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Phytoforensics tools: The degradation and detection of chlorinated solvents in integrated systems

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PHYTOFORENSICS TOOLS:

THE DEGRADATION AND DETECTION OF CHLORINATED SOLVENTS IN INTEGRATED SYSTEMS

by

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ABSTRACT

Due to decades of mismanaged pollutants entering groundwater, subsurface pollution of various compounds has become a widespread challenge. Chlorinated solvents are the most common groundwater contaminants that persist in aquifers, and remediation of these wide-spread plumes is difficult. Bioremediation, permeable reactive barriers, and phytoremediation are remedial technologies that have been developed and applied to chlorinated solvents in groundwater systems. This study integrates these technologies in different combinations to demonstrate the remediation potential of this approach. Zerovalent iron (ZVI) and bioaugmentation with a *Dehalococcoides sp.* (DHC) culture were applied separately and in combination for degradation of perchloroethene (PCE). *Salix pentandra* were planted in reactors and concurrently served as monitoring tools. Characteristics studied between reactor combinations included plant health, contaminant degradation rates, and water uptake. By creating an area of lower water potential, trees direct groundwater flow through the reactive zone and uptake the contaminated groundwater after contaminant degradation. Classroom experiential learning of this study was implemented to introduce phytoforensics to students. ZVI and DHC showed degradation of up to 92.0% and 99.3% reduction of PCE, respectively. Combined, ZVI and DHC increased PCE concentration reduction to 99.7%. Dichloroethene (DCE) was only found in all reactors containing DHC, but in no reactors without DHC. Plant sampling was shown to reveal degradation profiles and offer a low impact, low cost approach to monitoring PCE degradation processes in the subsurface. The degradation of PCE by DHC and ZVI was shown to occur through phytoforensics, and the specific mechanism was elucidated.

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NOMENCLATURE

Symbol	Description
BOD	Biological Oxygen Demand
CI	Confidence Interval
Cl-VOC	Chlorinated Volatile Organic Compound
C_{sat}	Saturation Concentration
DCE	Dichloroethene
DHC	*Dehalococcoides sp.*
DNAPL	Dense Non-Aqueous Phase Liquid
ECD	Electron Capture Detector
GC	Gas Chromatography
HRC	Hydrogen Release Compound
ID	Inner Diameter
K_{fg}	Fiber-Gas Partitioning Coefficient
K_{ma}	Material-Air Partitioning Coefficient
K_{ow}	Octanol-Water Partitioning Coefficient
K_{oc}	Organic Carbon Partitioning Coefficient
LTM	Long Term Monitoring
MB	Microbiota
MDL	Minimum Detection Limit
MNA	Monitored Natural Attenuation
PA	Polyacrylate

PCE	Perchloroethene
PRB	Permeable Reactive Barrier
PTFE	Polytetrafluoroethylene
SPME	Solid Phase Micro Extraction
SPS	Solid Phase Sampler
TCE	Trichloroethene
VC	Vinyl Chloride
VI	Vapor Intrusion
VOC	Volatile Organic Compound
VP	Vapor Pressure
ZVI	Zerovalent Iron

SECTION

1. INTRODUCTION

Chlorinated solvents have been identified as the most prevalent groundwater contaminants since the 1980s and have contributed to negative health effects in humans. Numerous chlorinated solvents are known carcinogens, and many more are suspected carcinogens (CDC, 2012). Many technologies have been developed to remediate chlorinated solvents in groundwater. However, each of these technologies presents some limitation, particularly *in-situ* technologies. Many *in-situ* technologies have been approved for groundwater cleanup sites, examples include: permeable reactive barriers, bioremediation, *in*-situ chemical oxidation, and phytoremediation (USEPA, 1993; USEPA, 1998; USEPA, 1999). Integrated systems that use multiple remediation technologies can increase the rate and efficacy of environmental cleanup.

