity of plants with the potential to treat them, remedial project managers must choose between a wide variety of phytoremediation techniques to solve the problem at hand.

Despite the diversity of phytoremediation technologies, its application is limited by a number of factors. Phytoremediation can only work at sites that are well suited for plant growth. This means that the concentration of pollutants cannot be toxic to the plants, and the pollution cannot be so deep in the soils or groundwater that plant roots cannot reach it. As a result, phytoremediation may be a good strategy for sites conducive to plant growth with shallow contamination, it may be a good secondary or tertiary phase in a treatment train for highly polluted sites, or it may not be a viable option for a site.

Table 1: Types of Phytoremediation Systems

Treatment Method	Mechanism	Media
Rhizofiltration	Uptake of metals in plant roots	surface water and water pumped through troughs
Phytotransformation	Plant uptake and degradation of organics	surface water, groundwater
Plant-Assisted Bioremediation	Enhanced microbial degradation in the rhizosphere	soils, groundwater within the rhizosphere
Phytoextraction	Uptake and concentration of metals via direct uptake into plant tissue with subsequent removal of the plants	soils
Phytostabilization	Root exudates cause metals to precipitate and become less bioavailable	soils, groundwater, mine tailings
Phytovolatilization	Plant evapotranspirates selenium, mercury, and volatile organics	soils, groundwater
Removal of organics from the air	Leaves take up volatile organics	air
Vegetative Caps	Rainwater is evapotranspirated by plants to prevent leaching contaminants from disposal sites	soils

Source: Adapted from Miller (1996) and Workshop on Phytoremediation of Organic Contaminants (1997)

Even though phytoremediation appears to have limited application, researchers in industry, academia, and government are looking into phytoremediation as a useful treatment technology. In fact, a number of companies that offer phytoremediation technologies have been started in the last few years, and many larger consulting firms are beginning to offer phytoremediation services as well. Appendix A lists many of these companies, but this list is not complete; there are likely many more companies who either currently offer phytoremediation services or will offer them in the near future. Again, since phytoremediation covers a broad spectrum of pollutants and treatments, many of these companies focus most of their attention on one niche of the phytoremediation field (e.g., metal extraction from soils, poplar tree buffers, etc.).

Most of the phytoremediation companies in Appendix A have already used, or are using, phytoremediation in the field. For example, four phytoremediation projects have been accepted for the Superfund Innovative Technology Evaluation (SITE) program. One project by Phytotech, Inc. involves the use of plants to extract metals from soils. The second project by Phytokinetics involves the use of poplar trees to treat PAH contaminated groundwater. Another project by Phytokinetics uses grasses to remediate surficial soils contaminated with PCP and PAHs. The fourth phytoremediation SITE project involves the use of cottonwoods to treat a plume of TCE in shallow groundwater at the Carswell Air Force base in Ft. Worth, TX. This last project will be detailed as a case study later in this report. In addition, a number of other pilot and field scale projects have taken place around the country. Appendix B summarizes some of these projects, including the types of contaminants present and treatment techniques employed at these sites.

1.1 Advantages and Disadvantages of Phytoremediation

Early research indicates that phytoremediation technology is a promising cleanup solution for a wide variety of pollutants and sites, but it has its limitations. Table 2 summarizes some of the advantages and limitations of phytoremediation. An examination of the table reveals that many of phytoremediation's limitations and advantages are a direct result of the biological aspect of this type of treatment system. Plant-based remediation systems can function with minimal maintenance once they are established, but they are not always the best solution to a contamination problem. One way to summarize many of phytoremediation's limitations is that the pollutant must be bioavailable to a plant and its root system. If a pollutant is located in a deep aquifer, then plant roots cannot reach it. If a soil pollutant is tightly bound to the organic portion of a soil, then it may not be available to plants or to microorganisms in the rhizosphere. On the other hand, if a pollutant is too water soluble it will pass by the root system without any uptake.

Table 2: Advantages and Limitations of Phytoremediation

Advantages of Phytoremediation	Limitations of Phytoremediation
in situ	Limited to shallow soils, streams, and groundwater
Passive	High concentrations of hazardous materials can be toxic to plants
Solar driven	Mass transfer limitations associated with other biotreatments
Costs 10% to 20% of mechanical treatments	Slower than mechanical treatments
Transfer is faster than natural attenuation	Only effective for moderately hydrophobic contaminants
High public acceptance	Toxicity and bioavailability of degradation products is not known
Fewer air and water emissions	Contaminants may be mobilized into the groundwater

Advantages of Phytoremediation	Limitations of Phytoremediation
Generate less secondary wastes	Potential for contaminants to enter food chain through animal consumption
Soils remain in place and are usable following treatment	Unfamiliar to many regulators

1.2 Performance

A major hurdle for innovative technologies to overcome is a lack of performance data, and phytoremediation is no exception. One of the current barriers to performance data is the length of time involved in a phytoremediation project. Plants can only grow so fast, so obtaining long term performance results is dependent on the rates of plant growth and activity. There are currently a number of pilot scale projects in existence, but they have not resulted in conclusive performance data at this time. These sites are being monitored and will report results over the next few years. Also, a number of firms have installed phytoremediation systems at polluted sites owned by private clients, so results from those sites are not publicly available.

Some performance data is included in the "Case Studies" section of this report. However, these studies are not complete, so the data presented is from early in those treatments. When looking at performance data, one needs to keep in mind that most data is very site specific, especially for a biological system like phytoremediation. For example, transpiration rates reported for a hot, arid region may not match those from a cooler climate. Performance may also be seasonal since many plants are dormant during the winter months. This is especially important when planning projects or comparing results of projects from different regions.

1.3 Cost

In addition to performance data, accurate cost data is often difficult to predict for new technologies. Most lab, pilot, and field scale tests include monitoring procedures far above those expected at a site with a remediation goal. This inflates the costs of monitoring at these test sites. As a result, it is difficult to predict the exact cost of a technology that has not been established through years of use. However, since phytoremediation involves the planting of trees or grasses, then it is by nature a relatively inexpensive technology when compared to technologies that involve the use of large scale, energy consuming equipment.

Phytoremediation costs will vary depending on the treatment strategy. For example, harvesting plants that bioaccumulate metals can drive up the cost of treatment when compared to treatments that do not require harvesting. Regardless, phytoremediation is often predicted to be cheaper than comparable technologies.

Tables 3 and 4 were included to outline some of the predicted costs of phytoremediation. Table 3 presents some estimates of phytoremediation's costs in relation to conventional technologies. This table represents some vague and variable estimates due to the current dearth of cost information. Since the bulk of this report deals with the use of poplar tree systems, Table 4 lists some of the costs listed by two companies who specialize in poplar designs. Keep in mind that costs of phytoremediation are highly site specific, so that any estimate found in these tables is merely a rough estimate of potential costs. Many of these estimates are speculative based on laboratory or pilot scale data.

Table 3: Estimates of Phytoremediation Costs Versus Costs of Established Technologies

Contaminant	Phytoremediation Costs	Estimated Cost using Other Technologies	Source
Metals	$80 per cubic yard	$250 per cubic yard	Black (1995)
Site contaminated with petroleum hydrocarbons (site size not disclosed)	$70,000	$850,000	Jipson (1996)
10 acres lead contaminated land	$500,000	$12 million	Plummer (1997)
Radionuclides in surface water	$2 to $6 per thousand gallons treated	none listed	Richman (1997)
1 hectare to a 15 cm depth (various contaminants)	$2,500 to $15,000	none listed	Cunningham et al. (1996)

Table 4: Ecolotree's and Applied and Natural Science, Inc.'s Cost Estimates of a Poplar Tree Phytoremediation System*

Ecolotree	
Activity	**Cost**
Installation of trees at 1450 trees/acre	$12,000 to $15,000
Predesign	$15,000
Design	$25,000
Site Visit	$5,000
Soil cover and amendments	$5,000
Transportation to site	$2.14/mile
Operation and Maintenance	$1,500/acre with irrigation $1,000/acre without irrigation
Pruning (not every year)	$500
Harvest (during harvest years)	$2,500
Applied Natural Science	
Activity	**Cost**
Treemediation program design and implementation	$50,000
Monitoring equipment	Hardware - $10,000
	Installation - $ 10,000
	Replacement - $5,000
Five-year monitoring	Travel and Meetings - $50,000
	Data collection- $50,000
	Annual reports - $25,000
	Sample collection and analysis - $50,000

* Estimates will vary with type of contaminant, goal of project (i.e., containment vs. removal), and location.

2. Phytoremediation of TCE with Trees in the Genus *Populus*

Researchers have been investigating the possibility of using trees in the genus *Populus* to hydraulically contain and ultimately remediate plumes of TCE in groundwater. In addition, managers at several Department of Defense and Superfund hazardous waste sites have planted *Populus* sp. in an effort to treat groundwater contaminated with chlorinated solvents. This section will focus on the available information about the use of *Populus* sp. to phytoremediate plumes of TCE. Included are background sections on TCE and poplar trees, followed by sections on mechanisms of TCE phytoremediation, other phytoremediation applications of poplars, and a comparison of conventional remediation methods to poplar systems.

2.1 Description of TCE

Trichloroethylene (TCE) is a common contaminant at many of the nation's hazardous waste sites. It can be found at 50% of Superfund National Priority List (NPL) sites with completed Records of Decision (RODs), and it is above action levels in the groundwater of 17% and soils of 16% of RCRA corrective action facilities (USEPA 542-R-96-005). TCE pollution became prevalent primarily through its use as an industrial degreasing agent. Other uses of TCE include its use as a solvent for dry cleaning, an anaesthetic for medical and dental use and as an ingredient in paints, inks, cosmetics, disinfectants, and cleaning fluids. Due to widespread TCE contamination, finding innovative ways to clean this pollutant has become a priority in the remediation field.

2.1.1 Physical and Chemical Properties of TCE

Examining the physical and chemical characteristics of a target compound is very important when choosing a remediation strategy. The physical characteristics of TCE, which are listed in Table 5, make it difficult to remove from the groundwater using traditional technologies. TCE is a dense non-aqueous-phase liquid (DNAPL), meaning that it is denser than water and therefore tends to exist in undissolved pools in the bottom an aquifer. This property makes it very difficult to treat TCE by methods such as pump and treat because it is almost impossible to tap into small pools of undissolved TCE that reside in the groundwater. Pump and treat methods can remove TCE that is in the aqueous phase, but since pools of non-aqueous TCE are in equilibrium with the groundwater, more TCE will dissolve into the aqueous phase as the groundwater is treated. This results in a continuous cycle of slow dissolution from the non-aqueous to the aqueous phase that could take many years and large amounts of money and energy to treat.

Table 5: Physical and Chemical Properties of TCE

Property	Value
Molecular weight	131.5
Boiling point	87° C
Melting point	-73° C
Specific gravity	1.4642 at 20° C
Solubility in water	1,000 mg/liter
Log octanol/water partitioning coefficient	2.29
Vapor pressure	60 mm Hg at 20° C
Vapor density	4.53

Source: Clement Associates (1985)

Many scientists and hazardous waste site managers, such as those involved with the three case studies included in this report, believe that it is more efficient to use trees as a solar driven pump and treat mechanism in a long term treatment process for TCE contamination. Others, however, remain skeptical. Cunningham et al. (1996) stated that TCE and perchloroethylene (PCE) are "a relatively poor choice of targets for phytoremediation" because they tend to form dense pools near the bottom of an aquifer, out of the reach of tree roots. Current studies will likely provide some answers to this debate.

