GREENHOUSE TREATABILITY STUDIES ON PHYTOREMEDIATION OF PERCHLOROETHYLENE (PCE) AND TRICHLOROETHYLENE (TCE) USING BLACK WILLOW AND EASTERN POPLAR

by

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ABSTRACT

Greenhouse studies were conducted to determine the effectiveness of black willow, eastern poplar, and duckweed to uptake, transform, and degrade perchloroethylene (PCE) and trichloroethylene (TCE). Results indicate that both black willow and eastern poplar are capable of decontaminating solution concentrations of up to 20 mg/L of PCE and 45 mg/L TCE. High concentrations of 45 mg/L PCE were shown to be toxic to the plants. Duckweed did not exhibit the ability to degrade these compounds. The results indicate that black willow and eastern poplar can transform and degrade these recalcitrant compounds and have the potential for application to remediate shallow, contaminated soil and groundwater.

INDEX WORDS: Phytoremediation, Perchloroethylene, Trichloroethylene, Groundwater, Contamination, *Populus*, *Salix*, *Lemnaceae*

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DEDICATION

To Heather

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CHAPTER I

INTRODUCTION

1.1 Problem Statement

Perchloroethylene (PCE) and trichloroethylene (TCE) are xenobiotic contaminants common at many of the nation's hazardous waste sites. The large-scale use and haphazard disposal of these contaminants has caused a worldwide pollution problem necessitating the development of effective and cost-efficient technologies to remediate these recalcitrant compounds from the environment. PCE and TCE can be found at 50% of Superfund National Priority List (NPL) sites with completed Records of Decision (RODs), and they exist at concentrations above action levels in 17% of groundwater and 16% of soils in RCRA corrective action facilities (USEPA 542-R-96-005, 1997). Widespread contamination by these pollutants is due mainly to their use as industrial degreasing agents and as dry cleaning solvents as they account for 80% to 85% of all dry cleaning fluid used. Fortunately, demand for PCE in the United States declined approximately 35% between 1989 and 1991 due to implementation of solvent recycling operations and reduced demand for chlorofluororcarbons. Demand should continue to decline as solvent alternatives and new industrial processes are applied (USEPA 749-F-94-020, 1994). Other commercial uses of these chlorinated solvents include anesthetic for medical and dental use and as an ingredient in paints, inks, cosmetics, disinfectants, and cleaning fluids (Chappell, 1997).

PCE and TCE are dense non-aqueous phase liquids (DNAPLs), meaning that in the subsurface, they are denser than water and tend to exist in undissolved pools perched on a confining layer or at the bottom of an aquifer (Table 1.1). DNAPL pools dissolve slowly into groundwater providing a pollutant source that may last for years to decades.

TABLE 1.1
Characteristics of tetrachloroethylene and trichloroethylene

Characteristic	Compound	
	Tetrachloroethylene (PCE)	Trichloroethylene (TCE)
Molecular Weight (g/mol)	165.83	131.39
Water Solubility (mg/L)	150	1100
Vapor Pressure (mmHg)	17.8	57.9
Density (g/mL)	1.63	1.46
Log Kow	3.19	2.64

These characteristics make the removal of these contaminants from groundwater extremely difficult (Chappell, 1997).

There are several existing technologies used for the remediation of media contaminated by chlorinated organic compounds, but they are not always cost-effective. These technologies include soil vapor extraction and groundwater extraction or "pump and treat", whereby contaminated groundwater is pumped to the surface where the contaminants may be stripped to the atmosphere, sorbed onto activated carbon, chemically destroyed or microbially broken down. *In-situ* remediation methods may involve enhanced natural attenuation, which is the stimulation of anaerobic and/or aerobic microbial systems in the aquifer, installation of chemically reactive zones, or *in-situ* stabilization. Conventional remediation technologies for aquifers contaminated by PCE and TCE are time and labor intensive and may be extremely costly to undertake (Newman, 1997).

1.2 Site History

The area under investigation is the Operable Unit (OU) 4 of the Naval Training Center (NTC) located in Orlando, FL. OU 4 is composed of Study Areas 12, 13, and 14 [Figure 1.1]. Study Area 13 includes Building 1100, a laundry and dry-cleaning facility under operation from 1943 to 1994, that has been identified as a site where hazardous material releases have occurred. The probable contaminant source and release mechanisms at OU 4 are:

1. Operational spills on the ground surface outside the building during the loading and unloading of containers of PCE;
2. Leaks associated with the collection and conveyance of waste from laundry and dry cleaning machines;
3. Spills inside the building transferred to the environment.

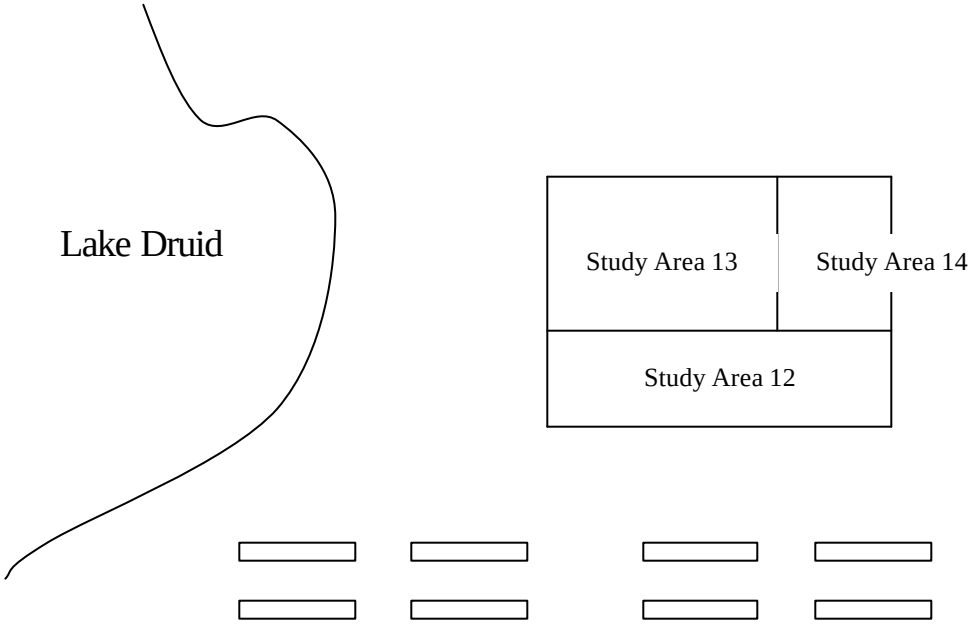


Figure 1.1
Location of Study Areas 12, 13, and 14

A ground-water plume of chlorinated solvents was found to be migrating from Building 1100 (Study Area 13) into Lake Druid. Volatile organic compounds (VOCs) detected in groundwater and surface water from Lake Druid included PCE, TCE, cis-1,2-dichloroethylene (DCE), trans-1,2-DCE, 1,1-DCE, and vinyl chloride (VC). The plume is approximately 4 to 45 feet below land surface with total VOC concentrations up to 30,000 µg/L in the source area northwest of Building 1100, and approximately 6000 µg/L between Building 1100 and Lake Druid. The water table varies seasonally from less than 1 foot to 4 feet below ground surface between Lake Druid and Building 1100 (Harding Lawson Associates, 1998).

1.3 Phytoremediation Overview

Vegetation has long been used for the restoration of disturbed areas, and tolerant vegetation is often found growing on contaminated soils. The use of plants to improve water quality in municipal and industrial water treatment systems is well-documented (Cunningham et al, 1997). Even though vegetative practices have existed for decades, the purposeful utilization of plants for the clean up of the environment has not received much attention despite the fact that plants play an important role in sustaining and restoring environments (Burken, 1996).

Phytoremediation is an emerging, innovative technology that promises to be an effective and inexpensive cleanup method of a wide variety of contaminants at certain hazardous waste sites (Table 1.2), (Schnoor et al., 1995). Phytoremediation can be defined as the use of green plant-based systems and their rhizospheric microbial communities to remove, degrade, or stabilize environmental contaminants from soils, sediments, surface and groundwater. Overall, the development and application of phytoremediation is being driven by its technical and potential economic advantages over conventional approaches (Flathman et al., 1998).

TABLE 1.2
Advantages and Disadvantages of Phytoremediation

Advantages	Disadvantages
in situ	Limited to shallow soils, streams, wetlands, and groundwater
Passive	High concentrations of hazardous materials can be toxic to plants
Solar driven	Longer cleanup times
Costs 10% to 20% less than mechanical treatments	Slower than mechanical treatments
Transfer is faster than natural attenuation	Only effective for moderately hydrophobic contaminants
High public acceptance / Aestheically pleasing	Toxicity and bioavailability of degradation products is not known
Fewer air and water emissions	Contaminants may be mobilized into the groundwater
Generate less secondary wastes	Potential for contaminants to enter food chain through animal consumption
Soil remains in place and are usable following treatment	Unfamiliar to many regulators

There are many types of phytoremediation systems in existence and depending on site-specific conditions, choosing the correct technology for application is critical (Table 1.3). Phytoremediation is best suited for sites with shallow contamination although certain plants are capable of remediating soils even beyond the reach of their root systems. Most plants have a high demand for water and some plants, such as poplars, can take up 200 L per day such that a dense population may actually lower the water table. The uptake of water by a plant may be accompanied by nutrients and contaminants that may result in the incorporation of metals and organics into the plant's tissue or transformation to other non-toxic compounds (Boyajian et al, 1997).

It has long been known that certain plants sequester unusually large amounts of metals such as lead, zinc, etc (Cunningham et al, 1997). Until recently, the majority of phytoremediation involved the use of plants to treat soils and waters contaminated with metals. This phytoremediation technique involves mechanisms such as phytoextraction, phytoaccumulation, and phytostabilization (Rennels, 1998). The plants used for phytoextraction are termed hyper accumulators because they have a tendency to concentrate certain elements within their tissues. Hyper accumulating plants can take up and translocate significant concentrations of heavy metals (Cunningham et al, 1997). These plants can be grown on contaminated sites, mechanically harvested, and dried. The biomass can then be burned to produce ash with a high content of metals that could then be recovered. Hyperaccumulators, due to the fact that the plants can uptake large amounts of metals, offer a better alternative to traditional methods of remediation (Rouhi, 1997).

Since the early 1990's, phytoremediation has extended into the use of plants to detoxify or degrade organic pollutants (Rennels, 1998). Recent investigations have demonstrated that many plants have tremendous potential to take up and transform toxic organic chemicals (Nzengung et al., 1999, 2001). Phytoremediation encompasses a

TABLE 1.3
Types of Phytoremediation Systems

Treatment Method	Mechanism	Media
Rhizofiltration	Uptake of metals in plant roots	Surface water
Phytodegradation/ Phytotransformation	Plant uptake and degradation or transformation of organics	Surface water and groundwater
Plant-Assisted Bioremediation	Enhanced microbila degradation in the rhizosphere	Soils, groundwater within the rhizosphere
Phytoextraction	Uptake and accumulation 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, and mine tailings
Phytovolatilization	Plant evapotranspirates selenium, mercury, and volative organics	Soils and 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

number of different methods that can lead to contaminant degradation, removal, or immobilization:

1) Degradation: For destruction or alteration of organic contaminants:
   A.) Rhizodegradation: Enhancement of biodegradation in the below-ground root zone by microorganisms,
   B.) Phytodegradation: Contaminant uptake and metabolism above or below-ground, within the root, stem, or leaves.
2) Accumulation: For containment or removal of organic and/or metal contaminants:
   A.) Phytoextraction/Phytoaccumulation: Contaminant uptake and accumulation for removal,
   B.) Rhizofiltration: Contaminant adsorption on roots for containment and/or removal.
3) Dissipation: For removal of organic and/or inorganic contaminants from soil or water and release into the atmosphere:
   A.) Phytovolatilization: Contaminant uptake and volatilization.
4) Immobilization: For containment of organic and/or inorganic contaminants:
   A.) Hydraulic control: Control of ground-water flow by plant uptake of water (Pivetz, 2001).

All plants have the ability to use their root systems to acquire nutrients and water from the subsurface (Nyer et al., 1996). Root uptake of organic compounds from soil is affected by three factors:

1) Physiochemical properties of the compound (i.e., K_{ow});
2) Environmental conditions;
3) Plant characteristics.

For given plant characteristics and environmental conditions, root uptake of xenobiotics from water has been shown to be directly proportional to the octanol/water partition coefficient (K_{OW}) for the chemical (Burken, 1996). For compounds of similar structure and molecular weight, it is known that uptake and translocation are greatest for compounds with log K_{OW} values ranging from 1 to 3.

The bioavailability of organic contaminants for plant uptake is primarily controlled by environmental soil factors such as organic matter content, pH, and moisture. Certain characteristics such as root surface area can alter contaminant absorption substantially. Surface area may be increased in plants with large root morphologies or in those with high densities of fine root hairs (Cunningham et al, 1997). Also, the phreatophytic tendencies of plants and their ability to tolerate targeted compounds are major considerations.

The ability of a plant to take up a chemical and translocate it through its shoots is described by a chemical's root concentration factor (RCF) and the transpiration stream concentration factor (TSCF). The RCF is a measure of the root concentration of a chemical versus the concentration in the external solution. The TSCF is a measure of the chemical concentration in the xylem sap in relation to the concentration in the external solution. These factors are chemical specific and vary directly with log K_{ow} (Chappell, 1997).

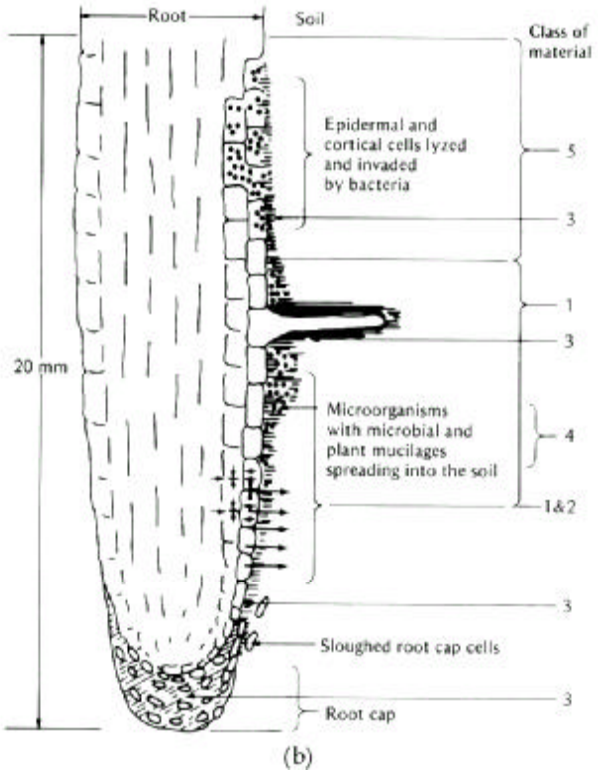
The ultimate fate of the contaminant is of paramount importance in phytoremediation. If a contaminant is translocated from the soil to the aboveground plant tissues and transpired into the atmosphere, the potential for ecological harm may be increased and phytoremediation may be an unacceptable treatment. However, if the compounds are shown to be detoxified the application of phytoremediation may be suitable (Burken, 1996).

1.4 The root zone and the rhizosphere effect

In certain situations, plant roots alone cannot extract organic compounds from the soil due to large amounts of organic matter present. The organic compounds are sorbed readily to the soil organic matter and are bound so strongly that the plant roots cannot extract them for uptake. This situation can be overcome to a degree by taking advantage of the symbiotic relationship between plant roots and microorganisms, which has long been known to exist. This relationship enables the plant and the microbes to work together to break down organic compounds and contaminants in the environment. The number of organisms in the rhizosphere may be as much as 100 times greater than elsewhere in the soil (Brady, 1990). The increased microbial activity associated with vegetated soils is termed the rhizospheric effect.

Living roots also affect the nutrition of soil microbes. Roots withdraw soluble nutrients directly from the groundwater and the surrounding soil but also release substances that influence nutrient availability. Significant quantities of organic compounds are exuded, secreted, or otherwise released at the surface of young roots [Figure 1.2]. For example, organic acids excreted by plant roots can solubilize nutrients and contaminants. Plants release amino acids and other simple carbon-containing compounds that stimulate microflora in the rhizosphere.

Also, organic compounds called mucilages are released or sloughed off from young plant roots. These compounds, when mixed with clay particles, form a gelatinous layer called mucigel, which surrounds the roots [Figure 1.2]. The mucigel enhances a root's ability to penetrate the soil during growth and is a major source of soil organic matter. The mucigel layer may facilitate root contact with the surrounding soil, especially during periods of moisture stress when the root proper may shrink in size and lose direct contact with the soil [Figure 1.3], (Brady, 1990). The mucigel, along with other organic cell excretions, constitute root exudate (Burken, 1997). This exudate



1. Simple *exudates*, which leak from plant cells to soil.
2. *Secretions*, simple compounds released by metabolic processes.
3. Plant *mucilages*, more complex organic compounds originating in root cells or from bacterial degradation.
4. *Mucigel*, a gelatinous layer composed of mucilages and soil particles intermixed.
5. *Lyzates*, compounds released through digestion of cells by bacteria.

Figure 1.2

Diagram of a root showing the origins of organic materials in the rhizosphere (Brady, 1990).

stimulates bacterial transformations and builds up the organic carbon in the rhizosphere. In addition, the rapid decay of fine root biomass becomes an important source of organic carbon in soils. This additional organic carbon increases microbial mineralization rates (Schnoor et al., 1995).

Plants aid in microbial transformations in the rhizosphere in many ways. For instance, roots can harbor mycorrhiza fungi, which metabolize organic pollutants (Schnoor et al, 1995). A mutually beneficial or symbiotic association between numerous fungi and the roots of higher plants is called mycorrhizae, a term meaning "fungi root". This association is now known to be widespread and to affect approximately 80% of plant species (Burken, 1996). This association greatly increases the availability of several essential nutrients to plants. The symbiotic relationship provides the fungi with sugars and other organic exudates for use as food and the fungi provide an enhanced availability of several essential nutrients, including phosphorus, zinc, copper, calcium, magnesium, manganese, and iron. These fungi provide unique enzymatic pathways that help degrade organics that cannot be transformed solely by bacteria (Shnoor et al, 1995). Some of the degradative enzymes are amylase, xylanase, mannase, polyphenol oxidase, protease, esterase, and cellulase.