Another approach for these technologies aim to enhance natural abiotic processes. An example of an abiotic technology for pollutant degradation is zerovalent iron (ZVI) which acts as a permeable reactive barrier (PRB). Permeable reactive barriers have a lower water potential than surrounding subsurface, so contaminated groundwater flows between the particles in the barrier. The particles act as reaction sites and reduce the contaminant in the groundwater to a lower oxidation level. This technique is effective in degrading a variety of chlorinated solvents (Gillham and Ohannesin, 1994). Many metals are effective at reducing oxidized organic compounds; iron is the most widely used because ZVI is readily available, is more cost effective, and has been more thoroughly researched. As a groundwater treatment technology, ZVI is advantageous because the

system is abiotic and passive with respect to energy and maintenance required, but ZVI can be disadvantageous due to the low reactivity caused by the passive layer, the small range of permissible pH, and the precipitation of metal oxides (Guan *et al.*, 2015). PRBs degrade with time and can lose functionality and reactivity with the contaminant of concern.

In most situations a single technology is administered to a site, but advantageous natural biodegradation usually occurs simultaneously (Löffler *et al.*, 2005). This advantageous natural biodegradation is called natural attenuation. Natural attenuation occurs when the natural microbial communities are shown to degrade the contaminant of concern at a sufficient rate to reach permissible contaminant levels in the required timeframe. If the natural microbial community is not sufficient to break down the contaminants, bioaugmentation or biostimulation can be performed to enhance the rates and degradation processes. Bioaugmentation is the addition of microbes to the system that do not occur naturally at a location. Biostimulation is the addition of nutrients to the system to encourage the growth and activity of the microbes desired for the site. Bioremediation has the advantage of being a relatively inexpensive, natural process with little energy expenditure, but low bioavailability of the contaminant and other present toxic compounds can cause bioremediation rates to languish (Dua *et al.*, 2002). The use of bioremediation is the best choice for some sites, but subsurface toxicological and permeability data for the site is needed to successfully implement a bioremediation project.

Phytoremediation is another biotic groundwater remediation technology. Vegetation is used as a natural pump to remove contaminated groundwater from the

subsurface through evapotranspiration (ET). Trees have deep roots that can tap into groundwater sources and access subsurface pollutants that most grasses and shrubs cannot reach. If the roots cannot reach the contaminated groundwater, phytoremediation is not useful for groundwater remediation. Also, phytoremediation alone may not be able to take-up the contaminant encountered due to chemical properties limitations which may not allow the contaminant to translocate across root membranes; this is often true of heavy metals. Other contaminants can be fatal to plants. Contaminated groundwater can be managed with phytoremediation, but the fate of pollutants must be considered. Volatile organic compounds (VOCs) can be released from plants to the atmosphere through diffused ET. Many pollutants are rapidly degraded in the atmosphere, but some may persist (Ma and Burken, 2002). Phytoremediation is advantageous for some sites by having inexpensive installation and maintenance costs while providing ecosystem services such as wildlife habitat and carbon sequestration, but can fail due to contaminant toxicity or environmental factors (Trapp and Karlson, 2001). The use of phytoremediation serves many benefits, but long term remediation times, potential plant mortality, and uncertain degradation rates detract from widespread application.

Plants have more uses than intended remediation. The field of phytoforensics uses trees in place of wells to gather data about contaminants in the groundwater. Trees act as natural monitoring wells by extracting the contaminated groundwater. They can also have interactions with soil vapors that may lead to contaminant uptake. Plant tissues can be taken from the tree over a plume for a fraction of the price, time, and environmental disturbance of drilling and monitoring a well. Although phytoforensics will not give the exact concentration in the subsurface, a semi-quantitative plume map can be created from

multiple sampled trees and the locations of highest concentration relate to the groundwater with the highest concentration of that contaminant. Placing multiple monitoring wells to locate the source of a contaminant plume is not cost effective. Phytoforensics can be used to delineate a contaminant plume and determine the best locations to drill wells saving both time and money as well as being less invasive to the environment. One approach to the long term monitoring (LTM) and assessment of dispersed plumes is phytoforensics. Phytoforensics has been shown to inexpensively provide detail on the relative concentration of groundwater contaminants. Phytoforensics can also provide a monitoring option for long term remedial options such as for permeable reactive barriers, bioremediation, and phytoremediation with minimal impact on the remedial action or environment (Limmer *et al.*, 2014).