2.1.2 TCE Availability to Plant Roots

In order for a plant to directly degrade, mineralize, or volatilize a compound, it must be able to take that compound up through its roots. The ability of a plant to take up a chemical from the soil and groundwater and translocate it to its shoots is described by a chemical's root concentration factor (RCF) and transpiration stream concentration factor (TSCF). The RCF is a measure of the root concentration of a contaminant versus the concentration in the external solution, while the TSCF is a measure of the concentration in the xylem sap in relation to the concentration in the external solution. Both of these factors vary directly with a chemical's water solubility, commonly expressed as its log K_{ow}. According to Briggs et al. (1982), contaminants in solution with the highest TSCF are moderately soluble compounds with a solubility in the range of log K_{ow} 1.5 to 2. However, several reviews expand this optimum range to include log K_{ow} values as low as 0.5 and as high as 3 (Schnoor et al. 1995, Schimp et al. 1993). Most chlorinated solvents, including TCE, fall within this expanded range, along with BTEX chemicals and short chain aliphatics. As a result, phytoremediation appears to be a viable option for treating dissolved TCE in groundwater.

Soils, on the other hand, pose a potential problem for plants because may they contain high levels of organic matter. In soils, the log K_{ow} for maximum TSCF can be shifted down to favor more polar molecules because of the competing process of sorption to soil organic matter (Cunningham et al. 1996). As a result, plant roots alone may have a difficult time extracting TCE from soils containing significant amounts of organic matter. However, microorganisms in the rhizosphere are capable of degrading TCE. Anderson and Walton (1995) found that TCE mineralization was significantly enhanced in the rhizoshpere of a number of plant species, such as *Pinus taeda* (loblolly pine) and *Lespedeza cuneata*, when compared with nonvegetated soils. The exact mechanisms of rhizospheric degradation will be discussed in a later section.

2.1.3 Toxicity of TCE to Animals

TCE has been found to be carcinogenic to laboratory mice in a number of studies reported by the National Institute of Environmental Health Sciences (T-2, TR-243). However, these studies did not find TCE carcinogenic to laboratory rats. TCE was found to be a mutagen when tested using microbial assay systems, and chronic inhalation exposure to TCE causes liver, kidney, neural, and dermatological reactions in animals (Clement Associates 1985). The EPA drinking water standard for TCE has been set at 5 parts per billion (ppb), and the EPA has also reported that drinking 1 part per million (ppm) TCE in water over a lifetime will cause 32 humans in a population of 100,000 to be at risk of cancer (EPA 540/R-94/044).

Vinyl chloride, which may result from the anaerobic breakdown of TCE by microorganisms, is regarded as a more potent human carcinogen than TCE. The EPA drinking water standard for vinyl chloride is 2 ppb, and the EPA estimates that drinking 1 ppm vinyl chloride over a lifetime will cause 9,570 cases of cancer in a population of 100,000 people (EPA 540/R-94/044). Since this microbial breakdown product is more chronically toxic than its parent compound, regulators are concerned with the exact fates of TCE in a remediation system.

2.1.4 Toxicity of TCE to Plants

While it is important to understand the concentrations of TCE that are toxic to plants, few studies report the phytotoxic effects of TCE. Gordon et al. (1997) reported that poplars were able to survive when grown in water containing 50 ppm TCE. Another experiment found TCE to be acutely toxic to a variety of crop plants at concentrations of about 2 mM in the gas phase (Ryu et al. 1996). The later study hypothesized that an increase in electrolyte leakage or interference with the photosynthetic system was the mechanism of acute toxicity in the plants, but the exact mechanism is not known.

2.2 Description of *Populus*

The genus *Populus* includes a number of species of trees such as poplars, cottonwoods, and aspens. *Populus* is a member of the *Salicaceae* family, which also includes willows. There are around 30 species of *Populus* distributed around the Northern Hemisphere, with eight species indigenous to North America and others that have been introduced. In addition, *Populus* sp. have the ability to cross within the genus both in the wild and through controlled breeding, so there are a large number of potential hybrids (Dickmann and Stuart 1983).

Due to their ability to readily form hybrids, poplars have been crossed by foresters for years in order to maximize growth rates and yield. Hybrid poplars were originally bred and grown as a cash crop for such uses as pulp wood and as a renewable energy source (Poplars and Willows on the WWW), but because of their rapid growth rates and high evapotranspiration rates, they make ideal candidates for phytoremediation. Table 6 summarizes many of the advantages of using poplars for phytoremediation.

Table 6: Advantages of *Populus* sp. in Phytoremediation

▸	Greater than 25 species worldwide
▸	Fast growing (3 to 5 meters/year)
▸	High transpiration rates (100 liters/day optimally for 5 year old tree)
▸	Not part of food chain
▸	Trees can be used for paper production or as biomass for energy
▸	Long lived (25-30 years)
▸	Grow easily from cuttings
▸	Can be harvested and then regrown from the stump

Source: Adapted from Gordon (1997) and Schnoor et al. (1995)

The goal of poplar hybridization is to achieve heterosis, which means that the genetic traits of hybrids exceed those of the parents (Dickmann and Stuart 1983). Two species of poplars, *Populus deltoides* (eastern cottonwood) and *Populus trichocarpa* (black cottonwood), are commonly crossed for use in phytoremediation. *Populus trichocarpa x deltoides,* pictured in Figure 1, have leaves that are about four times as large as the leaves of parent plants (ORNL 1996). Increasing leaf size increases the potential evapotranspiration rates of these trees due to increased total leaf surface area. Another common cross that has been used in phytoremediation studies is *P. deltoides x P. nigra* (black poplar). This cross is sometimes referred to as *P. x euramericana* due to the original distribution of the two species, the black poplar in Europe and the eastern cottonwood in North America. There are many other hybrids of poplars that have been developed, some of which have been or will likely be used in phytoremediation systems.

One piece of information that concerns most of those interested in poplar phytoremediation projects is the evapotranspiration rates of the trees. Poplars and cottonwoods are phreatophytic plants, which means that they can extend their roots to the water table and pump from the zone of saturation. For this reason, the presence of a number of cottonwood species in desert regions, such as *Populus fremontii* and *Populus wizlizeni*, has been historically used as an indicator of relatively shallow groundwater (Meinzer 1927). This ability to pump groundwater has earned poplars the name "solar driven pump and treat systems" in the phytoremediation field. Knowing how well these trees can act as a solar powered pump will aid in deciding whether or not they can be used to treat a particular site. A plot of trees with high evapotranspiration rates can cause a significant draw-down in the water table, which results in a hydraulic barrier to contaminant transport. Results of a number of studies indicate that a stand of poplars can cause a depression in the water table ranging from several inches (Workshop on

Phytoremediation of Organic Contaminants 1996) to several feet (see Aberdeen case study).

Unfortunately, since trees are solar driven and biological, as opposed to mechanical, pumping rates will vary with tree age and species as well as time of day, time of year, amounts of solar radiation, and other climatic and geographic factors. As a result, pumping rates will be highly site specific. Table 7 lists some reported evapotranspiration rates for individual poplar trees. This list includes a wide range of pumping rates, from 1.6 gpd/tree for a young tree to 53 gpd/tree for a five year old poplar.

Table 7: Estimates of Evapotranspiration Rates by Hybrid Poplars

Rate	Source
100 to 200 L/day/tree (~26 to 53 gallon/day) for 5 year old trees	Newman et al (1997)
100 L/day/tree for a 5 year old tree under optimal conditions	Stomp et al. (1994)
13 gallons per day (estimated) when trees are calculated as low-flow pumping wells	Sheldon Nelson - Workshop on Phytoremediation of Organic Contaminants (1996)
1.6 to 10 gpd/tree (observed) sap flow rates for young hybrid poplars at the Aberdeen Proving grounds in Maryland	Compton (1997)
10 - 11 kg/tree/day (observed) in early summer for 1-2 year old Eastern cottonwoods growing in Texas	Greg Harvey (personal communication)
40 gallons per day (observed) for 5 year old trees in Utah in the summer	Ari Ferro - Workshop on Phytoremediation of Organic Contaminants (1996)

Trees will transpire at different rates when grown together as a stand than they would when grown individually. This is because evapotranspiration varies with the total leaf surface area, whether it be for an individual tree or an entire stand. This means that tree density is an important consideration for phytoremediation. A dense stand will have less leaf surface area per tree, but the combined leaf surface area of a dense stand will be greater than the combined surface area of a thin stand. Some information on evapotranspiration is available for stands of poplar trees. For example, Gordon et al. (1997) reported that a stand of 5 year old poplars could cause a 140 cm/year draw-down in the water table when grown at a density of 1,750 trees/ha in the warm, arid conditions of eastern Washington state. Table 8 provides measured water uptake per hectare and per acre of poplars grown at a density of 2,170 trees/acre at varying tree ages. This table, along with Table 7, provides some good approximations of the pumping efficiencies of *Populus* sp. However, evapotranspiration rates will vary from site to site.

Table 8: Growth and Water Uptake Potential in Five Growing Seasons in Amana, IA for Poplars Planted at 2,170 Trees/acre Density*

Growing Season	Water Uptake (liter/hectare)	Water Uptake (gallons/acre)
1	437,545	46,766
2	2,99,035	319,795
3	8,329,440	890,258
4	9,957,364	1,064,264
5	21,845,073	2,334,847
5 year average	8,712,291	931,188

*The water uptake is calculated using 600:1 water to stem growth ratio

Source: Ecolotree, Inc.

2.3 Mechanisms of TCE Phytoremediation by *Populus* sp.

The following sections will discuss the phytoremediation mechanisms that have been reported when poplars are used to treat TCE. However, the use of these plants to degrade TCE is still a relatively new idea, so not all of the mechanisms are clearly understood at this time. Therefore, the following sections will outline the current body of knowledge on the subject of TCE remediation mechanisms by *Populus* sp. This section begins with a brief overview of *Populus* remediation mechanisms, followed by individual sections detailing what is known about those mechanisms. Also included are some results of controlled field trials of TCE phytoremediation using poplars.

2.3.1 Overview of Mechanisms

A recent study by Newman et al. (1997a) investigated phytoremediation of TCE using two varieties of hybrid poplars (*P. trichocarpa x P. deltoides* and *P. trichocarpa x P. maximowiczii*). These experiments were conducted using axenic poplar cell cultures and whole poplar trees grown in a greenhouse. The investigators reported the formation of TCE metabolites in hybrid poplar tree tissues. They confirmed that the trees were responsible for the metabolites in their tissue, and not microorganisms, by finding the same metabolites in sterile poplar cell cultures. They also found that TCE was evapotranspirated by the trees in the greenhouse studies, and that some TCE was incorporated into an insoluble residue within the trees. In addition, Walton and Anderson (1990) reported that TCE degradation by microbial organisms was enhanced in the rhizosphere of various plant and tree species. Results of these and other related studies of TCE fate in plants are summarized in Table 9.