There are two types of mycorrhizal associations of considerable practical importance to phytoremediation: ectomycorrhiza and endomycorrhiza. The ectomycorrhiza group includes hundreds of fungal species associated primarily with trees such as pine, birch, hemlock, beech, oak, spruce, and fir. These fungi, stimulated by root exudates, cover the surface of feeder roots with a fungal mantle. The hyphae of the fungi penetrate the roots and develop around the cells of the cortex but do not penetrate the cell walls [Figure 1.4].

The endomycorrhiza group, the most important of which are called vesicular arbuscular (VA) mycorrhizae, penetrates the root cell walls, enters the root cells, and

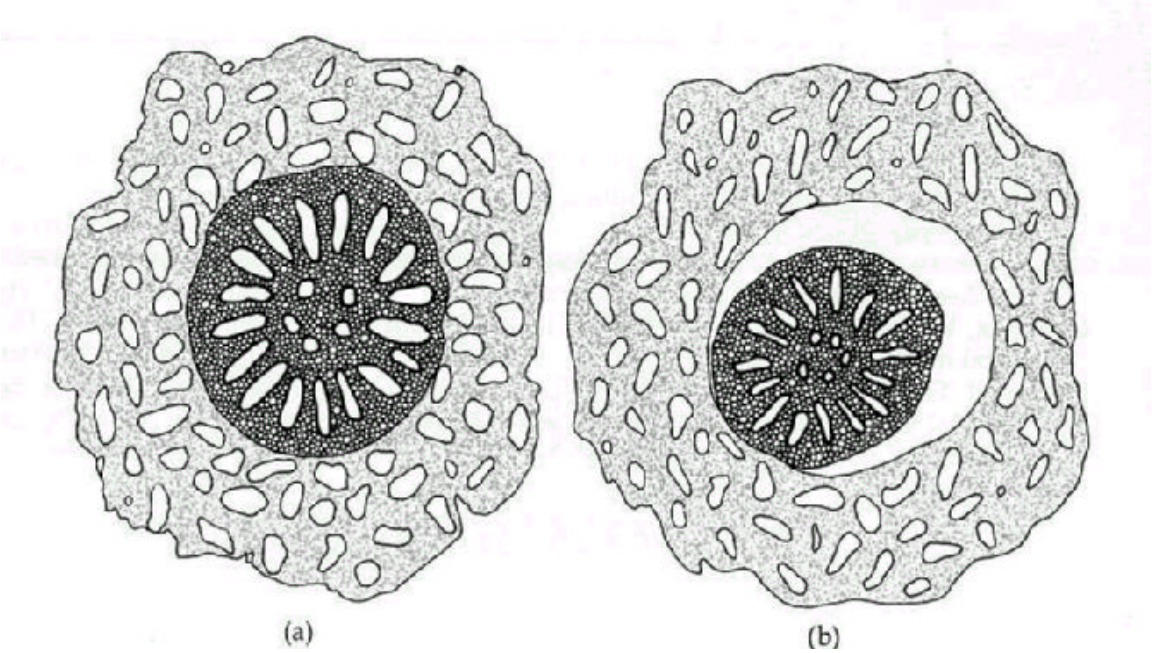


Figure 1.3
Cross section of a corn root surrounded by soil: (a) During periods of low soil moisture stress on the plant, the root completely fills the soil pore. (b) When the plant is under severe moisture stress the root can shrink, significantly reducing root-soil contact (Brady, 1990).

forms hyphal masses within the cells. This group is the most common and widespread of mycorrhizae. These VA mycorrhizae penetrate the root cortical cell walls of host plants with their hyphae. Inside the plant cell, the fungi form highly branched, small structures known as arbuscules. These structures are considered to be the sites of transfer of nutrients and compounds from the fungi to the host plants [Figure 1.4]. Other structures, called vesicles, serve as storage organs for the plant nutrients and other products.

The increase in nutrient availability provided by mycorrhizae is thought to result from the nutrient-absorbing surface provided by the fine filamentous hyphae of the fungi. The surface area of mycorrhizal infiltrated roots has been calculated to be as much as 10 times that of the uninfested roots. Also, the soil volume from which compounds are absorbed is greater for mycorrhizal associations. The very fine fungal hyphae extend up to 8 cm into the soil surrounding the roots, thereby increasing the absorption of nutrients and other compounds that do not diffuse readily to the roots (Table 1.4), (Brady, 1990).

1.5 Research Objective

The primary research objective was to evaluate the effectiveness of certain terrestrial and aquatic plants to uptake and biodegrade PCE and TCE and to identify and compare parent compound and metabolite concentrations from extracted laboratory and field plants. Specific objectives were as follows:

1. Determine sorption and degradation rates of PCE and TCE by live aquatic and terrestrial plants;
2. Determine the effect of varying concentrations of PCE and TCE on the reaction kinetics and to evaluate the toxicity caused to the plant;
3. Identify metabolites and conduct mass balances using both [^{12}C] and [^{14}C] PCE and TCE.

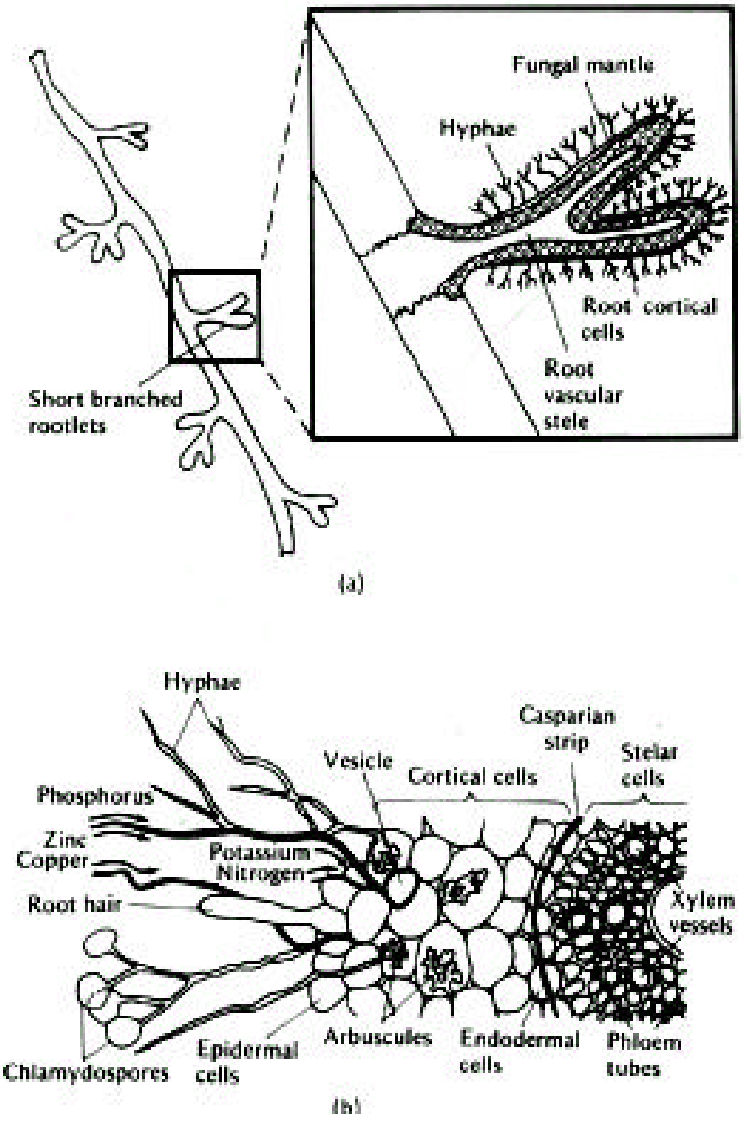


Figure 1.4

Diagram of ectomycorrhiza and vesicular arbuscular (VA) mycorrhiza association within plant roots: (a) The ectomycorrhiza fungi association. (b) The VA mycorrhizae (Brady, 1990).

TABLE 1.4
Effect of Inoculation with Mycorrhiza and of Added Phosphorus on the Content of Different Elements in the Shoots of Corn (Brady, 1990)

Expressed in micrograms per plant

Element in plant	No phosphorus		25 mg/kg phosphorus added	
	No mycorrhiza	Mycorrhiza	No mycorrhiza	Mycorrhiza
P	750	1340	2970	5910
K	6000	9700	17500	19900
Ca	1200	1600	2700	3500
Mg	430	630	990	1750
Zn	28	95	48	169
Cu	7	14	12	30
Mn	72	101	159	238
Fe	80	147	161	277

The aquatic plant selected for the study was duckweed (*Lemnaceae*). The primary terrestrial plants being investigated are black willows (*Salix nigra*) and eastern poplars (*Populus deltoides x nigra*). Duckweed is an excellent candidate for this study since it is already in use in certain wastewater treatment facilities, and it has shown a propensity to take up and phytodegrade numerous compounds. Willows and poplars were chosen because they can uptake large amounts of water through their extensive root systems, are very hardy, and have low maintenance requirements.

CHAPTER II

REVIEW OF LITERATURE

2.1 Previous Research

Schnabel et al. (1997) investigated the uptake and transformation of TCE by edible garden plants. Three different plants (carrots, spinach, and tomatoes) were dosed with [^{14}C] and [^{12}C] TCE. Radiolabled recoveries varied from 50% to 70%. 74% to 95% of TCE was volatilized through the leaves and 5% to 25% was sorbed to the soil. 1% to 2% was found in the plant material and could not be extracted. These findings suggest that the compound was taken up, transformed, and bound to the plant tissue.

Newman et al. (1997) used hybrid poplars to degrade TCE to several of its known metabolites. These poplars were also shown to transpire measurable amounts of TCE into the atmosphere. Tests were conducted with axenic poplar tumor cell cultures in addition to whole plant experiments to determine if poplar cells could metabolize TCE in the absence of soil and mycorrhizal flora. These "clean" cells produced the same metabolites as those containing microflora. Cells dosed with [^{14}C] produced low levels of radiolabeled carbon dioxide. Similarly, Walton and Anderson reported that TCE degradation by microbial organisms was enhanced in the rhizosphere of various plant and tree species (Chappell, 1997). In a later study, Newman et al. (1999) tested hybrid poplar trees to determine the fate of TCE. The poplars were grown in lined compartments for three years replicating field conditions. The trees were able to remove over 99% of the TCE during the growing season while only 9% of the removed 99% TCE was transpired through the leaves during the second and third years.

Burken and Schnoor (1997) found that hybrid poplars can uptake, hydrolyze, and dealkylate atrazine to less toxic metabolites. The metabolism of atrazine occurred in roots, stems, and leaves and increased with increasing residence time in the tissues. Fifty days after the initiation of the experiment, 21% of [^{14}C] labeled atrazine was present in the leaves, and at 80 days, the parent compound remaining in the leaves was 10%.

Burken and Schnoor (1998) also tested 12 common organic compounds found at hazardous waste sites for uptake by hybrid poplars. The whole-plant experiments were conducted hydroponically utilizing [^{14}C] labeled compounds to determine translocation and fate. Predictive relationships for common organic contaminants were established using log K_{ow}.

Thompson et al. (1998) examined the potential of hybrid poplars to remediate sites contaminated with trinitrotoluene (TNT). Both hydroponic and soil experiments were conducted. Studies showed that up to 75% of the contaminant was taken up and transformed in the roots while only 10% was translocated to the leaves. Two identifiable metabolites of TNT were found along with a number of unidentified compounds that were more polar than TNT.

Conger and Portier (1997) evaluated six tree species (black willow, yellow poplar, bald cypress, river birch, cherry bark oak, and live oak) to determine their ability to remediate groundwater contaminated with bentazon (an agricultural herbicide). Relative growth rates and the degree of phreatophytic behavior were determined during the first phase of the experiments. During the second period, two trial groups were dosed with bentazon. The first group had very high dosage concentrations of 2000 mg/L decreasing to 1000 mg/L due to toxic effects on the plants. The second group was dosed at 150 mg/L and was used as the final indicator test for determination of effectiveness. The river birch showed the best phreatophytic response although the black willows displayed very good transpiration given their smaller size. The black willows were determined to be

the best at degrading and mineralizing bentazon upon translocation to the plant leaves and was also the most tolerant specie of tree to bentazon exposure.

O'Niell et al. (1998) investigated the viability of using mixed-species microbial mats to degrade PCE and TCE. All experiments were conducted hydroponically with either sealed, batch-vials or with a continuous flow through growth chamber. Initial sorption of PCE was rapid and the partition coefficients were approximately twice that of TCE, which was attributed to the greater aqueous solubility of TCE. In experiments dosed with PCE, TCE usually appeared within seven days indicating dehalogenation. It was suggested that due to the absence of DCE and VC, daughter products of TCE, the degradation was not following a complete dehalogenation pathway.

Nzengung et al. (1999) utilized green, filamentous algae, parrotfeather, and waterweed to uptake and degrade PCE, carbontetrachloride (CTC), 1,1,2-trichloroethane (TCA), and hexachloroethane (HCA). It was observed that both live and dead algae and plant material transformed the chlorinated organic compounds suggesting that the algae and plants contain dehalogenase activity that persists after death. There was also an appreciable amount of the chlorinated organic compound that was taken up into the plants and irreversibly bound.

Research by Hughes et al. (1997) focused on the ability of *myriophyllum spicatum* and its surrounding microflora, axenic *myriophyllum aquaticum*, and axenic *catharanthus roseus* to degrade TNT. These plants were chosen for comparison between plant-microbe interactions leading to transformation vs. strictly plant-mediated transformation. The studies showed that the *myriophyllum* and the *catharanthus roseus* transform TNT rapidly regardless of the presence of microflora. No primary products of transformation such as aminonitrotoluenes were identified. Two aminated nitrotoluenes were the only identifiable transformation products observed in the experiments. In a similar study, Palazzo and Leggett used bush beans while Wolfe et al., used stonewort to take up and

degrade TNT (Hughes et al, 1997). Their research did not differentiate between plant processes and plant-microbe processes. Low levels of reduction products (e.g.,aminonitrotoluenes) were found in the leaves of the bush beans and in the aqueous medium of the stonewort.

Roper et al. (1996) compared the ability of minced horseradish roots vs. purified horseradish peroxidase (HRP) to decontaminate water polluted with chlorinated phenolic compounds. It has been established that HRP is an effective catalyst, especially when coupled with hydrogen peroxide (H_2O_2) for the cleanup of water contaminated with phenols and anilines. Horseradish roots, with the addition of H_2O_2, were shown to remove 99% of 27 of the 50 compounds tested. In the absence of H_2O_2, sorption was shown to be the main mechanism of substrate removal and was dependent on the pH of water.

Pradhan et al. (1998) conducted studies to determine the potential for phytoremediation of soils contaminated with polynuclear aromatic hydrocarbons (PAHs) using the plant species alfalfa, switch grass, and little bluestem grass. All three plants showed appreciable removal of both total PAHs and carcinogenic PAHs in soil. The total PAH decrease using switch grass, alfalfa, and little bluestem grass were 57%, 56%, and 47%, respectively. Respective decreases in the carcinogenic PAHs were 30%, 28%, and 28%. The control soil only had a 26% total PAH decrease and no carcinogenic PAH decrease was observed.

Halford (1998) conducted an assessment of the effects of phytoremediation on the site around area C at the Naval Training Center, FL. A three-layer finite-difference model was used to simulate ground-water flow through the surficial aquifer system. Halford found that under existing conditions, phytoremediation alone at area C would not stop the discharge of all contaminants into the nearest receptor, Lake Druid. This observation was determined from the evapotranspiration losses versus the ground-water

discharge into Lake Druid, 3.7 gal/min and 20.4 gal/min respectively. The addition of a drainage ditch to intercept flow from 0 to 40 feet below the water table and redirect it through the rooting zone of selected plants was thought to be a viable option for remediation of the site. The ditch could be manipulated to increase residence time or to slow the flow rate.

2.2 Proposed Mechanisms and Pathways

2.2.1 Degradation and Mineralization within the Plant

The processes involved in the transformation and degradation of compounds within plants are not well known. Numerous hypotheses have been proposed, and currently there are two major areas of research being conducted. They are similar in that both point toward an enzymatic process involved in oxidizing TCE to various metabolites. Also, there is always the possibility of more than one mechanism or pathway taking place within a plant (Chappell, 1997). Proposed plant-mediated degradation pathways for PCE and TCE are shown in Figures 5.1 and 5.2.

The first area of research being conducted by Newman et al., (1997), Chappell (1997), and Schnabel et al. (1997) suggested that transformation of TCE within plants may be similar to the mammalian breakdown of TCE. This theory is based on similar metabolites being observed in both plants and animals. Cunningham et al. (1996) stated that many of the enzyme systems present in mammalian metabolism of TCE are also present in plants (e.g., cytochrome p-450 and glutathione).