Permeable reactive barriers, bioremediation, and phytoremediation all have limitations. Among these issues is the LTM of impacts as these technologies are slow to degrade contaminants. By integrating bioremediation, PRBs, and phytoremediation, in this study, the volumetric rate of contaminated groundwater treated can be increased and phytoforensics can be used to develop degradation profiles. This integrated system shows potential to reduce the length of LTM and maintenance costs.

2. GOALS AND OBJECTIVES

Improved, passive systems for remediating groundwater will help to minimize cost for long term monitoring. The overall goal of this project was to provide proof of application and fundamental knowledge on integrated technologies including phytoremediation and a variety of *in-situ* reactive processes for remediation of groundwater chlorinated solvents. To accomplish this overall goal a set of specific objectives and hypothesis were formed:

Objective 1: Determine if plant sampling can be used as a surrogate for groundwater monitoring of chlorinated solvents downstream of other reactive technologies.

Hypothesis: Plants will be affected by the subsurface conditions imposed by the remediation technologies, but can still act as surrogates for groundwater monitoring of chlorinated solvent treatment rates.

Objective 2: Determine if metabolite profiles of chlorinated solvents can be sampled in plant tissues to give details to degradation mechanisms occurring in the subsurface.

Hypothesis: The degradation pathway for chlorinated solvents is dependent on the remediation technology being used. Being able to detect metabolites in plants will give insight to which degradation mechanism is being used and the effectiveness of that technology in reducing the target pollutants.

Objective 3: Promote a proof of concept for integrated *in-situ* degradation mechanisms followed with phytoremediation for enhanced groundwater treatment rate and extent of contaminant degradation.

Hypothesis: Integrated degradation mechanisms will increase the efficacy of contaminated groundwater treatment to decrease the transport of parent

compounds into plant systems, and phytoremediation will increase the volumetric treatment rate by increasing the flowrate of groundwater through the reactive zone.

Objective 4: Develop a lab scale experiment arrangement for classroom experiential learning that uses phytoforensics to determine subsurface conditions and contamination.

Hypothesis: Classroom experiments will provide useful insight for the students into the emerging field of phytoforensics, and convey interactions of complex processes such as mass transfer rates, partitioning, degradation pathways, and plant physiology.

3. LITERATURE REVIEW

3.1 CHLORINATED SOLVENTS

Chlorinated solvents are a type of organochloride that contain at least one chlorine atom covalently bonded to a carbon atom. Many chlorinated solvent species used range in physicochemical properties and toxicity. The smallest organochloride is chloromethane which has a molar mass of 50.49 g/mol, but many chlorinated solvents have multiple chlorine atoms significantly adding to their molar masses.

The prefix of the chlorinated ethenes (tetra-, tri-, di-) refers to the number of chlorine atoms attached to the carbons. Tetra- refers to having four chlorine atoms, whereas tri refers to three and di refers to two. The suffix at the end of the organochloride (-ene) refers to the number of bonds between carbon atoms; -ene refers to having a double bonded carbon while -ane refers to a single bonded carbon and –yne refers to a triple bonded carbon. Tetrachloroethene and perchloroethene are two names for the same compound. Per- refers to having the maximum number of reactive groups on a base compound. The chemical structure of PCE, TCE, and DCE can be found in Figure 3.1.