Table 9: Mechanisms of TCE Phytoremediation by the *Populus* sp.

Process	Product
Metabolism[a]	chloral hydrate, trichloroethanol di- and trichloroacetic acid
Incorporation[a]	Insoluble residue
Mineralization[a]	CO_2
Transpiration[a]	TCE vapor
Rhizospheric degradation via microorganisms	CO_2[b], dehalogenation metabolites such as *cis*-1,2-dichloroethylene[c], vinyl chloride[d], and others

a - Newman et al. (1997)
b -Walton and Anderson (1990)
c - Gordon et al. (1997)
d - Workshop on Phytoremediation of Organic Contaminants (1996)

2.3.2 Enzymatic Degradation and Mineralization in *Populus*

As stated in the previous section, poplar trees have been found to degrade TCE, but the exact metabolic mechanism or mechanisms of enzymatic degradation is currently under some speculation. Two lines of research have been reported to date. Both are similar in that they indicate an enzymatic process involved in oxidizing TCE to various metabolites. Regardless of the enzyme or enzymes involved, the oxidative process will ultimately mineralize the carbon in TCE to CO_2. Also, there is always the possibility of more than one mechanism taking place within a plant or between plant species.

The research of Newman et al. (1997a) suggests that TCE metabolism in poplars may be similar to the mammalian breakdown of TCE. This belief is based on the production of similar TCE metabolites in both plants and mammals. However, the exact mechanism was not determined by these researchers, only hypothesized based on the presence of the metabolism products (Table 9). According to a review by Cunningham et al. (1996), many of the enzyme systems involved in mammalian metabolism of TCE are also found in plants (e.g., cytochrome p-450 oxygenases and glutathione S-transferases), so this hypothesis seems possible.

Another line of research indicates that TCE metabolism in poplars is the result of a dehalogenase enzyme (Schnoor at al. 1995). According to Dr. Laura Carreira, who has isolated the enzyme, dehalogenase is an ethylene degrading enzyme (personal communication). It oxidizes alkanes, alkenes, and methanes and their halogenated analogues. Dehalogenase will ultimately mineralize TCE to CO_2 via an oxidative pathway. Dr. Carreira has developed an antibody assay for the ethylene degrading enzyme to use as an indicator of its presence in various plant species. This antibody technique can be used to predict the ability of a plant or tree species to degrade chlorinated solvents before that organism is chosen for use at a particular phytoremediation site. In the case of poplars, some species or hybrids can produce more of this enzyme than others, and some may not produce the enzyme at all. As a result, using different hybrids or species at various sites without assaying for the dehalogenase may yield contrasting results. A tree that manufactures large quantities of the enzyme will have the ability to degrade TCE, while plants that produce little or no quantities of the enzyme will tend to volatilize it.

A wide variety of plants can potentially produce the dehalogenase enzyme, so Dr. Carreira's antibody technique may be used to find species of ethylene degrading plants or trees that are native to a site. Table 10 lists some species that have been reported to produce dehalogenase enzyme and the half lives of hexachloroethane, a halogenated hydrocarbon, in the presence of these plants.

Table 10: Some Plant Species Containing Dehalogenase

Plant Species	**Half-life (hours) of Hexachloroethane**
Algae *Nitella* (stonewort)	90
Anthrocerotae sp.	120
Algae *Spirogyra*	95
Myriophyllium spicatum (parrot feather)	120
Populus sp.	50

Source: Adapted from Schnoor et al. 1995.

2.3.3 Enhanced TCE Degradation and Mineralization in the Rhizosphere

The root zone of plants provides an environment conducive to the growth and activity of microorganisms (Kunc 1989). These enhanced populations of microorganisms have been found to degrade TCE in the rhizosphere of a number of plant species growing on contaminated sites (Walton and Anderson 1990). In addition to bacteria, microrhizal fungi are capable of metabolizing chlorinated organics (Donnelly and Fletcher 1995). Initial research determined the plant species capable of promoting TCE degradation in soils (Anderson and Walton 1995). Recent studies have focused on determining the mechanisms of degradation within the rhizosphere.

A review on this subject by Davis et al. (1996) reports that microbial degradation of TCE can take place either aerobically or anaerobically. The aerobic process is an oxidative mechanism catalyzed by a mono-oxygenase enzyme. Methane mono-oxygenases (MMO) and alkene oxygenases are reported as the primary enzymes involved in the oxidative mechanism. Each enzyme uses either methane or an alkene as its primary substrate, but can also oxidize TCE in a fortuitous reaction. Plants may support this mechanism by transferring exudates to anaerobic sites, stimulating methanogens to produce methane. The methane in turn stimulates aerobic methanotrophs, who cometabolize TCE via the MMO enzyme. Products of this reaction include chloral, dichloroacetic acid, trichloroacetic acid, trichloroethanol, and ultimately CO_2.

The other microbial mechanism responsible for TCE metabolism is dechlorination catalyzed anaerobically via a dehalogenase enzyme. The review by Davis et al (1996) also describes this mechanism in the rhizosphere, which is primarily carried out by methanogens. Contaminants such as TCE have too low an energy yield to stimulate a population of methanogenic organisms, but plant exudates can supply enough carbon for use as a reductant by the microbes. Once a microbial population increases in density, it will begin to cometabolically dehalogenate TCE. The common products of this reaction are dichloroethylene, vinyl chloride, and eventually ethene and ethane.

2.3.4 Insoluble Residues of TCE

The research of Newman et al. (1997a) also found that a small percentage (3-4%) of TCE remained as an insoluble residue in the poplar tree cell. It is believed that this is due to abiotic binding of TCE to the cell walls of the plant, but the exact mechanism is still under investigation.

2.3.5 TCE Volatilization

Understanding TCE volatilization rates in poplar trees is critical for this technology to gain widespread acceptance amongst hazardous waste site managers and regulators. There may be concern if the trees are transpiring high concentrations of TCE into the atmosphere, where the pollutant becomes an air quality concern. Proponents of phytoremediation argue that VOCs will volatilize from the groundwater, through the soil, and into the air in the absence of trees. For example, plants will invade sites that are left unattended for extended periods of time, and invasive plants may evapotranspirate the contaminant. That being the case, there would be some evapotranspiration in the absence of a treatment strategy. Phytoremediation schemes would only accelerate the process of volatilization that occurs naturally. Still, volatilization concentrations are decreased by a number of factors, such as exclusion of nonpolar compounds at the roots. According to Davis et al. (1996), "Very few contaminants are sufficiently water soluble, non-toxic to plants, and volatile enough to reach atmospheric concentrations that would be of concern by [evapotranspiration]."

Despite the fact that evapotranspiration rates are still unclear, Davis et al. (1996) used energy input estimates to calculate a maximum transfer rate of TCE to the atmosphere. They predicted a maximum transfer rate of 10 $g/m^2/day$. This estimation assumes that the water is totally saturated with TCE at 1.5 g/L and the TSCF of TCE is 0.67, based on the equations of Briggs et al. (1982). Using a more realistic groundwater concentration of 1-15 mg/L TCE and a mixing height of 100-300 meters in the atmosphere, the investigators estimated that transfer to the atmosphere would be 4 to 6 orders of magnitude smaller than the maximum. The result is a very low air concentration of TCE downwind in a worst case scenario.

2.4 Field Trials

Investigations were conducted to follow up the work of Newman et al. (1997a) by conducting field trials of TCE phytoremediation in the state of Washington (Gordon et al. 1997, Newman et al. 1997b). These field experiments were conducted by Occidental Chemical Corporation along with researchers from the University of Washington and Washington State University. The state of Washington approved a two year field experiment where TCE was added to 3.7 x 6.1 meter cells that were 1½ meters deep and double lined with polyethylene. Hybrid poplars (*Populus tricocarpa x deltoides*, H-11-11) were planted in the experimental cells and soil was only added to the controls. TCE was added to the cells at a concentration of 50 ppm in the water. The investigators found that over 95% of the TCE was removed from the stream water in the plots with trees. During the first year of the trial, however, 65% of the added TCE was removed in the control plot without trees. This probably meant that a significant portion of the TCE was bound in the soils (Newman et al.1997b). At the end of the second growing season, 65-70% of the added TCE remained in the water stream in the control cells (Workshop on Phytoremediation of Organic Contaminants), indicating that the loss to the soil decreased substantially after the soil became saturated with TCE. Still, during the second year over 97% of the added TCE was removed from the water stream in the cells containing trees (Newman 1997c). The investigators also found products of anaerobic microbial dehalogenation, such as three isomers of dichloroethylene and small amounts of vinyl chloride, in the water streams (Lee Newman, personal communication).

2.5 Uncertainties of Phytoremediation with *Populus* sp.

Research indicates that phytoremediation of TCE using hybrid poplars will work. The question that remains to be answered is, "To what extent does it work?". Unfortunately, there is no answer to that question at this time. The case studies presented later in this report represent some of the most current data on pilot studies. However, these sites are still very new and phytoremediation is a long, slow process. Currently, managers at these sites do not know if poplars can clean TCE plumes to regulatory standards or how long it will take. Results of these studies are at least a year or two away, possibly more.

Another uncertainty surrounding poplar phytoremediation systems is the fate of the contaminant. Only a few mass balance studies of TCE fate in the field have been attempted. Mass balance predictions are further complicated by the fact that field conditions will vary greatly between sites and geographic regions. In addition, different species or hybrids of *Populus* sp. will have variable abilities to treat TCE in the groundwater. Again, results from the three case studies in this report as well as basic research currently taking place will address some of these uncertainties.

2.6 Other Types of *Populus* Phytoremediation Projects

Populus sp. have been used to treat contaminant problems other than TCE in groundwater. One project in Iowa investigated the ability of poplar strips to act as a buffer to protect water bodies from nutrient runoff (Paterson and Schnoor 1993). A similar study in Iowa looked at the ability of poplars to buffer triazine pesticide runoff from an agricultural field (Dhileepan et al. 1993). Both of these studies indicated that poplars could successfully act as a buffer, although they were less effective at buffering nutrients once the trees dropped their leaves in the fall. In addition, hybrid poplars are currently being used to act as a hydraulic barrier to contain a plume of gasoline and diesel fuel in the groundwater at a site in Ogden, UT (EPA/540/R-97/502). Another way that poplars have been used is as a component of vegetative caps for landfill facilities (Schnoor et al. 1995). Poplars are also being tested for their ability to phytostabilize metals such as lead, and they have been planted as a part of a constructed wetland design to treat explosives such as TNT and RDX in the soil (Schnoor 1997). There is also a report on the use of poplars to phytoextract zinc from soils (Gatliff 1994).

2.7 *Populus* Phytoremediation Versus Other Treatment Technologies

For those unfamiliar with groundwater treatment options, Table 11 lists many of the other technologies that have been used to treat plumes of chlorinated solvents. Table 11 includes the cost of cleaning a plume of chlorinated solvents using these technologies under an idealized set of conditions. Unfortunately, phytoremediation was not included in this economic analysis. However, phytoremediation would be somewhat more expensive than natural attenuation because it involves tree planting and maintenance as well as monitoring, and it would be significantly less expensive than pump and treat due to decreased energy needs. Therefore, the costs of a poplar phytoremediation system would likely fall somewhere in the middle of Table 11, and probably near the less expensive end of the spectrum.