The second area of research being conducted by Schnoor et al. (1995) proposed that TCE metabolism is the result of dehalogenase enzyme. Dehalogenase is an ethylene degrading enzyme that oxidizes alkanes, alkenes, methanes, and their halogenated analogues. An antibody assay has been developed to determine the presence of the dehalogenase enzyme in plants. This technique can be used to predict the ability of a

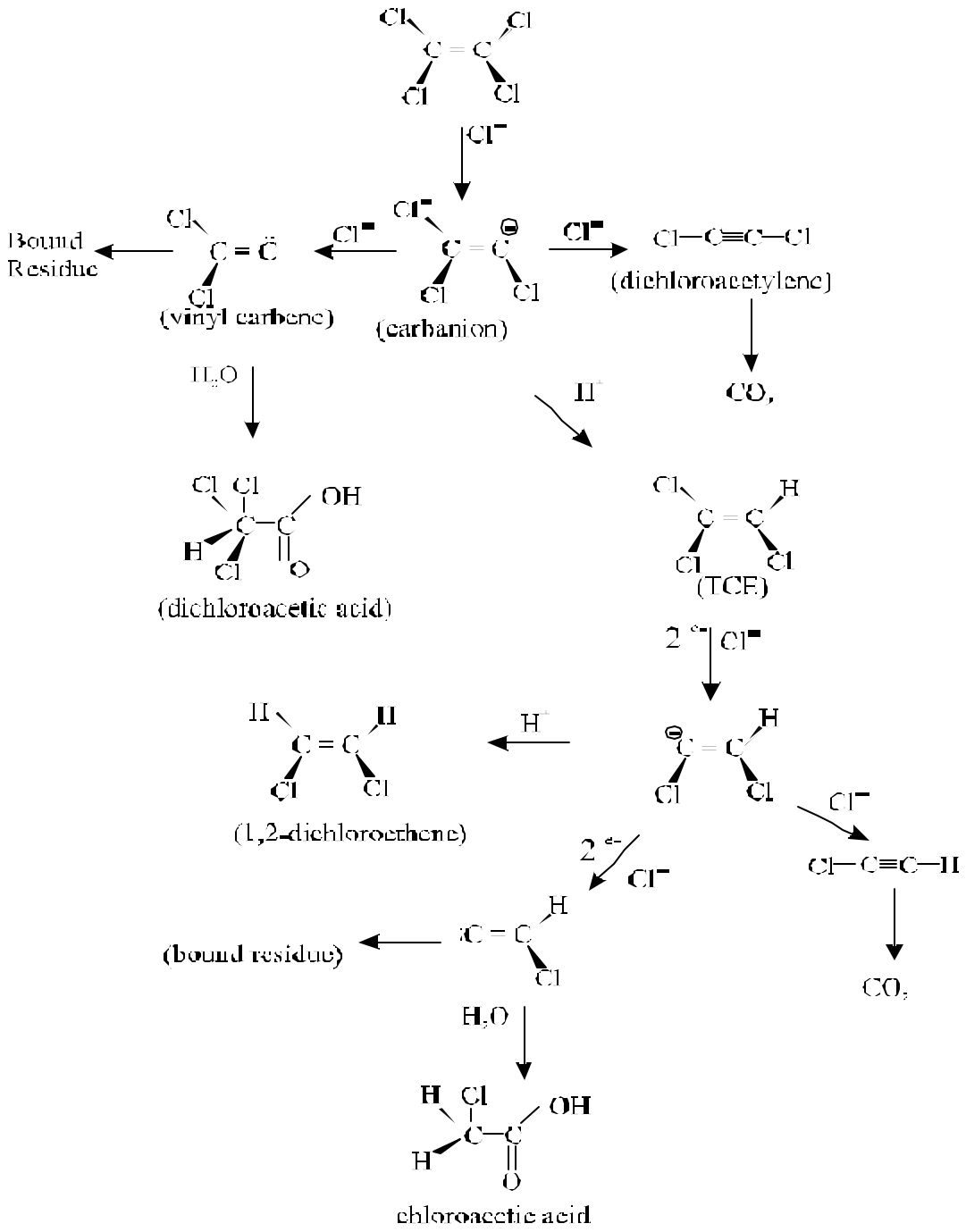


Figure 2.1
Proposed plant-mediated degradation pathway for PCE

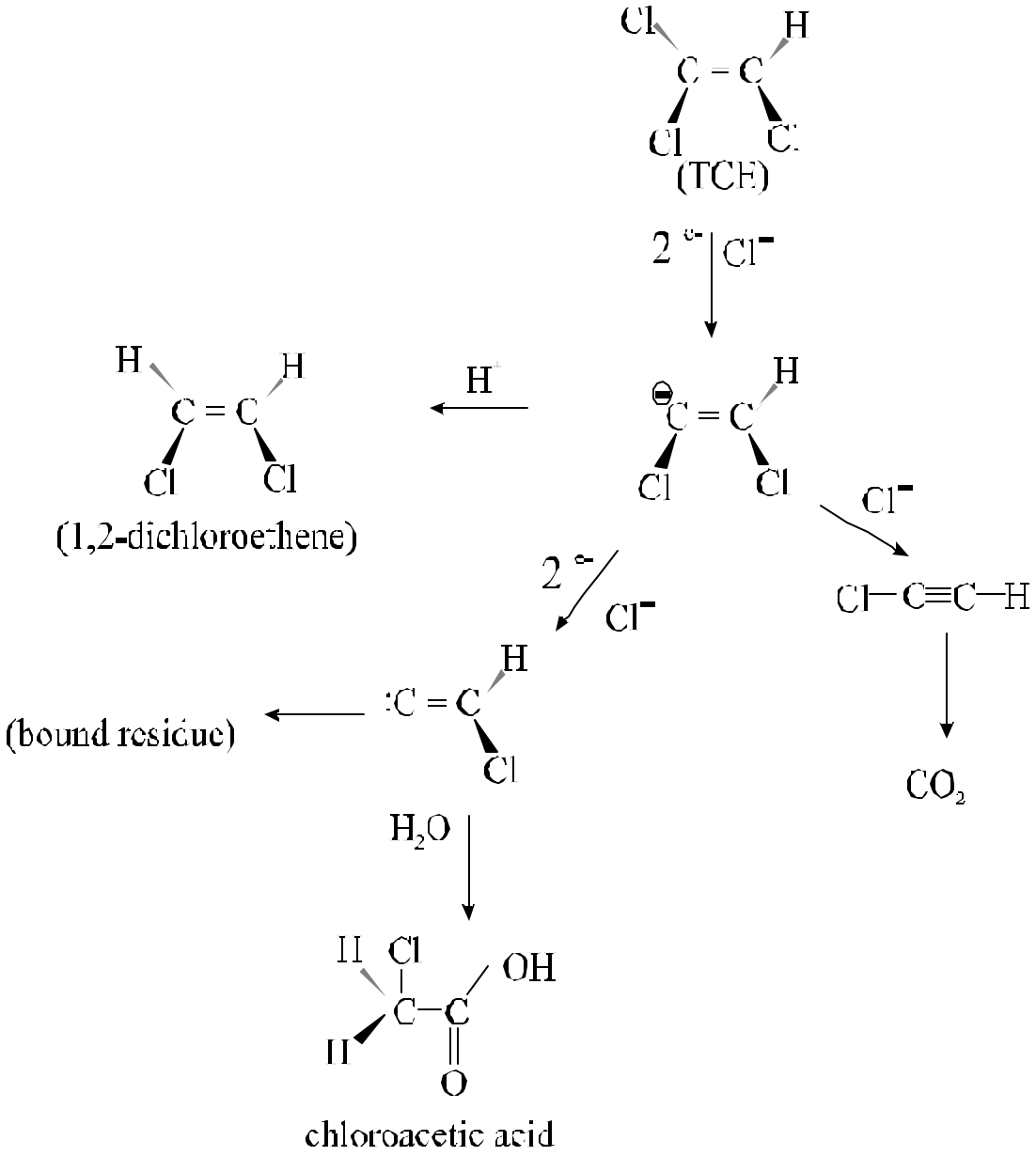


Figure 2.2
Proposed plant-mediated degradation pathway for TCE

plant or species to degrade chlorinated solvents and will aid in the selection of viable plants for a particular site (Chappell, 1997).

2.2.2 Degradation and Transformation in the Rhizosphere

Davis et al. (1996) reported that microbial degradation of TCE could take place either aerobically of anaerobically. The aerobic process is an oxidative mechanism catalyzed by a mono-oxygenase enzyme. Methane mono-oxgenases (MMO) and alkene oxygenases are the enzymes involved. Each enzyme uses either methane or an alkene as its primary substrate. Plants may transfer exudates to anaerobic sites that stimulate methanogens to produce methane. The methane then stimulates aerobic methanotrophs that cometabolize TCE via the MMO enzyme.

Another microbial mechanism being researched is dechlorination catalyzed anaerobically via a dehalogenase enzyme. Plant exudates supply carbon for use as a reductant by methanogenic microbes. This carbon source allows the microbial population to increase at which time it will begin to cometabolically dehalogenate TCE (Chappell, 1997).

CHAPTER III

MATERIALS AND METHODOLOGIES

3.1 Selection and harvest of plants

Lemnaceae, also referred to as duckweed, was the primary aquatic plant used for the experiments conducted in the laboratory. Four genera of duckweed occur in North America: *Lemna minor*, *Wolffia columbiana*, *Spirodela polyrhiza*, and *Wolffiella floridana*. *Lemna minor* and *Wolffia columbiana* were obtained for this study. The original duckweed was obtained from the White County Wastewater Treatment facility in Cleveland, GA. After collection, the duckweed was transferred to the UGA Riverbend Road Greenhouse where it was grown and maintained in a 20-gallon container. Periodically, specified amounts of duckweed were removed for use in the numerous experiments. This periodic removal also acted as a harvesting measure instigating new growth and relieving competitive stress. Table 3.1 gives a description of these two generas and their habitats.

Salix nigra, black willow, was one of two deciduous trees used in the experiments. Willow cuttings were obtained from different locations in Athens, GA and also from Carswell Air Force Base, Fort Worth, TX. Some of the cuttings collected in Athens were taken from the bank of a drainage ditch on Cedar Shoals Drive and others were taken from a wet, low lying area near the Parking Services Department at the University of Georgia. After collecting the cuttings, all the branches and leaves were removed and the main cuttings were reduced to approximately 24 inches in length. The cuttings were then taken to the Riverbend Road Greenhouse and placed in 2000 mL flasks with the addition of 30 % Hoagland solution and approximately 0.75 g of Root Tone® to each flask for the initiation of rooting. The pH was adjusted periodically to

TABLE 3.1

Characteristics of *Lemnaceae*

Scientific Name	Group	Description	Habitat	Distribution
Lemna minor L.	Monocotyledon	Small floating herb, frond green on both surfaces, flat, slightly longer than broad, nearly symmetrical, 2-5 mm long, with 3 veins. Rootlet 1 per frond.	Common floaters in freshwater habitat. Fronds may occur singly or grouped into colonies of 2-4.	Native in most regions of the world. Fronds are eaten by waterfowl and fish.
Wolffia columbiana Karst	Monotcotyledon	Very small floating herb, frond green, spherical, thick, to 1.2 mm in diameter, without veins. Rootlets absent.	Frequent species of lakes, ponds, and swamps. It may occur in such abundance as to obscure the surface of the water.	Common in North, Central, and South America. It provides a source of food for waterfowl and fish.

approximately six replicating the best growing environment for this particular plant. Table 3.2 gives a description of *Salix nigra* and its habitat.

Populus deltoides, eastern poplar, was the other deciduous tree used in the experiments. *Populus* was chosen because of its particular characteristics and due to the large amount of work being conducted using this tree in the phytoremediation field. The poplars were obtained from Carswell Air Force Base, Fort Worth, TX. The cuttings were sent at the specified length of 24 inches and upon arrival were prepared in the same manner as the black willows. Table 3.2 gives a description of *Populus deltoides* and its habitat.

3.2 Uptake and Transformation Experiments with [^{12}C] and [^{14}C] PCE and TCE

Individual uptake and transformation experiments were conducted with black willows, poplars, and duckweed. All experiments were replicated to verify initial results. Certain plants were dosed with a combination of [^{12}C] and [^{14}C] PCE or [^{12}C] PCE alone while others were dosed with a combination of [^{12}C] and [^{14}C] TCE or [^{12}C] TCE alone during separate experiments to determine degradation rates, reaction kinetics, and transformation products.

3.2.1 Duckweed

Six 2000 mL modified screw top Erlenmeyer flasks were set up, sealed, and dosed in the greenhouse [Figure 3.1]. Four treatment flasks contained both duckweed and DI water while two control flasks contained only DI water. All flasks contained 1600 mL of DI water. Approximately 30 g of duckweed was weighed and put into each of the four treatment flasks. The tops of the flasks were first sealed with parafilm and teflon disks manufactured at the UGA instrument shop were tightly screwed onto the flasks. These chambers were sealed to reduce the chance of volatilization of the contaminants.

TABLE 3.2

Characteristics of *Salix nigra* and *Populus deltoides*

Scientific Name	Group	Description	Habitat	Distribution
Salix nigra	Deciduous	Finely serrated, alternate leaves, 3-6 inches long and ½ inch wide, short trunked and spreading from 20-35 feet. 30-40 feet tall.	Prefers moist, wet soil but will grow under a wide range of conditions.	North America (Banks of streams, shores, rich low woods, etc.).
Populus deltoides	Deciduous	Leaves alternate, 3-7" long and wide, margins with 15 or more teeth per side, quaking effect, petiole flat. 50-80' wide and 80-100' tall. Broad, spreading, round-topped.	Prefers moist soil but will grow under a wide range of conditions.	North America (River banks, bottomlands, and rich woods, etc.).

Figure 3.1
Photograph of lemnaceae experimental setup

All reactors were dosed in the greenhouse. Two treatments and one control were dosed with [^{12}C] only while the other two treatments and control were dosed with [^{12}C] and [^{14}C] acting as a tracer. The [^{14}C] enables the tracking of the contaminant in the plant over time, the determination of mineralization of the compound by the plant, and produces a more accurate mass balance calculation.

Two separate duckweed experiments were conducted three months apart using the sealed flasks. The duckweed was allowed to acclimate for several days before dosing. In the first experiment, the aqueous phase was dosed with 10 mg/L [^{12}C] TCE and a [^{14}C] TCE tracer with specific activity of 7225.21 counts per minute (cpm) / mL. The second aqueous phase was dosed with 20 mg/L [^{12}C] PCE and a [^{14}C] PCE tracer with specific activity of 1476.25 cpm/mL.

In addition to the above experiments, four continuous air flow through plant growth chambers were set up to evaluate the degradation of PCE and TCE under simulated natural conditions. All four of the experiments were dosed with [^{12}C] PCE and one was also dosed with a [^{14}C] PCE tracer. Aqueous phase dosing concentrations ranged from 10 to 20 mg/L. The chambers were fitted with removable glass crowns that were sealed to the chambers using vacuum sealant. Three liters of 30% Hoagland solution and between 40 and 60 g of duckweed were added to the chambers, resulting in a headspace volume of 1.5 to 2 L. A sample port was located at the base of the chambers and air inflow/outflow tubes were attached to the top of the chambers. Traps used in the experiments included 10 g of granular activated carbon (GAC) and/or 50 mL ethyleneglycol monomethylether (EGME) to trap PCE, TCE and their metabolites. Sample ports were located at the base of the trap chambers also. When using radiolabled compounds, a trap containing 50 mL of 1 N NaOH was employed to capture [$^{14}CO_2$]. Blanks were used to accumulate condensation where appropriate. Airflow was maintained between 0.5-1.0 L/min through the upper compartments using a vacuum pump attached to the last trap. The inflow tube was suspended in a beaker of DI

water and moistened air entered the chamber through the inflow tube and traps were attached to the chamber headspace outflow tube. A magnetic stir bar was used to mix the liquid in the chamber prior to each sampling event. Aqueous phase samples were collected from the sample ports in the main chamber and traps as often as once per day, and were analyzed as described below.

3.2.2 Black Willow and Eastern Poplar

Black willow and poplar cuttings used in the experiments were selected according to their health and maturity. Duplicate runs were made with cuttings that came from the same tree and when possible, from the same branch. This provided consistency and reduced variation from plant to plant. Upon selection, the willows were transferred to modified 2000 mL Erlenmeyer flasks to acclimate for several days. A 30% Hoagland solution was placed in each flask and the pH was optimized to approximately 6 for each plant. The modified Erlenmeyer flask had a bottom sampling port and a larger feeding port near the top connected to a Pyrex® watering vessel by 3/8" Tygon® tubing.

After several tests, a reliable method of sealing these reactors was discovered. The willow cuttings were first placed in the bottom flask and marked at the point where it exited the top. All roots and branches were removed from this area and a layer of parafilm was wrapped around each willow cutting until it fit very snuggly inside of the Teflon-lined septum of the cap. Once the appropriate amount of parafilm was in place, a layer of aluminum foil was wrapped around the parafilm to minimize sorption of the compound. The cuttings were then placed inside the septum and the caps were screwed tightly onto the flasks. A thick layer of silicone sealant was placed on top of each cap to prevent leakage.

The foliage on the upper half of the plants were encased in inverted 5000 mL Erlenmeyer flasks with ports on the top and bottom. The upper flasks were sealed in the same manner as the bottom flasks as outlined above. Six-inch O-ring clamps that fit

outside of the flasks were secured to a metal stand to support the weight and keep the experiment upright. The top ports were attached to GAC safety traps, where the inflow of air occurred, to catch organic contaminants in any vapors that might escape from the main flasks and to catch any compounds that might be introduced from the outside. The bottom ports were attached to a series of traps that were used to capture any products that the plants transpired. The first trap was empty to catch any transpired water that built up during the duration of the experiment. The second trap contained EGME to capture any volatiles, the third trap contained 1M sodium hydroxide to capture any CO_2, and the fourth trap contained 10 g of GAC to capture all compounds. The last trap was connected to a vacuum pump maintaining a continuous airflow between 0.5-1.0 L/min through the entire system [Figures 3.2-3.4]. Silicone grease was used to seal all joints to ensure there was no leakage at the numerous connections. After data were analyzed for several of the willow experiments, the two solvent traps were omitted and another GAC trap was added with 15 g of GAC. The solvents did not trap a significant amount of the [^{14}C] while the GAC was a very effective trap for these compounds.

Twenty-two experiments using black willows were conducted, 11 using PCE and 11 using TCE. Radiolabeled [^{14}C] tracers were used in seven of the PCE experiments and five of the TCE experiments. PCE experiments were dosed with aqueous phase concentrations ranging from 10 to 45 mg/L. TCE experiments were dosed with aqueous phase concentrations ranging from 10 to 45 mg/L. All experiments dosed with [^{14}C] used traps while only certain [^{12}C] experiments used traps. The experiments using only the bottom Erlenmeyer flask encasing the root zone of the plants simulated natural conditions and allowed longer durations for data collection [Figure 3.5].

Five eastern poplar experiments were conducted. All poplar experiments used [^{12}C] PCE and four of the five utilized [^{14}C] PCE tracers. Dosing concentrations remained the same for all five experiments at 20 mg/L. All poplar experiments used both the bottom and top flasks including traps [Figures 3.2-3.4].