Table 11: Estimated Costs of Treating PCE in the Groundwater

(Assumes PCE plume averages 1 ppm, the remedial goal is 5 ppb, there is no pooled PCE in the aquatard, plume is in the aqueous phase, and the remediation time is 30 years)

Treatment Technology	Total Present Cost (x $1,000)	Cost / Pound PCE removed	Cost / 1,000 Gallons Treated
Pump and treat with air stripping and carbon absorption	$9800	$1600	$8.90
Iron reactive barrier	$3900	$640	$5.30
Biobarrier (substrate enhanced anaerobic bioremediation)	$3100	$520	$4.20
In situ bioremediation (substrate enhanced, recirculating source zone)	$1300	$220	$1.80

Treatment Technology	Total Present Cost (x $1,000)	Cost / Pound PCE removed	Cost / 1,000 Gallons Treated
Natural attenuation (intrinsic bioremediation)	$890	$150	$1.20

Source: Quinton 1997

Since poplar phytoremediation systems are primarily used as hydraulic barriers and solar powered pumps, the most closely related engineering technology is a pump and treat system. The major advantage of poplars over pumps is that poplars provide their own energy, where pumps often consume large amounts of electricity. This could save tremendous amounts of money over the course of a long remediation project. On the other hand, a major disadvantage of poplars is the fact that their pumping rates vary over the course of a year. In addition, poplar systems will only work at sites where the groundwater contamination is within reach of their roots.

Despite some limitations of poplars when compared to pump and treat, the potential economic advantages of this treatment system are tremendous. A report by the Environmental Security Technology Certification Program (ESTCP) estimates that poplar trees can be used to treat contaminants such as chlorinated solvents and petroleum hydrocarbons at 1,000 DOD cleanup sites around the world (ESTCP). This could save the government hundreds of millions of clean-up dollars.

3. Case Studies

Since poplars have been shown to remediate TCE under controlled experimental settings, several Department of Defense and Superfund sites are conducting pilot scale phytoremediation projects. The goals of these projects are to use poplars to remediate plumes of chlorinated solvents in the groundwater. Table 12 provides some general information about each site, including contacts. All three were still in early stages of sampling at the time of this writing, so very little performance data is available. However, results to date indicate that the phytoremediation systems are working. For example, several of the sites are reporting a depression in the water table beneath the plots and some contaminant volatilization from the trees. Future monitoring will help determine the extent of TCE removal that can be achieved using this technology.

Table 12: Case Studies Overview of Sites

Site	Size of Planting on Site	Number of Trees Planted	Species or Hybrid	Contacts
Aberdeen Proving Grounds - J Fields Site	~1 acre	183	*Populus trichocarpa x deltoides*, HP-510	Steve Hirsh EPA Region 3 (215) 566-3352 Harry Compton EPA ERT (908) 321-6751
Edward Sears Properties	~1/3 acre	118 deep rooted ~90 shallow rooted	*Populus charkowiiensis x incrassata*, NE 308	George Prince EPA ERT (908) 321-6649 Michael Moan Roy F. Weston/REAC (908) 321-4200
Carswell Air Force Base	~1 acre	660	*Populus deltoides*	Greg Harvey Acquisition and Environmental Management Restoration Division (513) 255-7716 x302 Steven Rock US EPA National Risk Management Laboratory (513) 569-7105

Tables 13 and 14 provide some meteorological data for the geographic regions of each site. While climate information will not be directly used in this report, it may be important in the future when people are comparing the effectiveness of treatment strategies at these three sites. The information in the following two tables only provides yearly averages, not data recorded at the sites. When data from these projects are analyzed, actual weather data taken during the treatments will be compared to determine the extent of climate's effect. Data in Tables 13 and 14 indicates that Edward Sears and Aberdeen, which are both located in the mid-Atlantic region, have similar temperature and rainfall averages. Carswell, which is located in Texas, has a warmer climate and less rainfall.

Table 13: Average Temperature in °F for Geographic Regions of the Case Study Sites

Site	Jan	Feb	May	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Year
Aberdeen	32	35	44	53	64	72	76	75	69	57	48	37	55
Ed Sears	31	33	42	50	60	70	75	74	67	56	47	37	53
Carswell	45	50	56	66	74	82	86	85	78	68	56	48	66

Source: Adapted from data found at http://www.worldclimate.com

Table 14: Average Rainfall in Inches for Geographic Regions of the Case Study Sites

Site	Jan	Feb	May	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Year
Aberdeen	2.0	3.9	3.7	3.5	2.6	6.2	2.1	0.8	2.7	1.8	5.1	2.9	37.2
Ed Sears	3.7	3.1	4.1	3.9	3.4	3.1	4.1	5.4	3.3	3.2	3.8	3.7	44.9
Carswell	1.8	2.2	2.8	3.5	4.9	3.0	2.3	2.2	3.4	3.5	2.3	1.8	33.7

Source: Adapted from data found at http://www.worldclimate.com

There is some cost information available for each site, but any cost data is skewed because these are pilot scale projects. The projected costs include monitoring and analytical procedures that exceed those normally associated with a remediation project. In addition, costs presented here are those that exceed the baseline investigations at a site. In other words, all sites have certain costs of sampling to determine the nature and extent of contamination. These initial costs would be the same regardless of the technology chosen.

A lack of cost and performance data is normal for innovative technologies at the pilot stage. That being the case, the goal of these case studies is to outline the information that is currently available. This information will hopefully provide a history of each site and the reasons for choosing phytoremediation as a treatment. In addition. these studies will provide a snapshot of the current status of these projects.

3.1 Aberdeen Proving Grounds - Edgewood Area J Fields Site (Edgewood, MD)

The Aberdeen Proving Grounds in Maryland began serving as a U.S. Army weapons testing facility in 1918. The installation is divided into two sections, the Edgewood Area and the Aberdeen Area, separated by the Bush River. Military weapons testing and past disposal activities over the years have caused extensive pollution throughout the soil and groundwater of the Proving Grounds. As a result, the entire Edgewood area of Aberdeen appears on the Superfund National Priority List (NPL). Today the Department of Defense (DOD) and the Environmental Protection Agency (EPA) are jointly funding pilot scale applications of innovative treatment technologies around the facility. At the J Fields Site in the Edgewood Area, the EPA's Environmental Response Team (ERT) coordinated the planting of hybrid poplars over a shallow plume of chlorinated solvents in an effort to hydraulically contain the contaminants and treat the groundwater.

3.1.1 Site Design, Monitoring, and Goals

The J Fields Toxic Pits Site had been used for many years as an open pit burning facility for munitions and chemical agents. During this process, large volumes of various chlorinated solvents were discharged. As a result, there is a plume of chlorinated solvents located in the aquifer below the burning pits. Table 15 lists the contaminants of concern at the J Fields site and their concentrations in the groundwater. Concentrations of total VOCs in the groundwater range from less than 20,000 μg/L in some areas to over 220,000 μg/L in others.

Table 15: Contaminants of Concern at Aberdeen Proving Grounds' J Fields Phytoremediation Site

Contaminant	Groundwater (μg/L)	Percent (%)
1,1,2,2-tetrachloroethane (1122)	170,000	65.9
Trichloroethene (TCE)	61,000	23.7
Cis-1,2-dichloroethene (c-DCE)	13,000	5.0
Tetrachloroethene (PCE)	9,000	3.5
Trans-1,2-dichloroethene (t-DCE)	3,900	1.5
1,1,2-trichloroethane (TCA)	930	0.4

Source: Tobia and Compton (1997)

Personnel from Roy F. Weston, Inc. were contracted to assess the J Fields site and conduct treatment activities. Several technologies were considered for cleaning the soil and groundwater at the site. Soil washing, vapor extraction, and capping were considered for soils, and pump and treat and air sparging were considered for the groundwater. These technologies were eliminated from consideration for a number of reasons. Technologies that involved a rigid installation design were eliminated because of a perched water table and the potential for unexploded bombs buried on-site. Pumping and treating the water would be difficult because of the high concentrations of contaminants and strict discharge regulations. In other words, the pump and treat system would need to remove high concentrations of contaminants from large volumes of groundwater, and then discharge the groundwater after it had been treated. Soil excavation was eliminated from consideration due to its high cost. After eliminating the other possibilities, project managers decided the J Fields site was a candidate for a pilot scale phytoremediation system (Tobia and Compton 1997).

Applied Natural Sciences, Inc. was subcontracted to design and install the phytoremediation system. The phytoremediation strategy employed at the J Fields site began in September of 1995 with an assessment for phytotoxicity of on-site pollutants and to determine any nutrient deficiencies that would hinder tree growth. In March and April of 1996, 183 hybrid poplars (*P. trichocarpa x deltoides* [HP-510]) were purchased from a tree farm in Pennsylvania and planted over the areas of highest pollutant concentration around the leading edge of the plume, totaling about one acre of trees. A sweetgum tree was growing on-site prior to installation of the phytoremediation system, so it was left standing. It will be monitored along with the poplars. See Figure 2 for a map of the site's layout.

In order to promote growth down to the saturated zone, each tree was planted with a plastic pipe around its upper roots. A long piece of rubber tubing was also added from the surface to the deeper roots in order to provide oxygen. A drainage system was installed in May 1996 to remove rainwater and therefore promote the plants' roots to seek groundwater.

Since the Aberdeen project involves a new treatment strategy, extensive monitoring is taking place to determine the fates of the pollutants, the transpiration rates of the trees, and the best methods for monitoring phytoremediation sites. The monitoring approaches are summarized in Table 16. The sampling design of the site involves collecting soils, transpiration gases, and tree tissues from the roots, shoots, stems, and leaves. Results will help determine the concentrations of contaminants and their metabolites along each step of the translocation pathway.

Table 16: Monitoring Approaches at the J Fields Site

Type of Analysis or Observation	Parameters Tested or Methods Used
Plant growth measurements and visual observations	Diameter, height, health, pruning, replacement
Groundwater and vadose zone sampling and analysis	14 wells and 4 lysimeters to sample for VOCs, metals, and nutrients
Soil sampling and analysis	Biodegradation activity, VOCs, metals
Tissue sampling and analysis	Degradation products, VOCs
Plant sap flow measurements	Correlate sap flow data to meteorological data
Transpirational gas sampling and analysis	Explore various methods

Source: Tobia and Compton (1997)

Eight monitoring wells were in place at the time of tree planting, and five additional wells were installed in November 1996. Two pairs of lysimeters were also installed on site. Tree sap flow rates are also being monitored in order to determine the pumping rates of the trees. An on-site weather monitor was used during sampling events to correlate tree evapotranspiration rates with weather fluctuations ranging from hourly to seasonal changes.

Several different protocols were used for transpirational gas studies to investigate the most accurate and efficient monitoring methods. All gas samples were collected in a 100 L Tedlar bag sealed over tree branches. Two of the sample collection media involved collection followed by analysis with a gas chromatograph (GC)/mass spectrometer(MS). One of these collection mediums was a Tennax/Carbon Molecular Sieve tube at collection rates ranging from 20-40 mL/min, and the other a 6 liter Summa canister. Perhaps the most accurate method involved a direct connection of Teflon tubing from the sample collection bag to a mass spectrometer/mass spectrometer quadrapole system. This method allowed real time analysis of the transpiration gases. However, this method involves expensive monitoring equipment, so it probably will not be practical at most cleanup sites.

3.1.2 Cost

The trees cost about $80/tree to install. This works out to roughly $15,000 for installation of 183 trees. Costs of monitoring are highly varied due to the numerous monitoring techniques that have been employed at the site.

3.1.3 Performance to Date

Sap flow rate data indicates that on a daily scale, maximum flow occurs in the morning hours. In addition, increasing amounts of solar radiation seems to increase sap flow rates, as would be expected in a tree. Groundwater monitoring data from May of 1997 indicates that the trees are pumping large amounts of groundwater. Data indicates that there is roughly a 2 foot depression in the water table beneath the trees in comparison to data from April of 1996 (Harry Compton, personal communication). Tree tissue samples indicate the presence of trichloroacetic acid (TCAA), a breakdown product of TCE. This correlates with the results of Newman et al. (1997a), who also found TCAA in plant tissues in both axenic poplars cell cultures and hybrid poplar tissues in a greenhouse scale study. Site managers at Aberdeen are also finding that chlorinated solvents (TCE and 1,1,2,2-tetrachloroethane) are being evapotranspirated by the trees. To date, no mass balance studies have been performed to quantitatively determine the different fates of chlorinated solvents in this treatment system. Future monitoring of the site will hopefully answer some of the questions about solvent fate. In order to accomplish this, additional types of monitoring will be employed, such as on-site infrared spectrometry and an on-site gas chromatograph/mass spectrometer.

One other piece of noteworthy information is that the J Fields site experienced about 10% tree loss during the first year. While some of this loss was due to the transplant process, many trees were damaged by deer rutting. In an attempt to keep deer away from the trees, the site managers hung bars of Ivory soap tied to string from the tree branches. The theory was that deer avoid the scent of humans, so they would think that there were humans nearby if they smelled soap. However, this did not completely deter the deer because there was still some rutting damage. Site managers plan to initiate some new strategies for the next fall. One possibility is placing metal fencing around the trees.

3.2 Edward Sears Property (New Gretna, NJ)

From the mid-1960's to the early 1990's, Edward Sears repackaged and sold expired paints, adhesives, paint thinners, and various military surplus materials out of his backyard in New Gretna, NJ. As a result, toxic materials were stored in leaky drums and containers on his property for many years. The soil and groundwater were contaminated with numerous hazardous wastes, including methylene chloride, tetrachloroethylene, trichloroethylene, trimethylbenzene, and xylene. After his death, no one could be found responsible for the site or its clean-up, so On-Scene Coordinators (OSC) from EPA's Region II Removal Action Branch were called in to remove the leaking drums of hazardous materials, including off-spec. paints and solvents. Soil sampling indicated that two areas, 35 x 40 feet and 15 x 20 feet, were very heavily contaminated with solvents. These soils were removed to 8 feet below ground surface (just above the water table). Further excavation could not be achieved without pumping and treating large volumes of ground water. The excavated area were backfilled with clean sand and the OSC activated the EPA's Environmental Response Team (ERT) of Edison, NJ to determine the extent of ground water and deep soil contamination.

Using innovative, hydraulic push ground water sampling techniques, the ERT investigation revealed localized, but highly contaminated ground water. Based on this information, a limited number of monitoring wells were installed to determine vertical contaminant migration and to conduct aquifer tests necessary to evaluate pump and treat options. A test pilot for a pump and treat system with air stripping and activated carbon was then conducted. The aquifer tests revealed a high yield aquifer, which required severe over pumping to create any substantial cone of influence around the pumping wells. Contaminants trapped in the silty-clay lens beneath the site would be difficult to extract in this manner because the transfer rate of contaminants into the groundwater is slow. As a result, large volumes of groundwater would need to be pumped to the surface for treatment, and this water would contain low concentrations of contaminant. Also, neighbors of the property would be disturbed by the noise created by a pump and treat system.

Based on these results, a pump and treat option would be expensive and inefficient for the Edward Sears site. Site managers then moved to a phytoremediation option. This site was judged as a potential candidate for a phytoremediation system due to the nature of the soils and groundwater. There is a highly permeable sand layer about 4-5 feet below ground surface, but below that exists a much less permeable layer of sand, silt, and clay from 5 to 18 feet bgs. This silt, sand, and clay layer acts as a semiconfining unit for water and contaminants percolating down toward an unconfined aquifer from 18 to 80 feet bgs. This unconfined aquifer is composed primarily of sand and is highly permeable. The top of the aquifer is about 9 feet bgs, which lies in the less permeable sand, silt, and clay layer. The top of the aquifer is relatively shallow and most of the contamination is confined from 5 to 18 feet bgs, so managers decided to plant hybrid poplars in order to prevent further migration of the contaminants and ultimately remove the contaminants from the groundwater.

Samples were taken from temporary well points throughout the site. Data from these sampling efforts indicated TCE concentrations in the groundwater ranged from 0 to 390 ppb. Most of the TCE was concentrated into a small area on-site. Seven monitoring wells were installed based on the information obtained from the temporary well points. Data from Monitoring Well 1 can be found in Tables 17 and 18. Keep in mind that these tables only provide information from Monitoring Well 1, and there were a total of 7 wells. Monitoring Well 1 was installed in the area of highest TCE contamination. There was little or undetectable TCE found in the groundwater samples from the other 6 wells. That does not mean that there were no contaminants found in those wells. Recall that the site was polluted with a wide variety of organic chemicals and metals due to the storage practices of Mr. Edward Sears. However, since this report focuses mainly on TCE, only data on TCE from Monitoring Well 1 was included.

Table 17: Concentrations of TCE Sampled from Groundwater in Monitoring Well 1 on Edward Sears Property

Sampling Date	TCE Concentration (ug/L)
12/8/95	28
8/8/96	1.2
8/19/96	2.3

Source: Roy F. Weston/REAC (1997)

Table 18: Concentrations of TCE in Soil Samples During Monitoring Well Installation of Monitoring Well 1 at Edward Sears Property

Feet bgs	1	4	6	8	10	11	12	14	16	18	20
ug/kg	130	18,000	540	270	120	140	48	17	35	180	8
Feet bgs	**21**	**27**	**32**	**37**	**42**	**47**	**52**	**57**	**62**	**67**	**72**
ug/kg	120	130	6	62	3	65	100	16	6	5	6
Feet bgs	**75**	**77**	**82**	**87**							
ug/kg	52	U	4	7							

U - Under Detection Limited

Source - Roy F. Weston/REAC (1997)

3.2.1 Site Design, Monitoring, and Goals

Roy F. Weston, Inc., under the Response Engineering and Analytical Contract (REAC), was tasked by the ERT to conduct a pilot phytoremediation test at the Sears site. The test is being conducted to determine whether hybrid poplar trees can be used to reduce soil and groundwater VOC contamination levels in the planted area and to prevent further offsite migration of contaminated groundwater. In October and November of 1996, the site was cleared of debris and a 4-inch clay layer was placed approximately 1 foot bgs to prevent penetration of rainwater into the upper root zone, thus promoting root growth into the underlying aquifer. This was followed by the replacement and grading of the native surface soil.

Thomas Consultants of Cincinnati, OH were subcontracted to layout the phytoremediation design. In December 1996, one hundred and eighteen hybrid poplar saplings (*Populus charkowiiensis x incrassata*, NE 308) were planted by ERT, REAC and Thomas Consultants personnel in a plot approximately one third of an acre in size. The trees were planted 10 feet apart on the axis running from north to south and 12.5 feet apart on the east-west axis. Figure 4 contains maps of the site's location and tree planting design.

The trees at Ed Sears were planted using a process called deep rooting. In deep rooting, the roughly 12 foot trees were buried nine feet under the ground so that only about 2-3 feet remained on the surface. Deep rooting the trees involved drilling 12 inch diameter holes to a depth of 13 feet. These holes were then back filled to 5 feet below ground surface with amendments such as peat moss, sand, limestone, and phosphate fertilizer. This backfill was installed to provide nutrients to the roots as they penetrated down through the soils. Waxed cardboard cylinders 12 inches in diameter and four feet long were installed to promote root growth down into the groundwater. These barriers settled about a foot into the planting holes, so 5 gallon buckets with the bottoms cut out were placed on top of the cylinders to create a 5 foot bgs root barrier. The trees were placed in the cylinders and the remaining five feet to surface was filled with clays removed during the boring process.

There were about 90 extra poplars left after the deep rooting was completed. These extra trees were planted along the boundary of the site to the north, west, and east sides of the site. These trees were only planted to a depth of 3 feet, or shallow rooted. The purpose of the shallow rooted trees was to prevent rainwater infiltration from off-site and to serve as a source of replacement trees in the event that there was a loss of some deep rooted trees. These trees were planted very close together (about 3 feet apart) under the assumption that natural thinning would take place over subsequent growing seasons. A surface water control system was then installed by planting grasses over the entire site. These grasses came from commercially available seeds purchased from a lawn and garden store.

ERT is conducting an ongoing maintenance and monitoring program at Ed Sears. Monitoring of the site includes periodic sampling of groundwater, soils, soil gas, plant tissue, evapotranspiration gas. Continued growth measurements will also be made as the trees mature. In the fall, the surface water control system will be replaced due to a summer drought that killed much of the grass. Site maintenance also involves the prevention of deer and insect damage. Bars of soap were hung from the trees to deter deer from rubbing their antlers on the trees. Some damage was inflicted by an insect larva known as the poplar leaf caterpillar. This caterpillar lives on poplar trees and makes its cocoon by rolling itself in a poplar leaf. A spray containing *Bacillus thuringesis*, a bacteria which produces toxins that are specific to various insects, was applied to the site. This spray has been effective in killing most of the caterpillars that were living on the trees.

3.2.2 Cost

Total cost of installation of the 118 deep rooted and 90 shallow rooted trees was about $25,000. Additionally, installation of the surface water control system and one year of on-site maintenance totaled about $15,000.

3.2.3 Performance to Date

The trees have been in the ground for less than one growing season, so as of now there is very little performance data available. Some sampling of evapotranspiration gas was conducted by placing Tedlar bags over entire trees. Data from these air samples suggests that the trees are evapotranspirating some VOC's. However, the VOC concentration in the Tedlar bags matches the background concentrations of VOCs in control samples. This could be due to VOCs volatilizing from the soils, or it could be due to evapotranspirated VOCs that may have gotten into the control samples. Future sampling designs will attempt to determine accurate background VOCs. Additionally, there is some tree growth data for the trees. They have grown about 30 inches above ground since planting. Figures 4 and 5 are photographs of the trees at Edward Sears taken in July 1997 showing their size after about 7 months of growing on site. Site managers plan to sacrifice one tree either after or during the next growing season to determine the extent of root growth.

3.3 Carswell Air Force Base (Ft. Worth, TX)

In Ft. Worth, TX, the U.S. Air Force planted Eastern cottonwoods *(Populus deltoides)* to investigate the ability of these trees to control and degrade a plume of TCE in a shallow aquifer. The plume is located near Air Force Plant 4 at the Naval Air Station Ft. Worth, which was formerly known as the Carswell Air Force Base (for the purpose of this case study, it will be referred to as the Carswell site). The initial funding and much of the ongoing support for this project was provided by the Environmental Security Technology Certification Program (ESTCP), a division of the Department of Defense. The Carswell site was chosen as an EPA Superfund Innovative Technology Evaluation (SITE) project in 1996 (EPA/540/R-97/502).