Figure 3.2
Photograph of *populus* and *salix* experimental setups

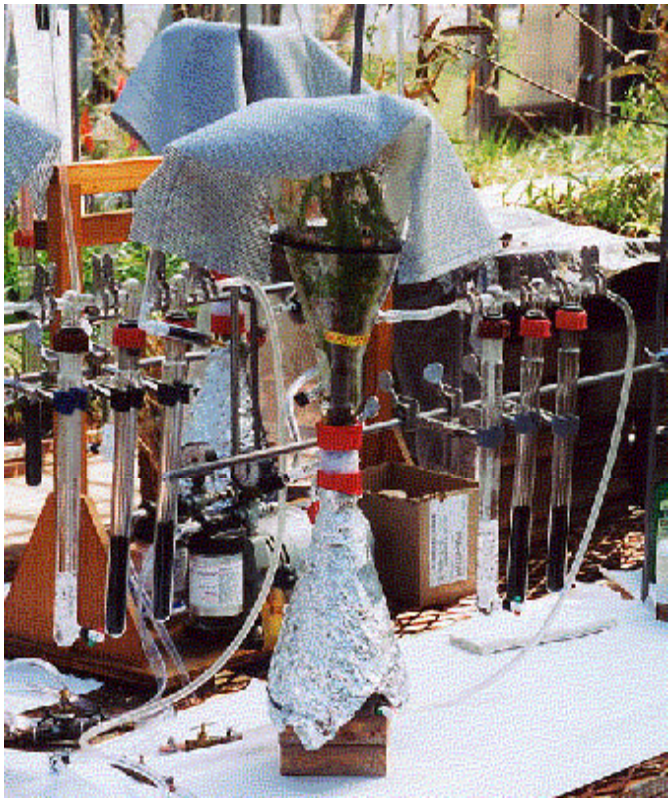


Figure 3.3
Photograph of *populus* and *salix* experimental setups

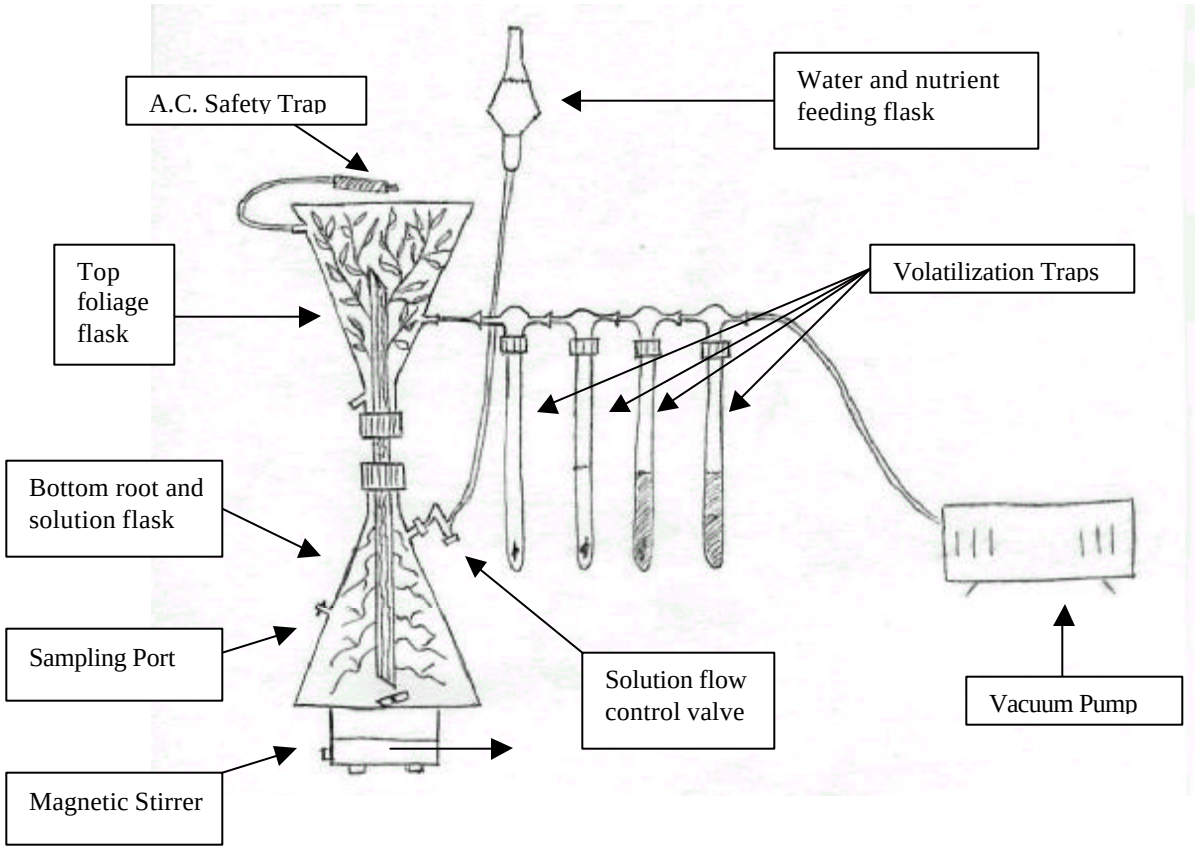


Figure 3.4
Diagram of populus and salix experimental setups

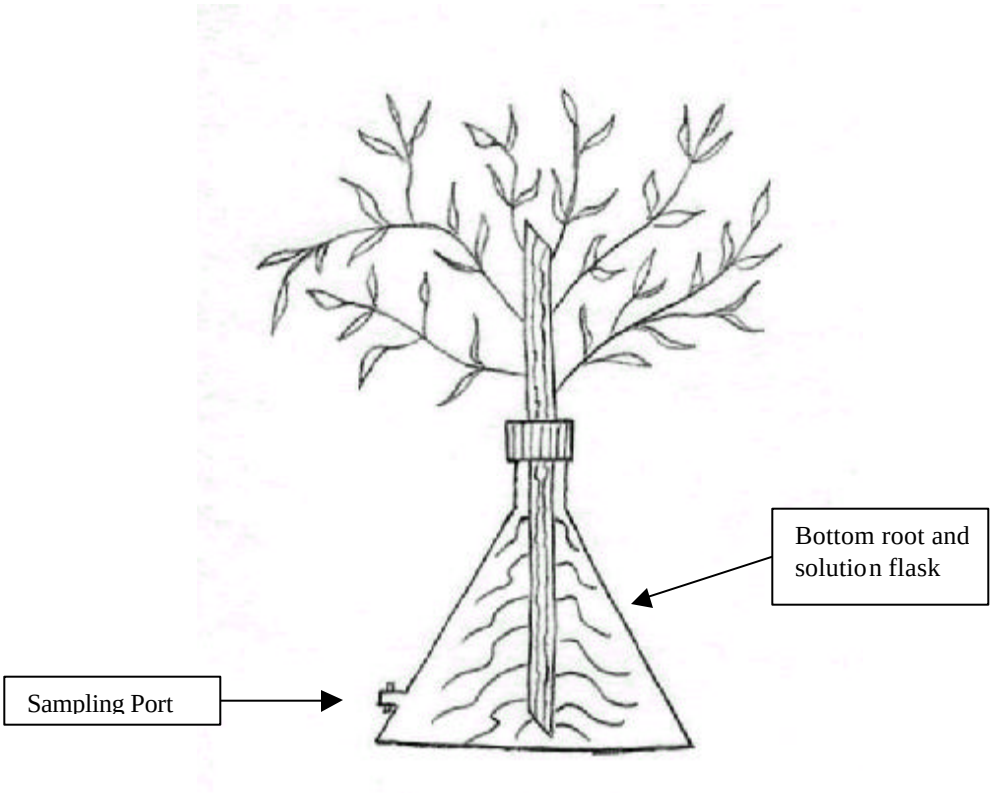


Figure 3.5
Diagram of populus and salix experimental setups

3.3 Reagents and Solvents

PCE and TCE were obtained from Aldrich Chemical Company (Milwaukee, WI). Labeled [^{14}C] PCE and TCE were obtained from Sigma Chemical Co. (St. Louis, MO). Analytical grade methanol (Fisher Scientific Co., Pittsburgh, PA) was the solvent used to prepare stock solutions of PCE and TCE. HPLC grade hexane (Fisher Scientific Co., Pittsburgh, PA) and methyl-tert butyl ether (MTBE) (Aldrich Chemical Co., Milwaukee, WI) were used for extraction of PCE, TCE, and their metabolites from both liquid and solid phases of samples. Trichloroacetic acid (TCAA) was in solid form and was obtained from Fisher Scientific Co. (Fair Lawn, NJ). Dichloroacetic acid (DCAA) was in liquid form and was obtained from Aldrich Chemical Company (Milwaukee, WI). Monochloroacetic acid (MCAA) was in solid form and was also obtained from Aldrich. All chemicals were greater than 99% purity, as confirmed by analysis using a gas chromatograph/ mass spectrometer (GC/MS). Chemicals were used as obtained without further purification.

Acetic acids were derivitized using diazomethane prior to GC/ECD analysis. A diazomethane/MTBE mixture used to derivatize polar chlorinated products and chloroacetic acids was prepared in-house using a diazomethane-generation apparatus (Aldrich Chemical Company, Milwaukee, WI). To generate diazomethane, approximately 0.2 g 1-methyl-3 nitro-1 nitroguanidine (MNN) (Aldrich Chemical Company, Milwaukee, WI) was added to the inner chamber of the generation appartus. Six to eight mL of MTBE was added to the outer chamber of the appartus. Three mL of 5M NaOH was slowly injected into the inner chamber to react with the MNN and form diazomethane gas that is absorbed by the MTBE. The diazomethane/MTBE mixture was removed, put into 2 mL sample vials and placed in the freezer at -4°C until used (or for a maximum of 14 days).

Derivitization was accomplished using the following procedure. A volume of 5 mL of each liquid phase sample was placed in 20 mL vials. One half mL of

diazomethane was added to the vial and then placed in the freezer for a minimum of 30 minutes. Derivitization by diazomethane transforms acetic acids into methyl esters. For each acetic acid, there is a corresponding methyl ester that is used to determine the concentration of that particular acetic acid in the extract. These methyl esters were then analyzed on the GC/ECD.

3.4 Analytical Methods

3.4.1 Gas Chromatography

A Shimadzu GC-14A gas chromatograph equipped with an electron capture detector and a Shimadzu CR501 Chromatopac integrator were used for quantitative and qualitative analysis of the PCE, TCE, and chloroacetic acids. All analyses were by direct splitless injection of 0.5 to 1 ? l of hexane or MTBE sample extract using a Shimadzu AOC 17 auto injector and AOC 1400 automatic sampler. The separation column used in the analyses was a DB-5 megabore * 0.53 mm * 1.5 ? m (methylpolysiloxane phase) manufactured by J.W. Scientific (Folsom, CA). Column lengths of 30 m and 15 m were used during the analyses. For the 30 m length, the column temperature was programmed at 35^{o}C for 2 minutes, followed by a temperature increase of 5^{o}C/min to 90^{o}C, and a subsequent temperature increase of 35^{o}C/min to 160^{o}C for 2 minutes. Nitrogen was used as the carrier gas with a combined flow rate of 40 mL/min and an in-column flow rate of 10 mL/min. For the 15 m length, the column temperature was programmed at 35^{o}C for 6 minutes, followed by a temperature increase of 5^{o}C/min to 90^{o}C, and a subsequent temperature increase at 35^{o}C/min. to 160^{o}C for 2 minutes. For both columns, the injector and detector temperatures were 200^{o}C and 340^{o}C, respectively. Calibration curves for PCE, TCE, and chloroacetic acids were prepared from standards weekly or when instrument conditions changed to ensure accurate quantitative results. One mg/L dichlorobenzene samples were analyzed as an external standard with each sampling event. Qualitive detection limits (DL) were calculated by running triplicate blanks and

low-level (1 ug/L to 15 ug/L) samples. A DL of 1 ug/L was observed for both PCE and TCE. A practical quantitation limit (PQL) was calculated using a signal to noise ration of three as the basis for determining the lowest concentration that can be accurately measured using this instrument. A PQL of 1 ug/L was calculated for PCE and a PQL of 5 ug/L was calculated for TCE.

A Hewlitt Packard 6890 gas chromatograph with a micro-electron capture detector was also used for quantitative and qualitative analysis of PCE, TCE, and chloroacetic acids in liquid and solid phase samples. All analyses were by splitless injection of 1 µL of hexane or MTBE sample extract using a HP 7683 Series automatic injector. The column used was a DB-VRX (J & W Scientific) with 450 µm inner diameter, 2.55 µm film thickness, and 75 m length. The column oven temperature was programmed to begin at 35°C and held for 12 minutes, followed by a temperature increase of 5°C/minute to 65°C and held for 1 minute, with a final ramp of 17°C/minute to 200°C that was held for 3 minutes. This program resulted in a total run time of 29.94 minutes per sample. Grade 5.0 helium was used as the carrier gas and grade 5.0 nitrogen was used as the makeup gas with an in-column flow rate of 3.3 mL/minute and 60 mL/minute, respectively. The injector temperature was set at 250°C and the detector temperature was set at 310°C. Calibration curves for PCE, TCE, and chloroacetic acids were prepared from standards weekly or when instrument conditions changed to ensure accurate quantitative results. One mg/L dichlorobenzene samples were analyzed as an external standard. Qualitive DLs were calculated by running triplicate blanks and low-level (1 ug/L to 15 ug/L) samples. A DL of 1 ug/L was observed for both PCE and TCE.

3.4.2 Biological Oxidation

A Packard 306 Sample Oxidizer was used to combust plant residue exposed to [^{14}C] labeled PCE and TCE and any CO_2 produced was captured. The CO_2 was captured in 8 mL Carbosorb and was then mixed with 12 mL Permafluor. The samples were

placed inside combustion cones for oxidation and 0.5 mL of Combust-Aid was added to samples that were hard to combust. Combustion times averaged 0.75 minutes for solid samples and 1.5 minutes for liquid samples. The Carbosorb/Permafluor mixture was analyzed via the liquid scintillation (LS) counter techniques described below in sections 3.4.3 and 3.4.4. Calibration and quench curves were prepared to ensure accurate results. A 98.4% recovery was calculated from the calibration curve.

3.4.3 Liquid Scintillation

A Beckman 5801 liquid scintillation (LS) counter was used to measure [^{14}C] activity in growth chamber experiment samples that were dosed with radiolabeled PCE and TCE. Background control samples were run for all experiments. A three minute run time for each sample was established by comparing recoveries ranging from two minutes to 20 minutes. Calibration curves were run periodically to ensure accurate results. Sample preparation techniques are described in section 3.4.4.

3.4.4 Sampling Procedures

At selected intervals, the solution phase of the growth chambers was sampled and the extract was analyzed by gas chromatography and/or liquid scintillation. The unlabeled aqueous samples for GC analysis were prepared as follows:

1. Twelve mL of hexane was measured into a 20 mL serum vial and the vial was sealed with an aluminum-faced septum;
2. One mL of aqueous sample was withdrawn from the growth chamber with a gas-tight syringe and injected into the hexane;
3. The mixture was manually shaken for 1 minute and centrifuged for 10 minutes;
4. Two mL of the hexane was withdrawn and transferred to a gas chromatography vial for analysis.

The samples for liquid scintillation assay were prepared as follows:

1. Fifteen mL of ScintiSafe 30% scintillation fluid (Fisher Scientific Co.) was added to a 20 mL scintillation vial and capped;
2. One mL of aqueous sample was withdrawn from the growth chamber with a gas-tight syringe and injected into the scintillation fluid;
3. The mixture was manually shaken for 15 seconds and then centrifuged for 10 minutes;
4. The samples were then run on the scintillation counter and were rerun after a 24-hour period to eliminate the effects of chemiluminescence.

The volatile contaminant traps used in certain experiments were tested periodically to determine the amount, if any, of parent compound and its metabolites that had been transpired by the plant. The [^{12}C] EGME and NaOH traps were sampled as follows:

1. Twelve mL of hexane was measured into a 20 mL serum vial and the vial was sealed with an aluminum-faced septum;
2. Twenty-five uL or 50 uL of sample was withdrawn from the trap with a gas-tight syringe and injected into the hexane;
3. The mixture was manually shaken for 1 minute and centrifuged for 10 minutes;
4. Two mL of the hexane was withdrawn and transferred to a 2 mL autosampler vial for GC analysis.

The [^{14}C] EGME and NaOH traps were sampled as follows:

1. Fifteen mL of ScintiSafe 30% scintillation fluid was added to a 20 mL scintillation vial and capped;
2. Fifty uL of sample was withdrawn from the trap with a gas-tight syringe and injected into the scintillation fluid;

3. The mixture was shaken for 15 seconds and then centrifuged for 10 minutes;
4. The samples were then run on the scintillation counter and were rerun after a 24-hour period to minimize the effects of chemiluminescence.

The activated carbon traps were extracted and sampled as follows for [^{14}C] and [^{12}C]:

1. The total 10 g of activated carbon from the trap was placed in a 50 mL serum vial;
2. Thirty mL of carbon disulfide was added to the vial to completely immerse the carbon and the vial was sealed with an aluminum-faced septum;
3. The vial was then sonicated for one hour;
4. The carbon disulfide extract was removed, measured, recorded, placed in a clean 50 mL serum vial and sealed with an aluminum-faced septum;
5. Steps 2-4 were repeated two more times;
6. For [^{12}C] PCE and TCE analysis, 15 uL of each extract was then mixed with 18 mL of hexane and shaken for one minute;
7. Two mL of the hexane was withdrawn and transferred to a 2 mL autosampler vial for GC analysis;
8. For [^{14}C] assays, 15 uL of each extract was placed in 15 mL of 30% Scintisafe and analyzed on the scintillation counter.

The plant matter used in these experiments was also extracted at the conclusion of the experiments to determine the amount of the parent compound bound reversibly to the plant. Whole aquatic plants were extracted while the terrestrial or woody plants were separated into leaves, roots, stems, upper bark, and lower bark before extraction. The procedure for extraction of plants exposed to the unlabeled chemicals for GC analysis was as follows:

1. The plant material was removed from the chamber and allowed to air dry;

2. A specified amount of plant material was weighed and then crushed in a mortar, the crushed plant was then placed in a 50 mL serum vial;
3. A small amount of MTBE (10 mL) was added and the vial was sealed with an aluminum-faced septa, sonicated for 30 minutes, and centrifuged for 10 minutes;
4. The top layer of MTBE was removed and measured and placed into a new 50 mL serum vial;
5. Steps 3 and 4 were repeated twice more and the final volumes of the MTBE extract were recorded;
6. Two mL of the MTBE extract from each extraction was withdrawn and transferred to a 2 mL autosampler vial for GC analysis.

The procedure for extraction of [^{14}C] activity from the plant phase was as follows:

1. The plant material was removed from the chamber and allowed to air dry;
2. A specified amount of plant material was weighed and then crushed in a mortar, the crushed plant was then placed in a 50 mL serum vial;
3. Depending upon the amount of plant material, 10-20 mL of mixed solvent (50:50 MeOH and EtOH) was added and the vial was sealed with an aluminum-faced septum, sonicated for 30 minutes, and centrifuged for 10 minutes;
4. The top layer of the mixed solvent was removed, measured, and placed into a new 50 mL serum vial;
5. Steps 3 and 4 were repeated twice more and the final volumes of the mixed solvent extract were recorded;
6. One mL of the mixed solvent extract from each extraction was placed in 15 mL of 30% Scintisafe and analyzed on the LS counter.