3.3.1 Site Design, Monitoring, and Goals

Greg Harvey of the U.S. Air Force Acquisition and Environmental Management Restoration Division and Steve Rock of the US EPA National Risk Management Research Laboratory carried out the design and implementation of the phytoremediation strategy at Carswell. In April of 1996, the USAF planted 660 cottonwoods in an effort to contain and remediate a plume of dissolved TCE located in a shallow alluvial aquifer (6 to 11 feet below grade). The species *P. deltoides* was chosen over a hybridized species of poplar because it is indigenous to the region. Therefore it has proven its ability to withstand the Texas climate, local pathogens, and other localized variables that may affect tree growth and health (Greg Harvey, personal communication).

Two sizes of trees were planted: whips and five gallon buckets. The whips were about the thickness of one's thumb and were about eighteen inches long at planting. The whips were planted so that about two inches remained above ground and the rest of the tree was below ground to take root. The five gallon bucket trees were about an inch in diameter and seven feet tall when planted. The five gallon bucket trees were estimated to have about twice as much leaf mass as the whips when planted, so they were expected to have higher evapotranspiration rates.

The layout for the project involved planting a separate plot of trees for the whips and the 5 gallon buckets, with both plots perpendicular to the contaminant plume. The plume is moving to the south and east, so the plots were laid out on a north and east axis. The whips section was planted to the north and west of the 5 gallon buckets, so that the plume would first travel through the root zone of the whips and then through the root zone of the 5 gallon buckets. A control area with monitoring wells was placed to the north and west of the whips, and another in between the whips and the five gallon buckets, along with monitoring wells throughout the treatment site. These control areas enable data to be collected on the amount of contaminant that enters each of the treatment areas (whips and five gallon buckets), so that a comparison of the performance of each type of tree can be made. Figure 6 contains the basic layout of this site.

One unique aspect of Carswell is that there is already a mature cottonwood growing on the site. This 70 foot tall tree is located just south and east of the planting area on the other side of a cart path. Groundwater monitoring wells were installed around this tree, and it will be sampled in a similar manner to the planted cottonwoods to see how well a mature tree functions in this phytoremediation system. Data from the first three groundwater samplings that have taken place indicate that the wells near the mature tree have lower concentrations of TCE. This observation is promising because it may be indicating that the older tree is treating the TCE in the groundwater at a higher rate than the younger trees. However, these observations are only speculative at this time.

3.3.2 Costs

Some rough estimates of cost for the Carswell site were provided by Mr. Greg Harvey. These estimates can be found in Table 19. Since this site involves an innovative treatment technology, these costs are substantially inflated due to the heavy monitoring taking place at the site. Also, there are no long term projected costs available or total costs for the project available because the time involved in remediating the site is uncertain. In addition to the costs in Table 19, $200,000 will be spent for extensive site monitoring that would not normally be associated with a phytoremediation system, so this amount was not included in the cost estimates.

Table 19: Rough Estimate Costs of Phytoremediation at Carswell Air Force Base

Activity	Estimated Cost
Wholesale cost of trees (does not include delivery or installation costs)	$8/tree for five-gallon bucket tree $0.20/tree for whips
29 wells (including surveying, drilling, and testing)	$200,000
Subsurface fine biomass	$60,000

Source: Greg Harvey (personal communication)

3.3.3 Performance to Date

Evapotranspiration rates at Carswell for May 13 and 15 and June 10, 11, and 12 of 1997 have been determined. Unfortunately, no quantitative evapotranspiration data was available to include in this report. Qualitatively, both types of trees were capable of evapotranspiring TCE, and the 5-gallon trees are evapotranspiring more water than the whips. This was to be expected because of the greater total surface area of the 5-gallon trees' leaves. In addition, the transpiration rates were generally higher in June than May, which is likely due to a combination of warmer weather and more fully developed leaves. There also appeared to be a midday decline in transpiration during June, indicating that the plants were experiencing water stress during the hottest part of the day in the summer months. In other words, the water demand for the tree exceeded the supply during that time. There was also a notable difference in transpiration rates between days in June, with cloudier days resulting in lower transpiration rates. In addition to evapotranspiration information, some tree growth data has also been collected. In 16 months the whips have grown about 20 feet, and the 5 gallon bucket trees have grown faster than the whips.

Groundwater samples were collected from the 29 monitoring wells and analyzed on three occasions to date. Concentrations of TCE, cis-DCE, and trans-DCE, and vinyl chloride were determined from these samples. They ranged from 2 to 930 ug/L TCE in the groundwater, with most samples falling in the 500-600 ug/L range. Average concentrations of the contaminants on the three sampling dates are provided in Table 20, with the exception of vinyl chloride. Vinyl chloride was only detectable in a handful of samples and generally in low levels, so an average concentration was not determined.

Table 20: Average Concentrations of TCE, cis-DCE, and trans-DCE at Carswell

Contaminant	**Average Concentration (ug/L)**		
	December 1996	**May 1997**	**July 1997**
TCE	610	570	550
cis-DCE	130	140	170
trans-DCE	4	2	4

Source: Steve Rock (unpublished data)

Some analytical work has been done on the tree tissues at the site, but this type of information is still in the early stages of collection. Data from November of 1996 indicated a TCE signature in the whips that were planted over an area where the groundwater was the shallowest. This indicates that the young trees were capable of evapotranspirating TCE after just one growing season. Now that the trees have been on site for more than an entire growing season, site managers at Carswell plan to increase monitoring at the site to include a whole suite of water, soil, air, and tree tissue sample analysis. Some of the more unique data they plan to collect (in relation to the other case study sites) are analyses of microbial populations and assays of TCE degrading enzymes in the trees.

4. The Future of Phytoremediation

The use of phytoremediation will most likely increase in the near future. As an example, Table 21 provides an analysis of the phytoremediation market for organics in groundwater through the year 2005. Assuming these predictions are accurate, phytoremediation will likely grow into a substantial market over the next decade. Of course, the results of the three case studies and other similar studies currently being designed and implemented may have an effect on these predictions.

Table 21: Estimated U.S. Phytoremediation Market Share for Organics in Groundwater

Market	Cost in Millions of Dollars		
	1997	2000	2005
Total Segment	2,600	2,600	2,600
Phytoremediation	2-3	6-12	20-45

Source: Glass (1997)

4.1 Future research

Table 22 outlines some of the research needs that the Remediation Technology Development Forum (RTDF) for Phytoremediation of Organics believes are needed in the near future. Because phytoremediation is such a new technology, most of the needs outlined in this list are still at a fundamental level. For example, very little is known about the mass balance of fates for many pollutants within plants and the degree that the rhizosphere can increase degradation.

Table 22: Basic Research Needs for Phytoremediation

- More basic data is needed on the phytoremediation process
  - -Validation of rhizosphere effects
  - -Determine the fate of a contaminant in phytoremediation systems
  - -Determine factors that affect mass balance, such as species, climate, soils, etc.
- Determine acceptable endpoints for phytoremediation
- Selecting a contaminant and site that will effectively convince regulators that phytoremediation is a valid technology

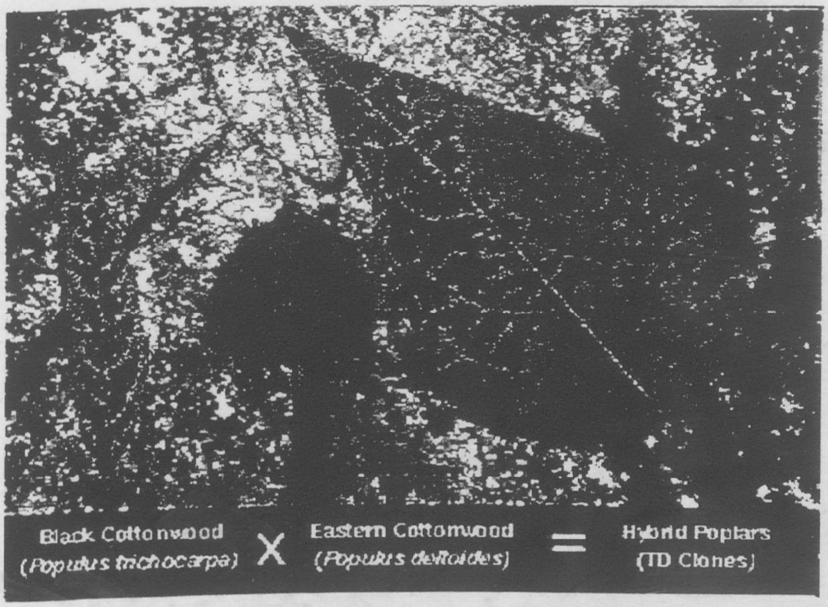
Source - Adapted from RTDF Phytoremediation Action Team Meeting, April 30, 1997

Once many of the basic questions have been answered, research will need to focus on optimization of phytoremediation for different clean-up situations and goals. This can be accomplished through a number of mechanisms, from complex studies such as genetic engineering to simply finding better ways to choose native plants. One way to accomplish this is to choose native plants that produce high concentrations of enzymes that are known to degrade a site's contaminants of concern. Table 1 of Appendix C outlines some of the enzymes known to work in phytoremediation systems and some of the plants that contain these enzymes. Researchers need to screen more plants for these and other enzymes potentially useful to phytoremediation (Appendix C Table 2) in order to create a catalogue of plants.

Today, trees such as poplars are commonly used in phytoremediation schemes because they can grow in a wide variety of climates and they are known to have high evapotranspiration rates. However, there may be plants that are better than poplars at degrading specific pollutants. For example, cypress trees and rice plants were found to contain much higher concentrations of dehalogenase than poplars, so in theory they are better equipped to degrade TCE than a poplar tree (Laura Carreira, personal communication). In addition, there may be certain trees native to an area that would be much heartier under local conditions and more resistant to local pathogens and parasites, so it is often beneficial to chose a native plant when designing a phytoremediation scheme (Greg Harvey, personal communication). Choosing native species and screening them for their ability to metabolize specific contaminants may be the key to optimizing phytoremediation in the future.

Currently, there is speculation as to how well phytoremediation works and under what conditions it will be useful. Many of these questions will be answered, at least in part, by pilot scale projects such as Aberdeen, Carswell, and Edward Sears, as well as the numerous other types of phytoremediation projects underway around the country (Appendix B). Early results from these current sites are promising, so it appears that phytoremediation will be an effective tool for cleaning hazardous waste in the future.