Upon completion of the [^{14}C] activity plant extraction, the same plant material used in the previous extraction was further analyzed. This remaining plant material was

used for the acidification and derivitization method to test for chloroacetic acids. The derivitization procedure was as follows:

1. Ten mL deionized water and 0.3 mL of sulfuric acid was added to the plant residue and sonicated for 30 minutes;
2. Small amounts of MTBE (6-10 mL) were added and the mixture was manually shaken for 1 minute, then centrifuged for 10 minutes;
3. The top layer of MTBE was removed and placed in a new vial;
4. Steps 1-3 were repeated;
5. The total MTBE extract was derivitized by the addition of diazomethane;
6. The mixture was placed in the freezer for a minimum of 30 minutes before transferring to 2 mL autosampler vials for GC analysis.

Upon completion of the [^{14}C] experiments, the solid phase of plants was oxidized to determine any unextracted metabolites or activity. The liquid phase was analyzed at selected intervals during the experiment and at the completion of the experiment. The procedures for solid and liquid phase sampling were as follows:

1. Two hundred mg of plant tissue was crushed and placed in a combusto cone or 0.5 mL of liquid was injected into a combusto cone;
2. The proper combustion time was set, 0.75 minutes for solids and 1.5 minutes for liquids;
3. The correct mixture of cocktail was determined to be 8 mL of Carbosorb and 12 mL of Permafluor;
4. Two 20 mL scintillation vials were placed in the vial carriage to collect the cocktail and the sample was placed in the heating coil;
5. The sample combusted to CO_2 that was mixed with the cocktail and injected into the vials. The cocktail vials was sealed and placed in the LS counter for analysis.

An acid-base test was conducted on the radiolabled liquid phase to differentiate between any hydrophilic, non-volatiles and CO_2. This procedure was as follows:

1. Three 20 mL vials were prepared; one contained 15 mL of ScintiSafe 30% scintillation fluid, one contained 0.3 mL of 1 N H_2SO_4, and one contained 0.3 mL of 1 N NaOH;
2. Three mL of aqueous solution was extracted from the chamber;
3. One mL was injected into the 15 mL of ScintiSafe 30% scintillation fluid vial and sealed;
4. One mL was injected into the 0.3 ml of 1 N H_2SO_4 vial;
5. The last mL was mixed with the 0.3 mL of 1 N NaOH by extracting the NaOH into the syringe where the aqueous phase and NaOH were mixed to trap any CO_2 that may have been formed during phytotransformation. The mixture was then injected into the vial;
6. The acid and base mixtures were then purged using argon gas for 10 minutes;
7. Once purged, 15 mL of ScintiSafe 30% scintillation fluid was added to the acid and base treated samples and the vials were sealed;
8. The samples were then counted on the LS counter.

The acid treated samples are used to quantify any hydrophilic compounds that might be present and the base treated samples are used to quantify any CO_2 that may have formed.

3.5 Kinetic data analysis

A zero order rate constant, k_s, was used to describe an initial, rapid reaction step attributed to sorption in four willow experiments which was then followed by slower degradation kinetics described by a psuedo-first order rate constant, k_{obs}. The psuedo-first order rate constant was used in all other experiments from initiation to completion to describe the degradation kinetics when there was not an initial sorption phase observed.

In order to differentiate between the sorption phase and the degradation phase, each experiment's kinetics were plotted graphically and the sorption and degradation phases were separated according to the slope of the curve (Appendix A).

All solute concentrations were measured as a function of time. Concentration measurements were initially made at time (t) = 0 (C_o) and at selected time intervals t (C_t), thereafter. In order to obtain first-order plots, ln (C_t/C_o) was plotted as a function of the reaction time. A psuedo-first order rate law was used to estimate the transformation rate constants and the half-lives of the parent compounds in the bioreactor experiments:

$$d[C]/dt = -k[C]$$

where:

k = psuedo-first order rate constant,

C = concentration of target compound, mg/L.

Integration of the psuedo-first order rate law yields a kinetic model of the observed curve:

$$[C]_t = [C]_o * e^{-kt}$$

where:

C_o = initial concentration at t = 0, mg/L,

C_t = concentration at time t, mg/L,

k = rate constant or slope from the linear regression of log C_t/C_o vs t.

The half-life ($t_{1/2}$) was then calculated using the following equation:

$$t_{1/2} = ln\ 2/k$$

The zero order rate equation used to determine the initial sorption reaction is as follows:

$$d[C]/dt = -k$$

where:

k = zero order rate constant,

C = concentration of target compound, mg/L.

Integration of the zero order rate law yields a kinetic model of the linear curve:

$$[C]_t = [C]_o - kt$$

where:

C_o = initial concentration at t = 0, mg/L,

C_t = concentration at time t, mg/L,

k = rate constant or slope from the linear regression of log C_t/C_o vs t.

CHAPTER IV

RESULTS

These studies focused on the use of *populus deltoides* (eastern poplar), *salix nigra* (black willow), and *lemnaceae* (duckweed) for the removal and/or transformation of the recalcitrant compounds PCE and TCE and any associated metabolites. All experiments were conducted in a controlled, greenhouse environment. Also, water uptake was recorded for comparison to degradation kinetics in order to establish a correlation between the two measurements.

Willow was the most intensively studied plant among those tested. Over twenty experiments were conducted with willows dosed with various concentrations of PCE and TCE. High aqueous phase concentrations (45 mg/L) of PCE were found to be phytotoxic and necrotic to all ages of willows but the plants performed best when dosed with concentrations of 20 mg/L or less PCE. Even though high concentrations of PCE were toxic to the plants, high concentrations of 45 mg/L TCE were easily tolerated. These findings were in agreement with actual field results seen on-site at the Naval Facility in Orlando, FL. Trees planted on-site and exposed to high concentrations of PCE are exhibiting signs of phytotoxicity and necrosis which confirms the viability of these experiments and the resulting data.

Unlike the large volume of phytoremediation research that focuses on TCE and poplar, this research focuses on phytoremediation of PCE using poplar and willows. While previous research has proven that these plants can tolerate higher concentrations of TCE, the results of this study indicated that PCE can be degraded at much lower initial concentrations of approximately 20 mg/L. A decrease in PCE concentration in solution was accompanied by the formation of TCE and trichloroethanol as primary daughter

products. Thus, both poplar and willow are potentially effective for phytoremediation of both PCE and TCE plumes.

Duckweed studies yielded degradation rates for both PCE and TCE experiments that were very similar to unplanted control experiment results. PCE and TCE were metabolized similarly in both planted and unplanted bioreactors. PCE degradation studies in the planted bioreactor and the unplanted control showed a small decrease in the target contaminant over time with the appearance of the daughter products TCE and trans-1,2-DCE in the plant experiments but not in the control. TCE degradation studies in the planted bioreactor and the unplanted control indicated a slightly higher degradation rate of the parent contaminant over time with the appearance of the daughter product Cl_3EtOH in the plant but not in the control. This suggests that duckweed may not be very efficient for remediation of PCE and TCE contaminated water.

Degradation and transformation rates were described by zero order (K_s) and psuedo first order (K_{obs}) kinetic models. During certain experiments, an initial sorption phase was observed and described by the zero order model. The pseudo first order transformation phase lasted up to 14 weeks depending on type of plant, health, maturity, size, root mass of each plant, and contaminant concentration.

Degradation rates were calculated and compared for all plant bioreactors dosed with both PCE and TCE. Comparisons of experiments dosed with both [^{12}C] and [^{14}C] labeled compounds were made. Terrestrial plants with foliage exposed to the atmosphere were dosed with [^{12}C] labeled compounds and had faster degradation rates than the plants whose foliage was encased in the top flask and dosed with [^{14}C] labeled compounds. The exposed plants dosed with [^{12}C] were able to take up more water due to their increased evapotranspiration ability, which in turn increased the plant's ability to take up contaminants.

4.1 Results of PCE Phytoremediation Experiments

The results of experiments conducted with willows showed for the first time that high doses of PCE were toxic to all ages of willows. Willows dosed at 45 mg/L PCE were necrotic within a month while the TCE dosed plants lived for over three months. Willows dosed with 20 mg/L PCE lived but did not show the same rapid degradation kinetics observed for TCE dosed plants. Also, experiments in which the foliage was enclosed in flasks generally showed slower degradation rates than for experiments with exposed foliage. The latter was especially true of the PCE experiments. Enclosing the foliage puts extreme stress on the study plants and shortens their lifespan. The increased stress is also thought to be a significant contributing factor to the lower removal and degradation rates. Selected willow experiments dosed with 20 mg/L PCE are shown in Figure 4.1 demonstrating PCE degradation over time. Degradation rates were noticeably different between [^{14}C] and [^{12}C] PCE experiments. The average half-life for willows dosed with aqueous phase concentrations of 45 mg/L [^{14}C] PCE was 64.5 days while the average half-life for willows dosed with aqueous phase concentrations of 20 mg/L [^{14}C] PCE was 24.8 days. The average half-life for willows dosed with aqueous phase concentrations of 20 mg/L [^{12}C] PCE was 17.5 days (Table 4.1).

Willow experiment number 20, conducted from May 25 to July 5, was dosed with an aqueous phase concentration of 20 mg/L PCE. The concentration of PCE decreased from 15.3 mg/L to 3.3 mg/L over 36 days. TCE and Cl_3EtOH concentrations approached detectable levels in the rhizosphere after 23 days and 28 days, respectively (Table 4.2). The degradation rate constant for PCE in this experiment was 0.041 days^{-1} yielding a half-life of 16.74 days with an R^2 of 0.96. The results of the tissue extraction and derivitization of the roots, leaves, and branches for willow experiment number 20 is shown in Table 4.2. Headspace analysis was performed on willow experiment number 20 and the results are shown in Table 4.3. The presence of reductive dechlorination products in the rhizosphere suggests that anaerobic conditions were created in the tree

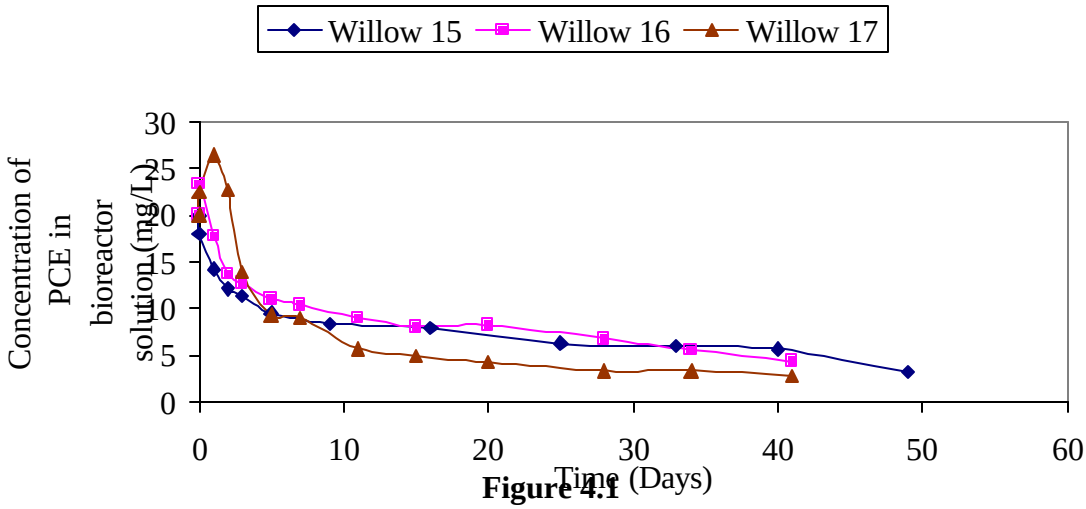


Figure 4.1

Representative experiments showing the rate of removal of PCE from hydroponic bioreactors by six month old rooted cuttings of willow trees. All experiments were dosed initially with 20 mg/L PCE.

TABLE 4.1
Willow Degradation Rate Constants and Half Lives

Plant No.	Initial Conc. (mg/L)	Final Conc. (mg/L)	Parent Chemical	Ks ($days^{-1}$)	R^2	Kobs ($days^{-1}$)	Half Life (Days)	R^2
Willow 8	45	24.7	[^{14}C] PCE	N/A	N/A	0.009	76	0.85
Willow 9	45	18.6	[^{14}C] PCE	N/A	N/A	0.013	53	0.98
Willow 13*	20	7.2	[^{14}C] PCE	3.579	0.79	0.023	30	0.98
Willow 14*	20	7.1	[^{14}C] PCE	2.172	0.76	0.022	31	0.97
Willow 15	20	3.2	PCE	N/A	N/A	0.024	18	0.92
Willow 16	20	4.4	[^{14}C] PCE	N/A	N/A	0.032	22	0.86
Willow 17	20	2.8	[^{14}C] PCE	N/A	N/A	0.045	16	0.80
Willow 20	20	3.3	PCE	N/A	N/A	0.041	17	0.96

* - Initial sorption due to large root mass area

N/A - Initial sorption not applicable due to lower root mass

TABLE 4.2
Distribution of PCE and its Metabolites in the Rhizosphere and Associated Plant Tissues of Willow No. 20 after 36 days

Products	Rhizosphere (mg/L)	Roots (mg/Kg)	Leaves (mg/Kg)	Branches (mg/Kg)
PCE	3.30	0.20	0.00	0.00
TCE	0.16	0.02	0.01	0.01
TCAA	0.00	0.12	0.23	0.26
MCAA	0.00	0.00	0.01	0.01
Cl_3EtOH	5.48	0.01	0.03	0.02

TABLE 4.3
Qualitative Headspace Analyses of the Rhizospheric Zone in Willow

Plant Type	Chemical	Metabolites Identified within Headspace
Willow nigra	PCE	PCE, 1,2-DCA, Trans-1,2-DCE, 1,1-DCE, Ethene, Ethane, Methane

rhizosphere. Unlike previous investigations that have mostly ignored rhizospheric processes, this study shows that chlorinated ethenes are removed from the rhizosphere by rhizoreduction.

Willow experiment number 15, conducted from March 4 to April 22, was dosed with 20 mg/L PCE. PCE concentrations decreased from 18.0 to 3.2 over 49 days. TCE and Cl_3EtOH appeared after 33 days. The degradation rate constant was 0.024 $days^{-1}$ yielding a half-life of 17.59 days with an R^2 of 0.92.

Two [^{12}C] PCE phytoremediation experiments using poplar trees were performed. The plants used in these experiments were dosed with 20 mg/L and were exposed to the atmosphere. These experiments lasted from 7 to 15 days. One tree was initially dosed with [^{12}C], but after 7 days was dosed with [^{14}C] in order to obtain further radiolabeled data. The half-life for the one long term [^{12}C] experiment was 11 days (Table 4.4). Selected poplar experiments are shown in Figure 4.2 demonstrating PCE degradation over time. PCE decreased in solution phase from 20 mg/L to 7.4 mg/L in 15 days. Detectable concentrations of the daughter product TCE were observed after one day, while Cl_3EtOH was observed after six days. Upon extraction of each plant section, these same compounds were observed. After extraction and derivitization, TCAA was the primary acetic acid found and small amounts of MCAA were also detected (Table 4.5).

Twelve duckweed experiments were conducted using enclosed, sealed chambers to reduce the chance of volatilization. Headspace analyses were conducted on selected experiments. The duckweed were dosed with 20 mg/L [^{12}C] or [^{14}C] PCE and were maintained for 56 days. The average pseudo-first order rate constant of [^{12}C] PCE degradation was 0.004 $days^{-1}$ and for [^{14}C] PCE, the degradation rate was 0.002 $days^{-1}$. [^{12}C] PCE half-lives ranged from 136 days to 217 days in these experiments and [^{14}C] PCE half-lives ranged from 231 days to 1155 days (Tables 4.6).

Duckweed experiment number 10, conducted from December 13, 1999 to February 7, 2000 was dosed with 20 mg/L [^{12}C] PCE. PCE aqueous phase concentrations

TABLE 4.4
Poplar Degradation Rate Constants and Half Lives

Plant No.	Initial Conc. (mg/L)	Final Conc. (mg/L)	Parent Chemical	Kobs ($days^{-1}$)	Half Life (Days)	R^2
Poplar 1	20	13.6	[^{14}C] PCE	0.045	15	0.83
Poplar 2	20	9.2	[^{14}C] PCE	0.083	8	0.81
Poplar 3	20	9.5	[^{14}C] PCE	0.037	19	0.83
Poplar 4	20	9.2	[^{14}C] PCE	0.065	11	0.87
Poplar 5	20	6.9	PCE	0.066	11	0.87

TABLE 4.5
Distribution of PCE and its Metabolites in the Rhizosphere and Associated Plant Tissues of Poplar No. 5 after 15 days

Products	Rhizosphere (mg/L)	Roots (mg/Kg)	Leaves (mg/Kg)	Branches (mg/Kg)
PCE	6.94	3.21	0.00	0.09
TCE	0.03	0.00	0.00	0.00
TCAA	0.00	0.44	0.55	0.48
MCAA	0.00	0.32	0.00	0.02
Cl_3EtOH	0.74	0.01	0.02	0.00

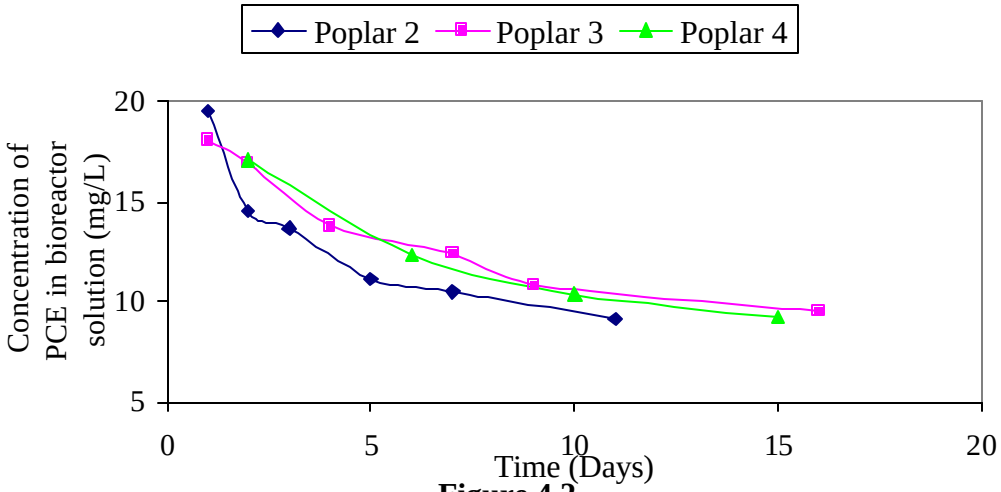


Figure 4.2

Representative experiments showing the rate of removal of PCE from hydroponic reactors by six month old rooted cuttings of poplar trees.

TABLE 4.6
Duckweed Degradation Rate Constants and Half Lives

Plant No.	Initial Conc. (mg/L)	Final Conc. (mg/L)	Parent Chemical
Duckweed 10	20	12.9	PCE
Duckweed 11	20	13.3	PCE
Duckweed 12	20	13.1	[^{14}C] PCE
Duckweed 13	20	7.9	[^{14}C] PCE
Duckweed Control [^{12}C]	20	14.7	PCE
Duckweed Control [^{14}C]	20	11.6	[^{14}C] PCE

TABLE 4.7
Distribution of PCE and its Metabolites in the Rhizosphere and Associated Plant Tissues of Duckweed No. 10 after 56 days

Products	Rhizosphere (mg/L)	Plant Tissue (mg/Kg)
PCE	13.29	0.02
TCE	0.18	0.00
Trans-1,2-DCE	9.82	0.00
DCAA	0.00	7.20
MCAA	0.00	0.07
Cl_3EtOH	0.00	0.02

decreased from 18.2 mg/L to 13.3 mg/L over 56 days. TCE and trans-1,2-DCE appeared after 43 days. PCE, TCE, and Cl_3EtOH were also detected in the plant tissue. After derivitization, DCAA was the primary acetic acid detected and small amounts of MCAA were also found (Table 4.7).

A plantless control experiment containing only DI water was conducted concurrently from December 13 to February 7 with duckweed experiment numbers10 and 11 dosed with 20 mg/L [^{12}C] PCE. PCE aqueous phase concentrations decreased from 18.3 mg/L to 14.7 mg/L over 56 days. TCE and trans-1,2-DCE appeared after 43 days. A comparison between the control experiment and the dosed plant experiment is shown in Figure 4.3. The similarity indicates that there was little degradation that is attributable to the plant processes.

4.2 Results of TCE Phytoremediation Experiments

Rooted willow cuttings dosed with high concentrations of [^{12}C] TCE (45 mg/L) survived while those dosed with PCE at the same concentration did not. Certain TCE dosed plants lived in excess of three months while no PCE dosed plants lived past one month. Also, plants dosed with 20 mg/L TCE exhibited much more rapid degradation kinetics than the PCE dosed plants. As with the PCE experiments, enclosing the foliage in an air-flow through chamber slowed the phytokinetic reactions and also placed much more stress on the plant resulting in a shorter life span and less water uptake.

Degradation rates were not significantly different between [^{14}C] and [^{12}C] TCE experiments. The half-life range for the [^{14}C] TCE experiments was 25.29 days to 34.14 days while the half-life for the [^{12}C] TCE experiments ranged from 19.04 days to 63 days (Tables 4.1 and 4.8).

Three [^{12}C] TCE willow experiments were conducted. Plants were dosed with 45 mg/L TCE and were exposed to the atmosphere. Experiment durations ranged from 44 days to 103 days with half-lives ranging from 19.04 to 63 days (Table 4.8). Selected

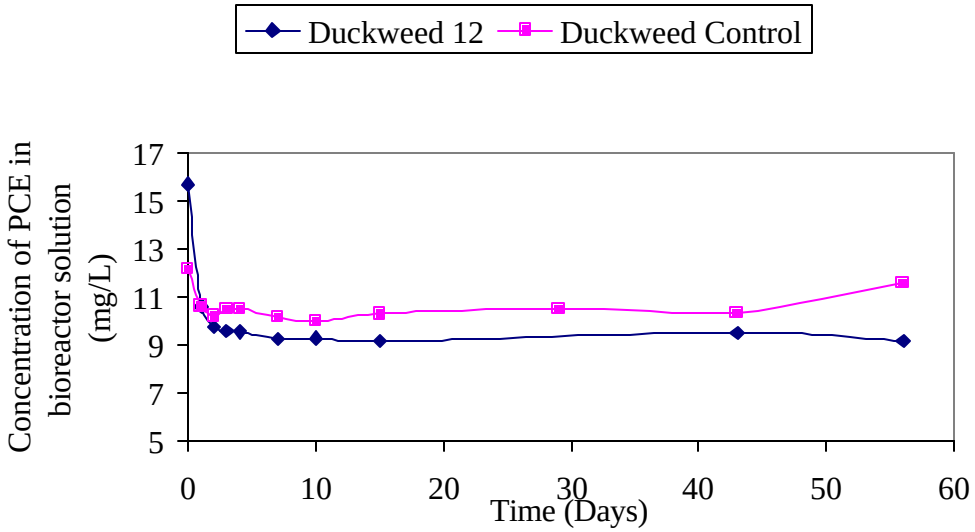


Figure 4.3
Representative experiments showing the rate of removal of PCE from hydroponic reactors by duckweed plants.

TABLE 4.8
Willow Degradation Rate Constants and Half Lives

Plant No.	Parent Chemical	Final Conc. (mg/L)	Initial Conc. (mg/L)	Ks ($days^{-1}$)	R^2	Kobs ($days^{-1}$)	Half Life (Days)	R^2
Willow 7	[^{14}C] TCE	11.7	45	N/A	N/A	0.020	34	0.98
Willow 10	TCE	0.8	45	N/A	N/A	0.034	19	0.97
Willow 11*	TCE	14.3	45	0.177	1.00	0.013	52	0.90
Willow 12*	TCE	14.1	45	0.183	0.98	0.011	63	0.96
Willow 18	[^{14}C] TCE	9.3	20	N/A	N/A	0.027	25	0.84
Willow 19	[^{14}C] TCE	9.6	20	N/A	N/A	0.026	26	0.92

* - Initial sorption due to large root mass area

N/A - Initial sorption not applicable due to lower root mass

TABLE 4.9
Distribution of TCE and its Metabolites in the Rhizosphere and Associated Plant Tissues of Willow No. 10 after 103 days

Products	Rhizosphere (mg/L)	Roots (mg/Kg)	Leaves (mg/Kg)	Branches (mg/Kg)
TCE	0.84	0.03	0.16	0.05
TCAA	0.00	0.53	0.38	0.31
MCAA	0.00	0.02	0.04	0.01
Cl_3EtOH	0.02	0.02	0.06	0.01

TABLE 4.10
Qualitative Headspace Analyses of the Rhizospheric Zone in Willow

Plant Type	Chemical	Metabolites Identified within Headspace
Willow nigra	TCE	TCE, Trans-1,2-DCE, Ethene, Ethane, Methane

willow experiments are shown in Figure 4.4 demonstrating TCE degradation over time. Willow experiment number 10, conducted from January 10 to April 22, was dosed with 45 mg/L TCE.

The TCE concentration decreased from 35.8 mg/L to 0.8 mg/L over 103 days. Cis-1,2-dichloroethene (DCE) and trans-1,2-DCE appeared after 8 days and 15days, respectively. Cl_3EtOH appeared after 59 days. The pseudo-first order degradation rate constant was 0.034 $days^{-1}$ yielding a half-life of 19.04 days with an R^2 of 0.97 (Table 4.8).

Willow experiment numbers 11 and 12, conducted from January 10 to February 23, were dosed with 45 mg/L TCE. TCE concentration in the rhizosphere of willow 11 decreased from 40.7 mg/L to 14.3 mg/L over 44 days. Cis-1,2-DCE appeared after 8 days and trans-1,2-DCE appeared after 15 days. The degradation rate constant was 0.013 yielding a half-life of 51.72 days with an R^2 of 0.92. TCE concentration in the rhizosphere of willow 12 decreased from 47 mg/L to 14 mg/L over 44 days. Cis-1,2-DCE appeared after 8 days and Trans-1,2-DCE appeared after 15 days. The degradation rate constant was 0.011 yielding a half-life of 63 days with an R^2 of 0.96 (Table 4.8).

Headspace analyses performed on willow experiment number 10 showed the presence of TCE, trans-1,2-DCE, ethene, ethane, and methane as phytovolatilization products in the rhizosphere (Table 4.10). The results of the tissue extraction and derivitization of the roots, leaves, and branches for experiment number 10 are shown in Table 4.9. The presence of reductive dechlorination products in the rhizosphere suggests that anaerobic conditions were created in the tree rhizosphere. Unlike previous investigations that have mostly ignored rhizospheric processes, this study shows that chlorinated ethenes are removed from the rhizosphere by rhizoreduction.

Twelve experiments were conducted to investigate the removal of TCE from aqueous solution by duckweed. The experiments were conducted in sealed glass

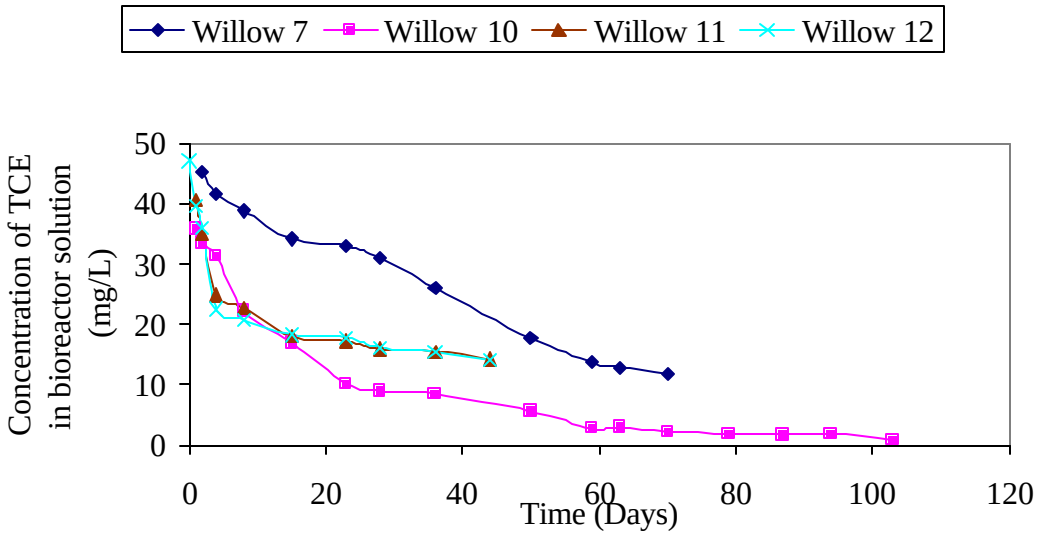


Figure 4.4

Representative experiments showing the rate of removal of TCE from hydroponic bioreactors by six month old rooted cuttings of willow trees.

chambers to reduce the chance of volatilization. Headspace analyses were conducted on selected experiments. TCE dosed plants were dosed with 10 mg/L [^{12}C] or [^{14}C] TCE and were maintained for 77 days (Table 4.11). The removal of TCE from solution by the duckweed was very small and not described by any of the kinetics models.

Duckweed experiment number 7, conducted from September 19 to November 22, was dosed with 10 mg/L [^{12}C] TCE. TCE aqueous phase concentrations decreased from 10 mg/L to 4.7 mg/L over 64 days. Cl_3EtOH was observed in solution after 8 days. Upon tissue analysis, TCE and Cl_3EtOH were detected in the plant tissue. After derivitization, DCAA was the primary chloro-acetic acid detected and small amounts of MCAA were also found (Table 4.12).

A plantless control experiment containing only DI water was conducted in parallel from September 19 to November 22 with duckweed 6 and 7, and was dosed with 10 mg/L [^{12}C] TCE. TCE aqueous phase concentrations decreased from 10 mg/L to 5.5 mg/L over 64 days. No daughter products were observed. Losses were attributed to chemical partitioning to the headspace air phase in the bioreactor. A comparison between the control experiment and the dosed plant experiment is shown in Figure 4.5. Although the decrease in solution concentration in plant and control experiments are the same, transformation products were identified in the plant bioreactor and not in the control.

4.3 Mass Balance

PCE and TCE mass balances were determined for all three plants use in this study: willow, poplar, and duckweed. Radiolabeled [^{14}C] PCE and TCE were utilized to increase the accuracy of the mass balance calculations. Mass balances were not calculated for the [^{12}C] experiments, as they would not be as precise and accurate as the [^{14}C] determinations. However, [^{14}C] recoveries from each plant region were compared with [^{12}C] recoveries in the same region of a similar plant.

TABLE 4.11
Duckweed Degradation Rate Constants and Half Lives

Plant No.	Parent Chemical	Initial Conc. (mg/L)	Final Conc. (mg/L)
Duckweed 6	TCE	10	4.7
Duckweed 7	TCE	10	4.7
Duckweed 8	[^{14}C] TCE	10	5.4
Duckweed 9	[^{14}C] TCE	10	5.2
Duckweed Control [^{12}C]	TCE	10	5.5
Duckweed Control [^{14}C]	[^{14}C] TCE	10	6.3

TABLE 4.12
Distribution of TCE and its Metabolites in the Rhizosphere and Associated Plant Tissues of Duckweed No. 7 after 64 days

Products	Rhizosphere (mg/L)	Plant Tissue (mg/Kg)
TCE	4.73	0.00
DCAA	0.00	2.12
MCAA	0.00	0.05
Cl_3EtOH	0.00	0.00

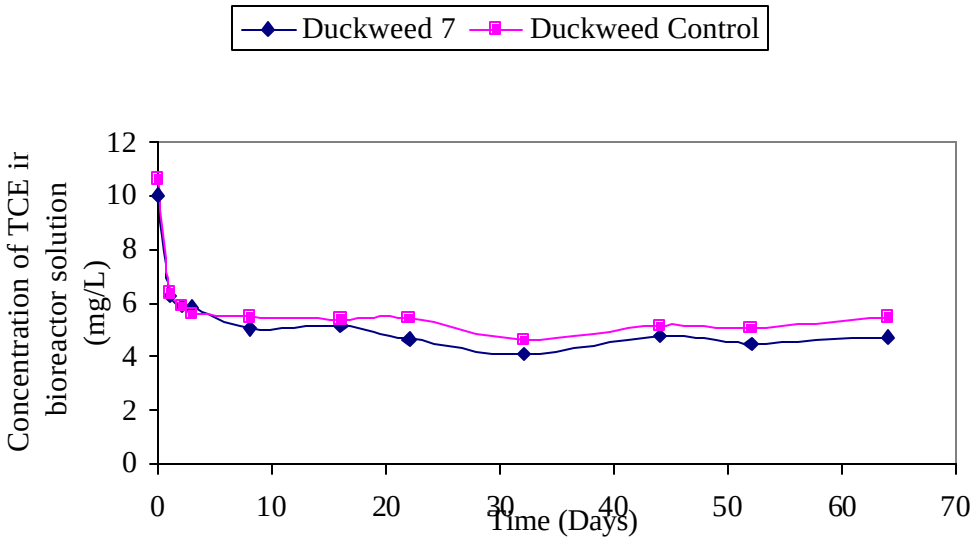


Figure 4.5
Sample experiments showing the rate of removal of TCE from hydroponic reactors in selected duckweed. Transformation products were observed in the plant but not in the control.

Radiolabeled [^{14}C] PCE was utilized in six willow experiments. Radiolabeled [^{14}C] TCE was utilized in three willow experiments. The willows were dosed with aqueous solutions of either 20 mg/L or 45 mg/L PCE or TCE. All [^{14}C] PCE or TCE dosed plants were enclosed with an air-flow through glass enclosure to trap any volatiles. The experiment durations for the radiolabled PCE plants ranged from 41 days to 49 days. Durations for the radiolabeled TCE dosed plant experiments ranged from 27 to 70 days.

[^{14}C] PCE recoveries for willows averaged 64.55% +/- 15.7 SE. Plants yielding the highest recoveries were dosed with 45 mg/L of [^{12}C] and [^{14}C] to achieve a specific activity of 3543 cpm/mL. Plants having the lowest recoveries were dosed with 20 mg/L [^{12}C] and [^{14}C] TCE to obtain a specific activity of 6012.4 cpm/mL. The percent CO_2 in solution ranged from 2.85% to 8.81% for these experiments indicating mineralization of the compound occurred (Table 4.13). The mineralization of chlorinated ethenes was documented for the first time in these experiments. The mineralization of PCE and TCE in the root zone of these trees confirms that rhizodegradation is an important fate process. Also, the higher recoveries observed in the 45 mg/L dosed plants is attributed to a significant portion of PCE in solution being excluding from uptake due to its toxicity and lower TSCF which is discussed in a later chapter.

The mass recoveries for three willow trees dosed with [^{14}C] TCE averaged 65.69 % +/- 11.99 SE. The percent CO_2 varied from 0.55% to 3.18% and is an indication of TCE mineralization in the root zone of willow trees. The highest recoveries were realized in experiments in which the willow trees were dosed with 20 mg/L with a specific activity of 6309.7 cpm/mL and the lowest recovery was obtained from trees dosed with 45 mg/L with a specific activity of 6500.9 cpm/mL (Table 4.13).

The disparity in the mass recoveries may be attributed to numerous factors: (1) the heterogeneous distribution of metabolites in plant compartments make it impossible

TABLE 4.13

Willow Mass Balances

Plant Fraction or Reactor Compartment	**% Activity Recovered**						
	[^{14}C] PCE				**[^{14}C] TCE**		
	Willow 8 45 ppm	**Willow 9** 45 ppm	**Willow 16** 20 ppm	**Willow 17** 20 ppm	**Willow 7** 45 ppm	**Willow 18** 20 ppm	**Willow 19** 20 ppm
Leaves	0.05	0.12	0.15	0.22	0.61	0.06	0.14
Branches	0.01	0.21	0.13	0.47	0.09	0.05	0.12
Upper Bark	0.06	0.08	0.26	0.06	0.39	0.26	1.06
Lower Bark	0.18	0.31	0.81	0.40	2.35	0.56	0.23
Stem Pith	3.77	1.70	1.50	1.12	2.22	1.70	1.40
Roots	0.43	0.69	1.88	5.62	3.05	6.88	6.23
Act. Carbon 1	12.80	59.96	9.24	20.26	8.99	6.97	18.76
Act. Carbon 2	NA	NA	0.32	0.50	NA	3.11	9.68
A.C. Safety Trap	0.00	0.11	0.21	0.06	0.00	0.04	0.01
Caps/Sealant	0.68	0.50	0.30	0.36	0.35	0.28	0.26
Solution	54.90	41.21	21.94	13.94	26.07	46.72	48.09
EGME Trap	0.35	0.28	NA	NA	0.23	NA	NA
NaOH Trap	0.01	0.05	NA	NA	0.12	NA	NA
% CO_2 in Solution	3.66	3.24	2.85	8.81	3.18	0.55	1.01

N/A - Not available

Average % recovery in PCE dosed willows = 64.6 % +/- 15.7

Average % recovery in TCE dosed willows = 65.6 % +/- 12

to obtain a true representative sample for biological oxidation to directly quantify the bound fraction; (2) VOC partitioning to the plant cuticle or tissue caused losses due to volatilization during plant preparation; and (3) the residence time of VOCs in the carbon traps may have been too short and not absorbed for the complete removal of the gas phase solutes.