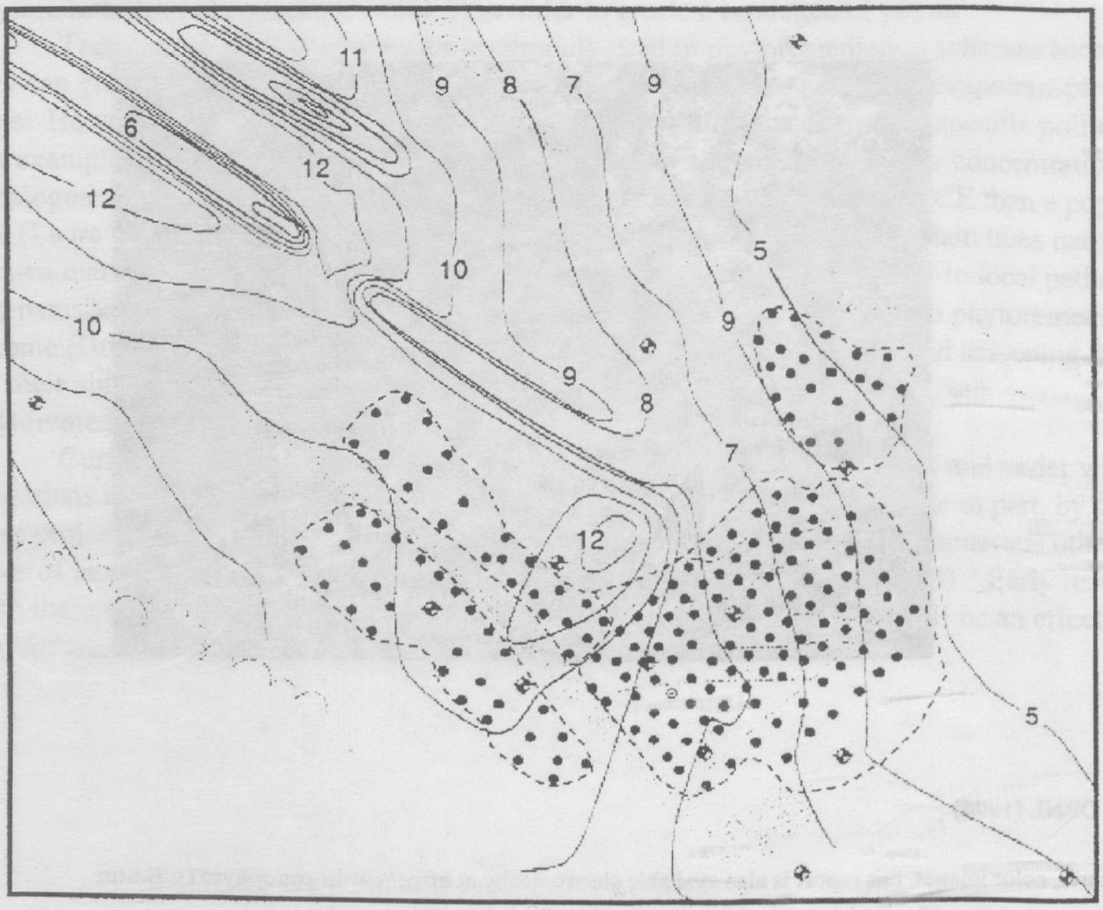
Figure 1 - Image of Leaves from Parent Poplar Species (*Populus tricocarpa* and *Populus deltoides*) and Hybrid Offspring*

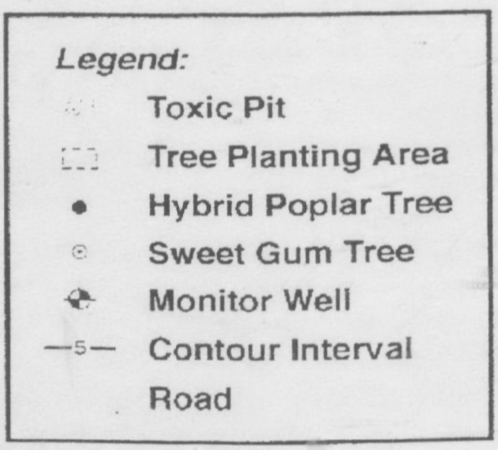


Source: ORNL (1996)

*For clearer, color images, this report is also available electronically at **http://clu-in.com/phytoTCE.htm**

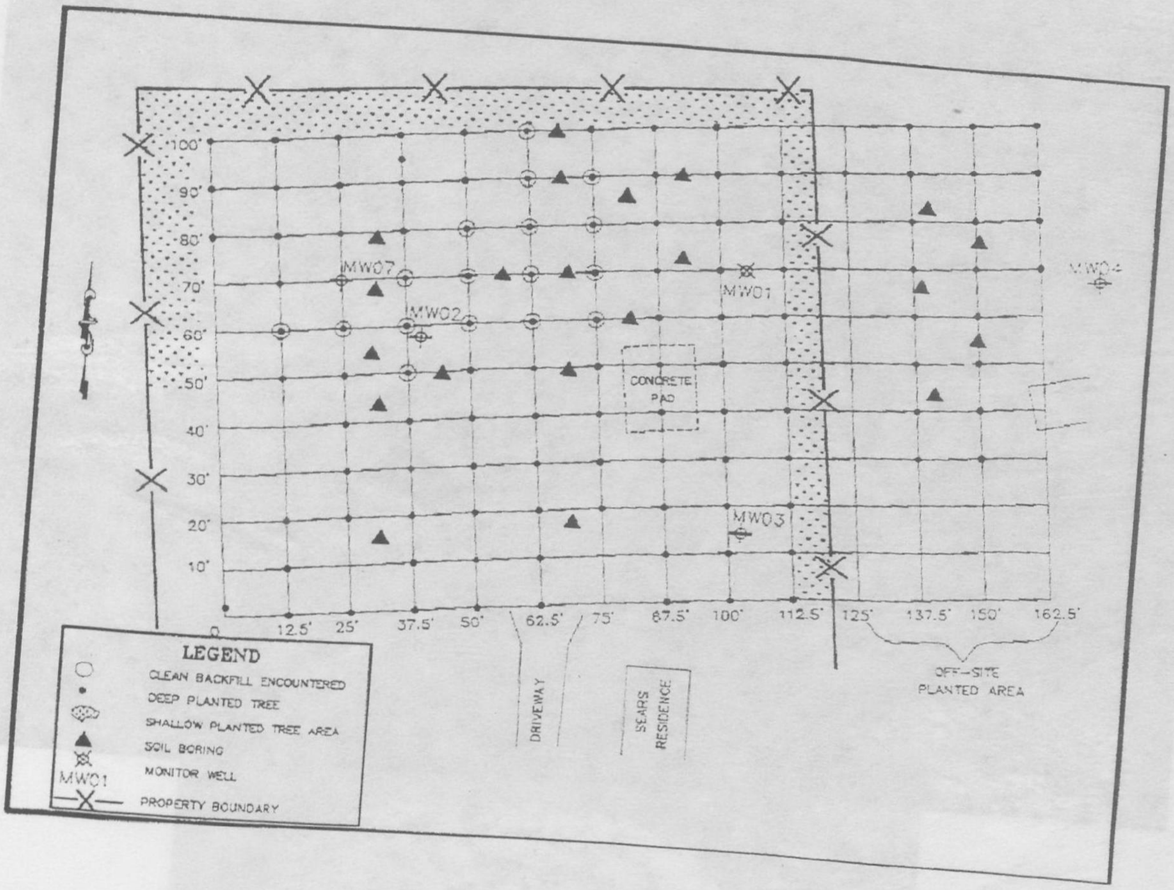
Figure 2 - J Fields Phytoremediation Tree Planting Area Map, Aberdeen Proving Grounds -Edgewood, MD





Source: Tobia and Compton (1997)

Figure 3 - Edward Sears Property Tree Planting Layout



Source: Roy F. Weston (1997)

Figure 4 - Photograph of Hybrid Poplar Field at Edward Sears Property

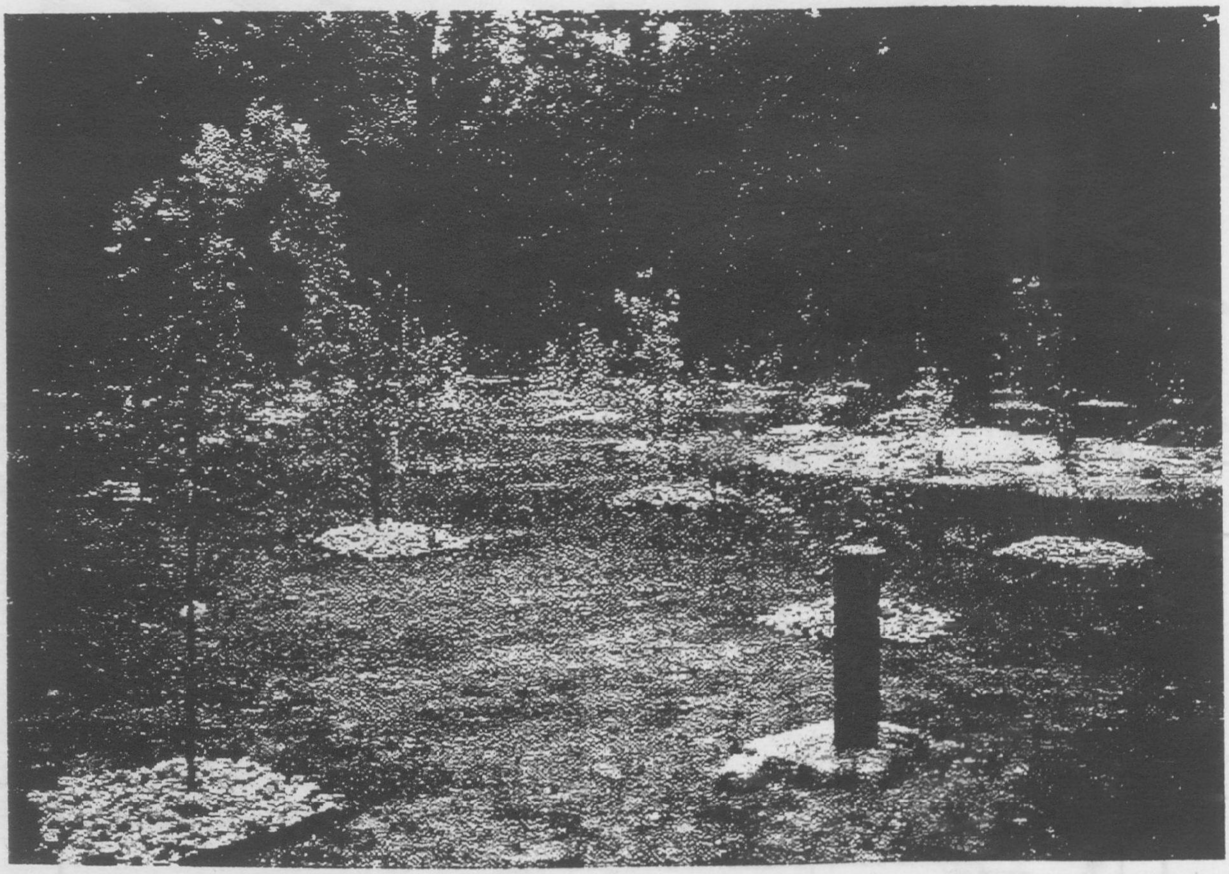


Figure 5 - Photograph of Hybrid Poplar Tree at Edward Sears Property

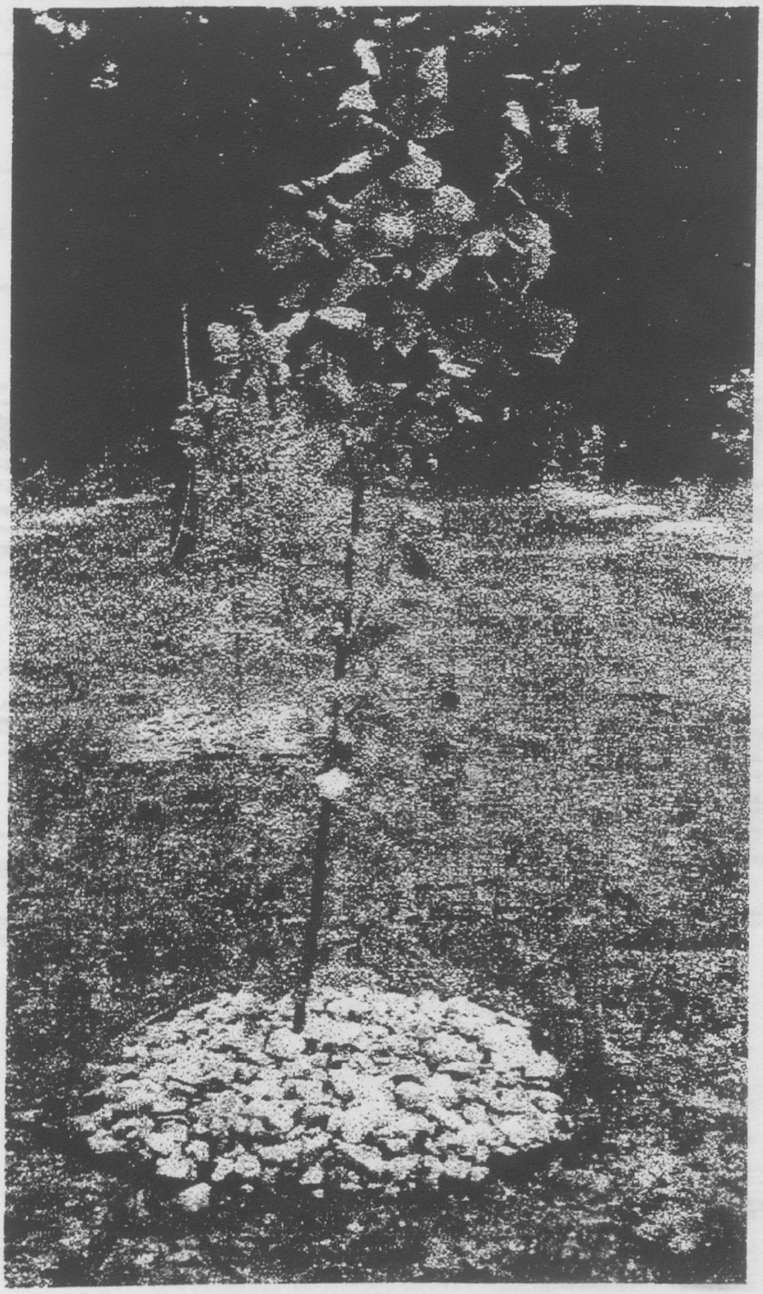
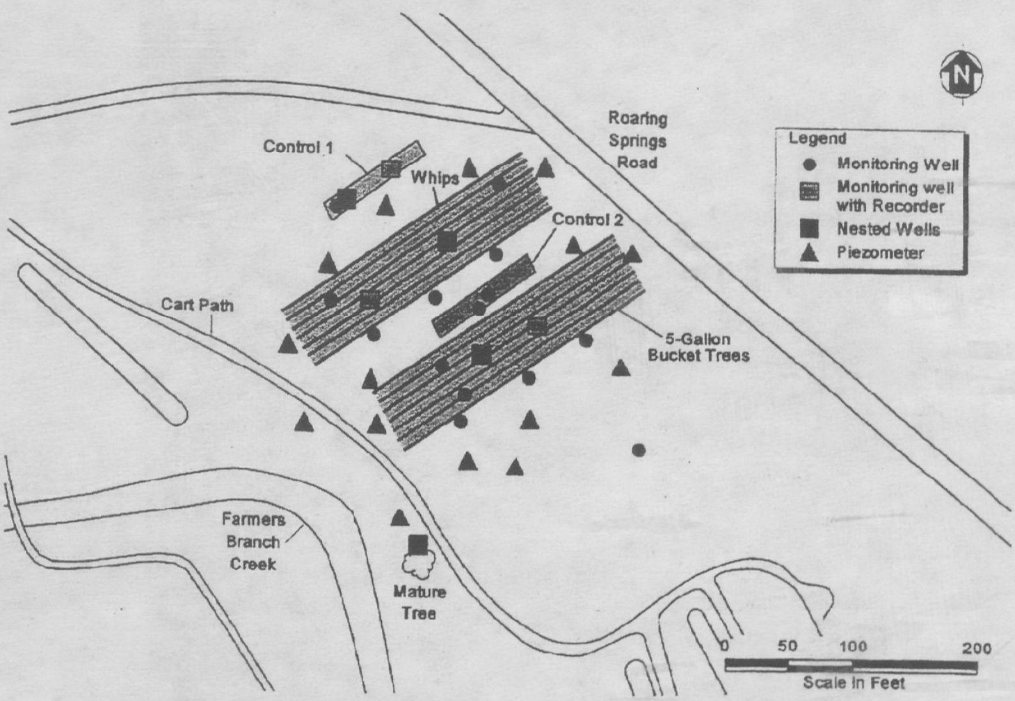


Figure 6 - Air Force Plant 4 Phytoremediation Site Layout, Carswell Air Force Base - Ft. Worth, TX



Source: EPA/540/R-97/502

Appendix A - Companies Specializing in Phytoremediation Technologies

Company Name	Types of Treatment	Contact
Applied Natural Sciences, Inc. Dayton, OH	Treemediation, hybrid poplars and grasses used to treat contaminants such as chlorinated solvents and zinc	Edward Gatliff, President
BEAK Guelph, ON office	Tree buffers to contain arsenic in groundwater, PAHs and PCBs using grasses, legumes, and trees, phyto as a polishing step in treatment	Bob Tossell
Ecolotree Iowa City, IO	Ecolotree Cap (vegetative cap), Ecolotree Buffers - poplars and grasses used as a hydraulic barrier	Louis Licht, President
D. Glass Associates, Inc. Needham, MA	Phyto- and Bioremediation market analyzation and technology transfer	David Glass
Phytotech Monmouth Junction, NJ	Metals, radionuclides	Burt Ensley, President
Phytokinetics North Logan, UT	plants such as poplars and alfalfa used to contain petroleum hydrocarbon plumes, toluene spills, and excess nitrate and ammonia in groundwater	Ari Ferro, President
PhytoWorks Athens, GA	Phytoremediation of mercury, plant enzyme antibodies, phytoremediation of organics	George E. Boyajian, Principle, Science and Technology
Thomas Consulting Cincinnati, OH	Poplar projects	Paul Thomas

Appendix B - Some Representative Examples of Phytoremediation Projects

Name and Location	Party Conducting Treatment	Type of Contaminant	Type of Treatment
Aberdeen Proving Grounds Aberdeen, Maryland	DOD, EPA ERT	TCE in groundwater	Poplars used to contain the movement of the plume
Carswell Air Force Base Ft. Worth, TX*	DOD, EPA	TCE in groundwater	Cottonwoods to contain the movement of the plume
Chernobyl Nuclear Power Plant Chernobyl, Ukraine and a DOE site in Ashtabula, OH	Phytotech, Inc	Radionuclides	Rhizofiltration in a continuous flow system
Chevron Ogden, UT*	Phytokinetics, Inc., EPA (monitoring)	Petroleum hydrocarbons	Poplars used to contain the movement of the contaminant plume
Edward Sears New Gretna, NJ	EPA ERT	Solvents in groundwater	Poplars used to contain the movement of the contaminant plume
Lakeside Landfill Beaverton, Oregon	Ecolotree	Landfill cap	Poplar tree cap used to prevent landfill from leaching
Metal plating facility in Findlay, OH*	Phytotech, Inc.	Metals in soils (lead, chromium, nickel, zinc, and cadmium)	Plants used to extract metals from soils.
Milan Army Ammunition Plant Tennessee	DOD	Explosives in groundwater (TNT, RDX, HMX, DNT)	Constructed wetland containing nitrogen reducing species of plants

* SITE demonstration project

Appendix C: Phytoremediation Enzymes

Table 1: Some Enzymes Found to be Involved in Phytoremediation

Enzyme	Pollutant Degraded	Some Plants Known to Produce Enzyme
Dehalogenases[1,2]	chlorinated solvents, ethylene containing compounds	*Populus* sp. (Hybrid poplars), *Myriophyllium spicatum* (parrot feather), Algae *Nitella* (stonewort), Algae *spirogyra, Anthrocerotea* sp.
Lactase[1]	oxidative step in munitions degradation	Algae *Nitella* (stonewort), *Myriohyllium spicatum* (parrot feather)
Nitroreductase[1]	munitions (TNT, RDX, etc.)	*Populus* sp. (Hybrid poplars), *Myriohyllium spicatum* (parrot feather), *Lemna minor* (duckweed),Algae *Nitella* (stonewort), plus more
Nitrilase[3]	herbicides	
Peroxidases[3,4]	phenols	*Armoracia rusticana* (Horseradish)

1 - Schnoor et al. (1995)
2 - Laura Carreira, personal communication
3 - Workshop on Phytoremediation of Organics (1996)
4 - Cunningham et al. (1996)

Table 2: Plant Enzymes Believed Probable for Phytoremediation but Not Tested

Enzyme	Contaminants Potentially Degraded
Phosphatase	organophosphates
Aromic Dehalogenase	chlorinated aromatics (DDT, PCB's, etc.)
o - demethylase	pendimethaline, alachlor, metolachor

Source: PhytoWorks, Inc.

Appendix D - Phytoremediation Web Sites

Page Name	Address
Bioresource Engineering - Oregon State University	www.bre.orst.edu
Dr. Ilya Raskin's Laboratory	cook~college.rutgers.edu/~halpern/index.html
Envirobiz - Chevron Grows New Remediation Technology: Alfalfa and Poplars	www.envirobiz.com/newsdaily/960502e1.htm
Environmental Security Technology Certification Program - Cleanup Projects page	scaffold.walcoff.com/estcp2/projects/cleanup/index.html
Ground-Water Remediation Technologies Analysis Center: Phytoremediation - Technology Overview	www.gwrtac.org/html/tech_over.html#PHYTOREM
HSRC's Phytoremediation page	www.engg.ksu.edu/HSRC/phytorem
Hyperaccumulators and Phytoremediation	bob.soils.wisc.edu/~barak/soilscience326/agres.htm
Phytoremediation at Utah State University	www.usu.edu/~cpl/phytorem.html
Phytotech, Inc.	www.phytotech.com
Poplars and Willows on the World Wide Web	poplar1.cfr.washingtion.asedu
The RTDF Phytoremediation of Organics Action Team	www.rtdf.org/phyto.htm
USDA Economic Research Service - Industrial Uses of Agricultural Materials page	www.econ.ag.gov/epubs/pdf/IUS6/INDEX.HTM

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