Four experiments were conducted with poplar trees dosed with [^{14}C] labeled PCE. All plants were dosed with 20 mg/L PCE and were enclosed with an air-flow through glass chamber connected to the traps to trap any volatiles evapotranspired through the leaves. Poplar experiments lasted for 11 days to 16 days. One poplar was initially dosed with [^{12}C], but after 7 days was dosed with [^{14}C] in order to obtain further radiolabeled data. Unlike the willow trees, degradation rates for [^{14}C] and [^{12}C] dosed poplars was not significantly different. The half-life for the [^{14}C] PCE experiments ranged from 8.32 days to 18.53 days (Table 4.4). The mass recoveries for the poplar experiments ranged from 57.69% to 80.48%. No CO_2 data was determined for the poplar experiments (Table 4.14). Since data is available only for PCE dosed poplars, comparisons between PCE and TCE dosed poplars cannot be made.

Mass recoveries for the [^{14}C] PCE in duckweed experiments ranged from 46.01% to 60.44%. Mass recoveries for the [^{14}C] TCE in duckweed experiments ranged from 63.63% to 66.53% (Table 4.15). Qualitative analyses of the headspace were performed on certain experiments. Low mass balances are attributed to headspace losses and volatilization losses of VOCs that partitioned to the plant cuticle or tissue during preparation for biological oxidation. The bound fraction of the VOCs should partition to the gas phase during sample processing resulting in poor mass recoveries.

4.4 Transpiration Stream Concentration Factor

The amount of water taken up by each plant for their normal functions was recorded and compared to the degradation kinetics over time. Age, size, sunlight,

TABLE 4.14

Poplar Mass Balances

Plant Fraction or Reactor Compartment	**% Activity Recovered [^{14}C] PCE**			
	Poplar 1	**Poplar 2**	**Poplar 3**	**Poplar 4**
Leaves	0.03	0.04	0.02	0.18
Branches	0.05	0.02	0.07	0.05
Upper Bark	0.21	0.34	1.32	2.03
Lower Bark	6.42	11.46	0.17	1.06
Stem Pith	1.38	1.17	1.49	1.51
Roots	1.09	1.01	0.68	1.00
Activated Carbon 1	2.95	7.48	5.94	19.37
Activated Carbon 2	0.02	0.02	0.14	0.32
A.C. Safety Trap	0.01	0.01	0.01	0.03
Caps and Sealant	0.31	0.32	0.30	0.33
Solution	67.99	45.82	47.55	46.19

Average % recovery in PCE dosed poplar = 69.5 +/- 4.7

TABLE 4.15
Duckweed Mass Balances

Plant Fraction or Reactor Compartment	% Activity Recovered					
	[^{14}C] PCE, 20 mg/L			[^{14}C] TCE, 10 mg/L		
	DW 12	DW 13	Control	DW 8	DW 9	Control
Plant Tissue	5.24	4.92	NA	12.16	12.06	NA
Solution	45.93	39.42	57.98	53.71	51.52	62.86
Headspace	1.95	1.67	2.46	0.66	0.64	0.77
% CO_2 in Solution	NA	NA	NA	0.22	0.41	2.06

NA - Not available

Average % recovery in PCE dosed duckweed = 53.2 +/- 4.2
Average % recovery in TCE dosed duckweed = 64.8 +/- 0.9

temperature, and root mass were the main factors controlling water uptake. Of these, sunlight and root mass were identified as the two most important factors. On overcast days, the plants did not take up as much water as when it was sunny. The large root mass of terrestrial plants enabled these plants to take up more water [Figures 4.6 and 4.7].

The use of the upper Erlenmeyer flask to encase the foliage also made a significant difference in the amount of water taken up by the plants. Once the flask was in place, the plants discontinued the uptake of large quantities of water. In natural environments, plant foliage is exposed to the atmosphere where conditions are conducive for growth and there is lower humidity. When the plant foliage is placed inside a flask with only one direction of air-flow, the humidity rises significantly. Under these conditions, the stomata of plants do not open. These stomata regulate transpiration and gas exchange. The plants cannot transpire water to their immediate environment because the air is already saturated with moisture due to the high humidity. Light intensity, temperature, humidity, and the CO_2 concentration regulate plant transpiration. Tests showed that a higher airflow rate contributed to lowering condensation amounts that in turn, lowered the humidity level within the enclosure.

The specific mechanisms and factors affecting the translocation of chemicals from roots to shoots are poorly understood. Organic contaminant uptake by plants is believed to be a passive process partly related to the lipophilicity of the contaminant as described by the octanol/water partition coefficient (K_{ow}).

The transpiration stream concentration factor (TSCF) has been widely used to model organic contaminant uptake and translocation by different plant species. Because xylem sap concentrations are difficult to measure directly for intact plants, TSCFs are often determined from measured shoot concentrations, where the shoot tissue concentration is normalized to the amount of water transpired during exposure to the chemical (Burken, 1996). The majority of TSCF data available has been obtained from

laboratory environments due to the difficulty of controlling natural field conditions. The TSCF is defined as the concentration of the analyte in the transpiration stream divided by the concentration in the bulk solution from which uptake is occurring:

TSCF = Xylem sap concentration (mg/L) / Root-zone solution concentration (mg/L)

In our studies, the TSCF was calculated by obtaining the concentrations in the leaf tissue and the concentrations in the activated carbon that had been volatilized and adding them together. The summation of the tissue and carbon concentrations was then substituted for the xylem sap concentration in the earlier formula and the new formula is:

TSCF = (Leaf tissue concentration + Activated carbon concentration (mg/L)) / Root-zone solution concentration (mg/L)

Compounds may be actively or passively taken up by plants or may be excluded. Active uptake (TSCF > 1.0) generally occurs with nutrient cations (N, P, and K) and requires the expenditure of metabolic energy. With the possible exception of some hormone-like chemicals (2,4-D), there is no evidence of active uptake of anthropogenic chemicals. Passive uptake (TSCF = 1.0) occurs when a chemical is taken up directly with water because of the water potential gradient resulting from evapotranspiration. A chemical is thought to be excluded (TSCF < 1.0) when uptake is not directly proportional to water uptake, although the mechanism of uptake is still thought to be a passive process (Trapp, 1995).

TSCF values were calculated for four poplar experiments and two willow experiments dosed with [^{14}C] PCE. Also, TSCF values were calculated for three willow experiments dosed with [^{14}C] TCE. Uptake values were measured by [^{14}C] activity found to be translocated in plant leaf tissues and the [^{14}C] captured in the volatilized air stream.

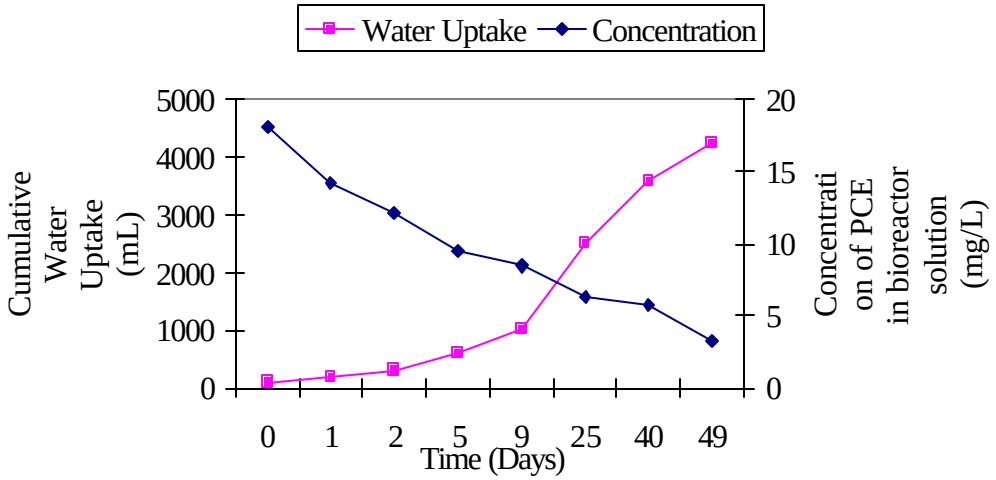


Figure 4.6

Cumulative water uptake by willow tree dosed with 20 mg/L of PCE. Upon reaching a concentration of approximately 8 mg/L, water uptake by the plant began to increase.

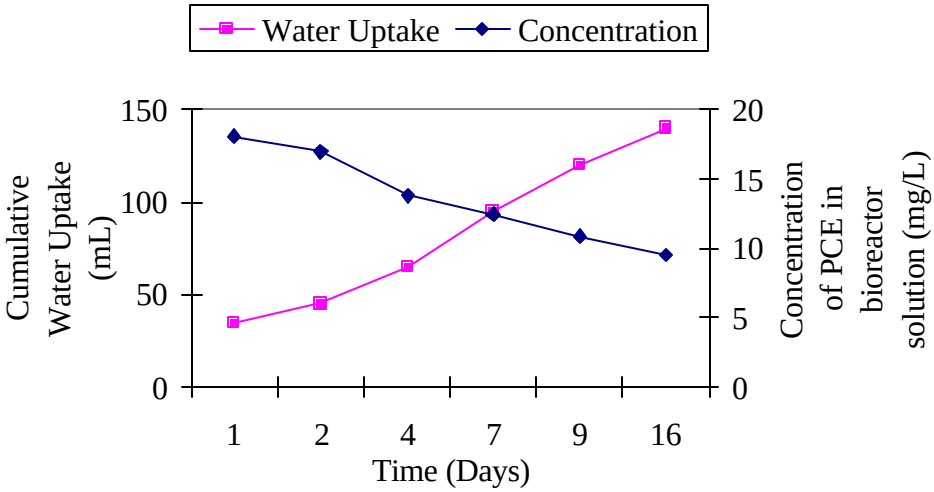


Figure 4.8

Cumulative water uptake by poplar tree dosed with 20 mg/L PCE.

Average TSCF values for the [^{14}C] PCE dosed poplar and willow were 0.66 and 0.75, respectively. The average TSCF value for the [^{14}C] TCE willow was 0.96. These studies indicate that organic chemical uptake is an exclusionary passive process. An example of the calculations to determine the TSCF is shown in Appendix B. When dosed with PCE, both poplar and willow exhibited TSCFs significantly less than one. When willow was dosed with TCE, the TSCF was also less than one, but the margin between exclusion and passive uptake was much smaller indicating the TCE uptake by plants may be more proportional with water uptake. Based on the TSCFs obtained for PCE and TCE in these experiments, the results indicate that PCE tends to be excluded by poplar and willow trees to a greater extent than TCE. This finding is in agreement with the levels of toxicity observed; PCE is more toxic to the plants than TCE.

4.5 Sorption

A rapid initial decrease in the concentration of the parent compound was observed in four willow experiments immediately after dosing. This rapid, initial decrease was attributed to sorption and sequestration as reported in previous research by Nzengung et al. (1997, 2001). If this initial decrease of the parent compound were due to transformation, a corresponding increase in the daughter metabolites would be detected in the plant and/or the surrounding media, which was not observed.

The four willow trees that exhibited the initial, rapid decrease in concentration possessed larger root masses which may explain the significantly higher sorption than was observed for willows that did not possess large root masses. Of these four experiments, two willows were dosed with aqueous phase concentrations of 20 mg/L PCE and two were dosed with aqueous phase concentrations of 45 mg/L TCE. PCE is more readily sorbed to organic matter due to lower aqueous solubility than TCE. These differences may explain the short sorption half lives of 0.32 and 0.19 days for PCE versus

the longer sorption half-lives of 3.92 and 3.78 days for TCE. Comparisons between experiments exhibiting the initial sorption phase versus the non-sorption phase are shown in Figures 4.8 and 4.9. An example of the data calculations is shown in Appendix A.

Poplar trees dosed with PCE and TCE did not show the rapid decrease due to initial sorption, as did four willow trees. Only PCE dosed poplar tree experiments were conducted, so there could be no comparisons between the sorption rates of PCE and TCE. However, since PCE is expected to sorb more strongly than TCE, one may conclude that sorption was not an important fate process in TCE experiments. It should be noted that the root mass for poplars was much less than for willow trees of the same age. The relatively insignificant contribution of sorption in experiments conducted with poplar trees is thus attributed to their small fraction of root mass.

Duckweed experiments did not show the initial rapid sorption phase as seen in certain willow experiments. A consistently slow removal of PCE and TCE by duckweed was observed. Given the large surface area of duckweed, sorption was expected to contribute more significantly to the removal of PCE and TCE from the hydroponic reactors, but this was not observed.

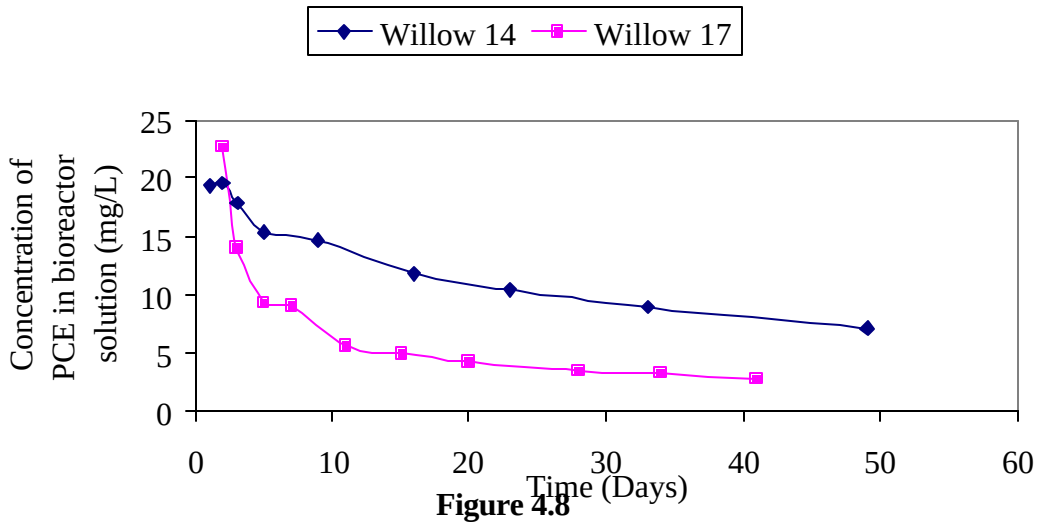


Figure 4.8

Relative rate of removal of PCE initially through sorption by a willow tree with a larger root mass (Willow 17) compared to a willow tree with a smaller root mass (Willow 14).

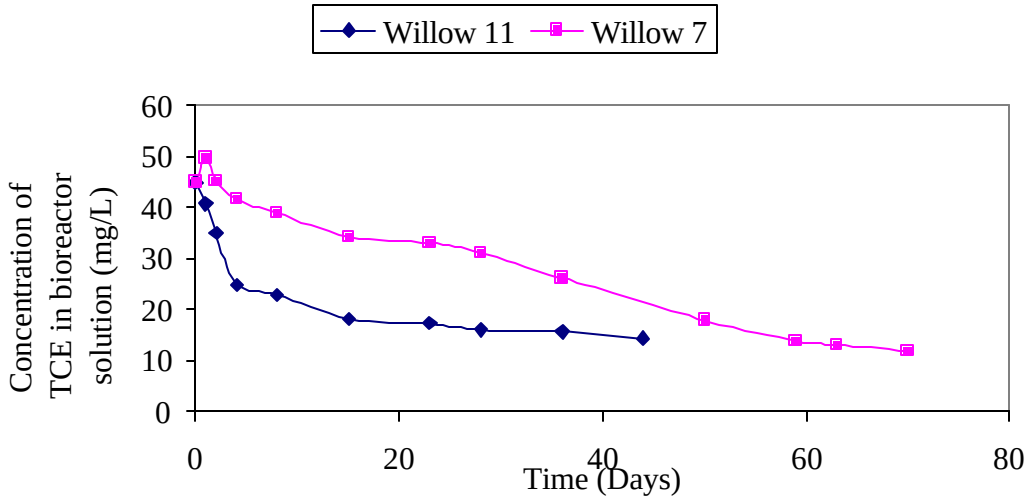


Figure 4.9

Relative rate of removal of TCE initially through sorption by a willow tree with a larger root mass (Willow 11) compared to a willow tree with a smaller root mass (Willow 7).

CHAPTER V

CONCLUSIONS

Willow and poplar trees were used to remove recalcitrant chlorinated organics from groundwater. A fraction of the parent PCE and TCE were degraded over time in controlled, greenhouse experiments. The results of these experiments indicate that dissolved PCE and TCE will partition from the aqueous phase to the rhizospheric root zone where it is either sorbed or taken up by the plant. Also, in all willow experiments, PCE was shown to be phytotoxic resulting in necrosis at aqueous phase concentrations approaching 45 mg/L. Lower concentrations of 20 mg/L PCE were tolerated by both willow and poplar trees. In an update to these findings, poplar trees planted on-site at the Naval Facility in Orlando, FL exhibited the same phytotoxic, necrotic behavior to high concentrations of PCE and its less chlorinated products as the greenhouse experiments conducted in these studies.

Both willow and poplar trees were able to tolerate and degrade aqueous phase concentrations of 20 mg/L PCE and 45 mg/L TCE. Willow and poplar proved capable of reducing aqueous phase PCE concentrations of 20 mg/L by 86 % over 41 days and 65.5 % over 11 days, respectively. Willow also reduced aqueous phase TCE concentrations of 45 mg/L by 97.7 % over 103 days.

Mineralization of PCE and TCE by willow trees indicates the importance of rhizodegradation during phytoremediation of chlorinated ethenes. For the first time, chlorinated ethenes were documented as being mineralized to CO_2 in the rhizosphere solution in these studies. The average percent CO_2 in willow planted bioreactors dosed at concentrations of 45 mg/L and 20 mg/L PCE was 3.45 and 5.83, respectively. The average percent CO_2 in willow planted bioreactors dosed at concentrations of 45 mg/L

and 20 mg/L was 3.18 and 0.78, respectively. This is a very important finding in that prior studies did not indicate the degradation of these contaminants in the rhizosphere.

Uptake, assimilation, and transformation were found to be the most important processes in the degradation of these compounds. Reductive dechlorination and phytooxidation of these compounds were the main phyto-transformation processes identified. Quantifiable amounts of the parent compounds and their metabolites were reversibly and irreversibly bound to the plant tissues. A direct relationship between water uptake and degradation rates was established as the plants increased their uptake of water, the removal rate of the parent compound increased.

A phytoreductive pathway was confirmed by the detection of TCE in samples dosed with PCE, and a phytooxidative pathway was confirmed by the detection of chloroacetic acids in terrestrial experiments. The occurrence of multiple degradation pathways minimizes the production of more hazardous metabolites of PCE and TCE such as vinyl chloride and DCE that may occur if reductive dehalogenation were the only degradation mechanism.

TSCF ranges for PCE and TCE were calculated and were found to be in agreement with results from these studies regarding plant toxicity and actual solution uptake. When dosed with PCE, both poplar and willow exhibited TSCFs significantly less than one. When willow was dosed with TCE, the TSCF was almost one, indicating the TCE uptake by plants is more proportional with water uptake. Based on the TSCFs obtained for PCE and TCE in these experiments, the results indicate that PCE tends to be excluded by poplar and willow trees to a greater extent than TCE. This finding is in agreement with the levels of toxicity observed; PCE is more toxic to the plants than TCE.

Duckweed was observed to be ineffective in the removal of either PCE or TCE from aqueous solution. Even though small amounts of metabolites were seen in certain experiments, the overall plant removal of PCE and TCE was not statistically different from the plantless control experiments. This indicates that duckweed may not be

effective for the remediation of these particular compounds. Even though duckweed proved ineffective for removing PCE or TCE, it has proven its applicability to remove inorganics and further testing to degrade other contaminants of concern is warranted.

Phytoremediation of TCE and PCE by willow and poplar trees is limited by high contaminant concentrations, especially PCE, that can be phytotoxic causing chlorosis and necrosis in these plants. Source areas containing high concentrations of these contaminants would have to be treated with other technologies and phytoremediation used as a polishing step for the lower contaminant concentrations. Phytoremediation requires more effort than simply planting vegetation and assuming that contaminants will disappear. It requires an understanding of the different phytomechanisms, the plants selected, and the maintenance requirements to ensure plant growth. Given the great number of plant candidates, a relatively limited number of plants have been investigated. Extrapolation of results from hydroponic or greenhouse studies to actual field situations will require caution and further field studies will be necessary. Applicability of phytoremediation will be site-specific and is not suited for every situation but phytoremediation has the potential to provide a lower-cost, environmentally friendly alternative to conventional remedial technologies in many situations.

REFERENCES

Boyajian, George E., and Deborah L. Deredjian, Phytoremediation: It grows on you, Soil and Groundwater Research, 22, February/March 1997.

Brady, Nyle C., The Nature and Properties of Soils, 264-267, 1990.

Burken, J. G., Uptake and Fate of Organic Contaminants by Hybrid Poplar Trees, University of Iowa, Iowa City, PHD Thesis, 1-18, 1996.

Burken, Joel G., Jerald L. Schnoor, Predictive Relationships for Uptake of Organic Contaminants by Hybrid Poplar Trees, Environmental Science and Technology, Vol. 32, No. 21:3379-3384, 1998.

Burken, Joel G., Jerald L. Schnoor, Uptake and Metabolism of Atrazine by Poplar Trees, Environmental Science and Technology, Vol. 31, No. 5:1399, 1997.

Chappell, Jonathon, Phytoremediation of TCE using Populus, Status Report prepared for the USEPA Technology Innovation Office under a National Network of Environmental Management Studies Fellowship, 2-14, August 1997.

Conger, Robert Mark, Ralph J. Portier, Phytoremediation Experimentation with the Herbicide Bentazon, Remediation, Spring:19-36, 1997.

Cunningham, S.D., Anderson, T.A., Schwab, A.P., Hsu, T.C., Phytoremediation of Soils Contaminated with Organic Pollutants, Advances in Agronomy, Vol. 56:55-114, 1996.

Cunningham, S. D., J. R. Shann, David E. Crowley, and Todd A. Anderson, Phytoremediation of Contaminated Water and Soil, Phytoremediation of Soil and Water Contaminants, Ellen L. Kruger, Todd A. Anderson, and Joel R. Coats (Editors), 2-15, 1997.

Davis, L.C., Banks, M.K., Schwab, A.P., Muralidharan, N., Erichson, L.E., Tracy, J.C.,

Plant Based Bioremediation, Bioremediation, Sikdar and Irvine (Editers), Technomics Publishing Co., 1996.

Flathman, Paul E., and Guy R Lanza, Phytoremediation: Current Views on an Emerging Green Technology, Journal of Soil Contamination, 7:415, 1998.

Halford, Keith J., Assessment of the Potential Effects of Phytoremediation on Ground-Water Flow Area C at Orlando Naval Training Center, Florida, Water-Resources Investigations Report 98-4110, United States Geological Survey, 1998.

Harding Lawson Associates, Remedial Investigation, Operable Unit 4, Study Areas 12, 13, 14 (Area C), Naval Training Center, Orlando, Florida, Department of the Navy, Southern Division, Naval Facilities Engineering Command, September 1998.

Hughes, Joseph B., Jacqueline Shanks, Mindy Vanderford, John Lauritzen and Rajiv Bhadra, Transformation of TNT by Aquatic Plants and Plant Tissue Cultures, Environmental Science and Technology, Vol.31, No. 1:266-270, 1997.

Newman, Lee A., Stuart E. Strand, Nami Choe, Hames Duffy, Gordon Ekuan, Martin Ruszaj, B. Brook Shurtleff, Jodi Wilmoth, Paul Heilman, Milton P. Gordon, Uptake and Biotransformation of Trichloroethylene by Hybrid Poplars, Environmental Science and Technology, Vol. 31, No. 4:1062,1066-1067, 1997.

Newman, Lee A., Xiaoping Wang, Indulis A. Muiznieks, Gordon Ekuan, Martin Ruszaj, Remi Cortellucci, David Domroes, George Karscig, Tim Newman, Robert S. Crampton, Ram A. Hashmonay, Michael G. Yost, Paul E. Heilman, James Duffy, Milton P. Gordon, and Struart E. Strand, Remediation of Trichloroethylene in and Artificial Aquifer with Trees: A Controlled Field Study, Environmental Science and Technology, Vol. 33, No. 13:2257-2265, 1999.

Nzengung, Valentine A., N. Lee Wolfe, Laura H. Carreira, Steven C. McCuttcheon, Phytotransformation of Halogenated Aliphatics Using Aquatic Plants, Environmental Science and Technology, Vol. 33:1470-1478, 1999.

Nzengung, Valentine A., Peter Jeffers, Sequestration, Phytoreduction, and Phytooxidation of Halogenated Organic Chemicals by Aquatic and Terrestrial Plants, International Journal of Phytoremediation, Vol. 3, No. 1:13-40, 2001.

Nyer, Evan K., Donald F. Kidd, Peter L. Palmer, Tom L. Crossman, Sami Fam, Frank J. Johns II, Gary Boettcher, Suthan S. Suthersan, In Situ Treatment Technology, 308, 1996.

O'Neill, Walter L., Biosorption and Transformation of Tetrachloroethylene and Trichloroethylene Using Mixed Species Microbial Mats, University of Georgia, Athens, GA, PhD Thesis, 1999.

USEPA Publication 540-S-01-500. Pivetz, Bruce E., Ground Water Issue: Phytoremediation of Contaminated Soil and Ground Water at Hazardous Waste Sites, Environmental Protection Agency, Office of Research and Development, Washington D.C., February 2001.

Pradhan, S.P., J.R. Conrad, J.R. Paterek, V.J.Srivastava, Potential of Phytoremediation for Treatment of PAHs in Soil at MGP Sites, Journal of Soil Contamination, Vol. 7(4):467-480, 1998.

Rennels, Darrell Eugene, Aquatic Plant-Mediated Transformation of Halogenated Aliphatic Organic Compounds, University of Georgia, Athens, Master's Thesis, 2-3, 1998.

Roper, J. Chadwick, Jerzy Dec, and Jean-Marc Bollag, Using Minced Horseradish Roots for the Treatment of Polluted Waters, Journal of Environmental Quality, Vol. 25:1242.

Rouhi, A. Maureen, Plants to the Rescue, Science/Technology, 21, January 13, 1997.

Schnabel, William E., Annette C. Deitz, Joel G. Burken, Jerald L. Schnoor, Pedro J. Alvarez, Uptake and Transformation of Trichloroethylene by Edible Garden Plants, Water Resources, Vol. 31, No. 4:816-822, 1997.

Schnoor, Jerald L., Louis A. Licht, Steven C. McCutcheon, N. Lee Wolfe, Laura H.

Carreira, Phytoremediation of Organic and Nutrient Contaminants, Environmental Science and Technology, Vol. 29, No. 7:318-320, 1995.

Thompson, Phillip L., Liz A. Ramer, Jerald L. Schnoor, Uptake and Transformation of TNT by Hybrid Poplar Trees, Environmental Science and Technology, Vol. 32, No. 7:975-980, 1998.

Trapp, Stefan, and J. Craig McFarlane, Plant Contamination, 48-51, 1995.

USEPA Publication 542-R-96-005. Cleaning Up the Nation's Waste Sites: Markets and Technology Trends, Environmental Protection Agency, Washington D.C., April, 1997.

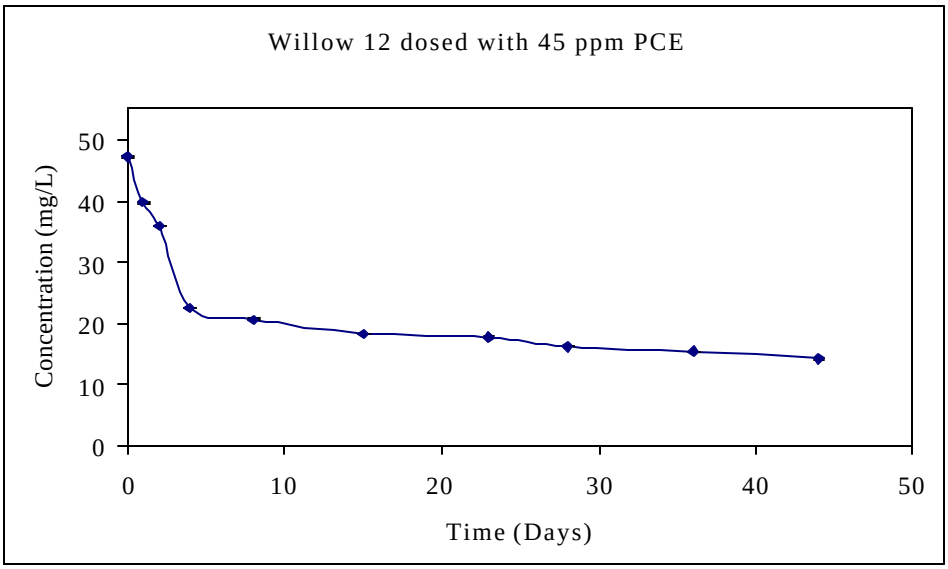
USEPA Publication 749-F-94-020. Chemicals in the Environment: Perchloroethylene (CAS No. 127-18-4), Office of Pollution Prevention and Toxics, Environmental Protection Agency, Washington D.C., August 1994.

Walton, B.T., and T.A. Anderson, Microbial Degradation of Trichloroethylene in the Rhizosphere: Potential Application to Biological Remediation of Waste Sites, Applied Environmental Microbiology, Vol. 56:1012-1016, 1996.

APPENDIX A

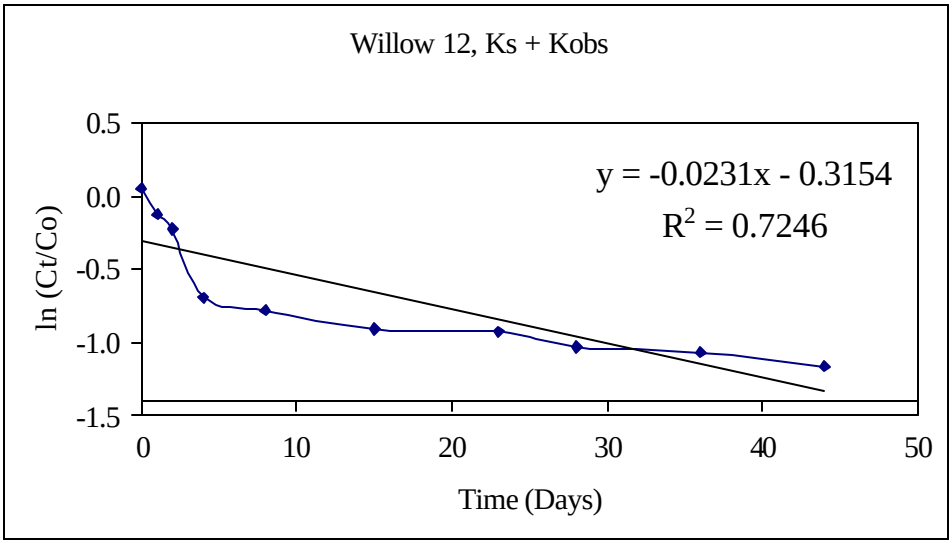
Data Analysis Using Zero Order and Psuedo-First Order Rate Constants
Descriptions of data analyses are provided in Section 3.5. The following are examples of graphs used for calculations performed to determine kinetic rates and to separate the initial sorption phase from the slower degradation phase.

Graph 1



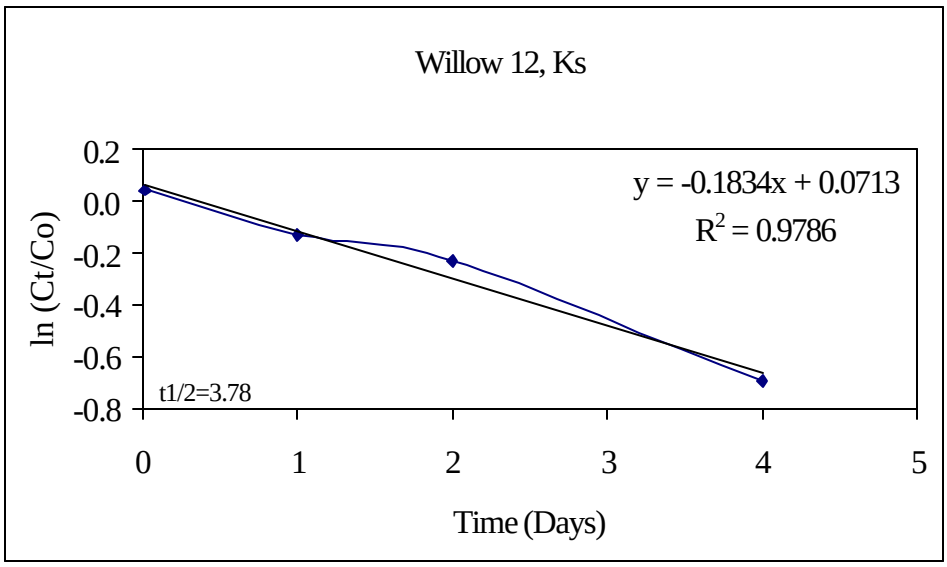
Illustrates the initial, rapid sorption phase followed by the slower degradation phase.

Graph 2



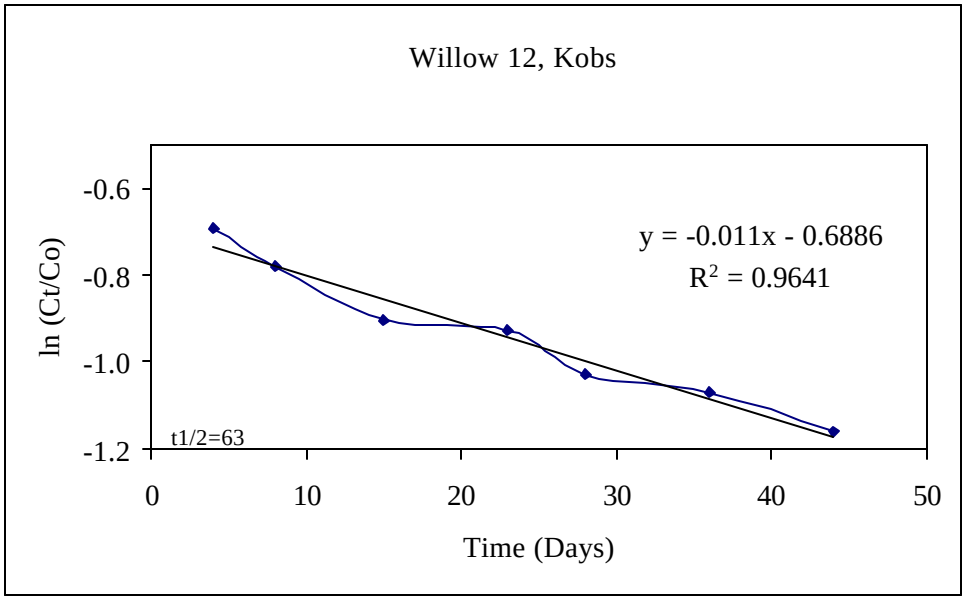
Illustrates the natural log of final and initial concentrations over time. Notice the difference in the slope of the graph at approximately 4 days. At 4 days, the curve was separated graphically to determine sorption rates versus degradation rates. Trendlines were added to show the R^2 of each curve.

Graph 3



Sorption phase from 0 to 4 days yielding a half-life of 3.8 days and an R^2 of 0.98.

Graph 4



Degradation phase starting at 4 days until experiment completion. Half-life was 63 days and the R^2 was 0.96.

APPENDIX B

Example of Transpiration Stream Concentration Factor

Willow 19 Plant Tissue	Total Activity Recovered
Leaves	18,114.4727
Branches	16,063.6304
Roots	810,624.3051
Upper Bark	138,207.7670
Lower Bark	29,489.6222
Stem Pith	185,651.2602
A. C. Trap 1	2,438,812.1117
A. C. Trap 2	1,258,554.3792
A. C. Safety Trap	1,146.4938
Caps and Parafilm	33,200.0000
Ending CPM in solution	6,252,000.0
Total	11,181,864.0424

Water uptake from initiation to completion = 600 mL.

Add the total activity from the leaves, activated carbon traps 1 and 2, and the activated carbon safety trap = 3,716,622 cpm.

Then correlate the total activity to the amount of water that had been taken up over the duration of the experiment by dividing total activity by water uptake = 6194379 cpm/mL.

Then divide the leaf and transpired concentration by the ending activity in solution (cpm/mL) to obtain the TSCF = 0.99.

Table of TSCF final calculations

Poplar Exp. (*PCE*)	**TSCF**		
1	0.31		
2	1.14		
3	0.48		
4	0.7	Avg. TSCF->	0.6575

Willow Exp.	**TSCF**		
PCE			
16	0.27		
17	1.23	Avg. TSCF->	0.75
TCE			
7	1.34		
18	0.54		
19	0.99	Avg. TSCF->	0.956667