Many chlorinated solvents have effective cleaning properties which make them useful for degreasing fats and oils. For this reason, chlorinated solvents are commonly used in dry cleaning applications, specifically perchloroethene (PCE), tricholoroethene (TCE), and dichloroethene (DCE). In 1980, 347,000 metric tons of PCE and 121,000 metric tons of TCE were produced in the United States (USGS, 2015). Chlorinated volatile organic compounds (Cl-VOCs) are the most prevalent pollutants found in the

groundwater at sites with some form of groundwater contamination. Most chlorinated solvents, including PCE, TCE, and DCE, are denser than water and are termed dense non-aqueous phase liquids (DNAPLs). Non-aqueous phase liquids are liquids that when mixed with water separate out into two distinct phases based on their solubility. The K_{ow} of a compound is the partitioning coefficient between octanol and water for a compound, which indicates the lipophilic property of a compound. The larger the partitioning coefficient of a compound the less that compound will dissolve into water. Chlorinated solvents that classify as a DNAPL slowly dissolve into the water and degrade at slow rates (CDPHE, 2014). The slow degradation of chlorinated solvents results in long-term plumes.

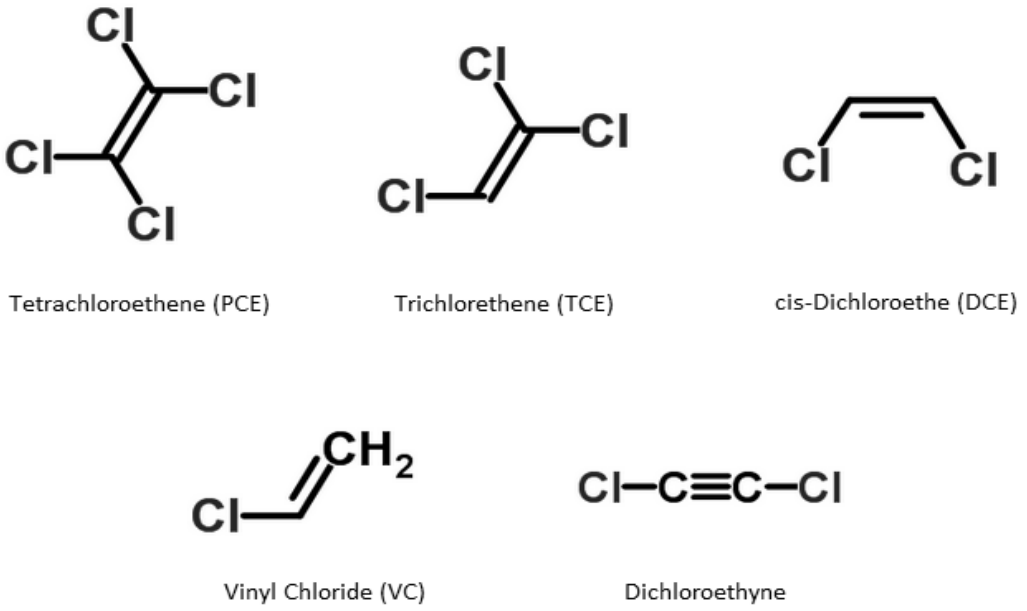

Figure 3.1 – Parent Compound PCE and anticipated chlorinated solvents. Adapted from (ChemSpider, 2015)

Most organochlorides have high vapor pressures and volatilize readily at standard temperature and pressure which can lead to vapor intrusion (VI) problems for structures. Chlorinated solvent vapors have been shown to be able to penetrate residential slabs (Henry *et al.*, 2013; Erdogan and Hsieh, 2014). The ability to penetrate slabs enables VI to be a leading exposure pathway for VOC exposure in many communities (Provoost *et al.*, 2008). With the limited airflow of many structures, Cl-VOCs from a single source are often found at higher indoor concentrations than outdoor concentrations (Dodson *et al.*, 2009). Due to the variability of indoor air concentrations of VOCs, groundwater concentrations do not serve as adequate surrogates when measuring VI potential and assessing risk (Folkes *et al.*, 2009). Properties of the PCE and select PCE metabolites are found in Table 3.1.

Table 3.1 - Chemical Properties of Perchloroethene and Select Byproducts (DeLassus and Schmidt, 1981; Horvath *et al.*, 1999; USEPA, 2015)

Contaminant	Mol. Mass (Da)	log K_{ow}	log K_{oc}	C_{sat} (mg/L)	VP (mm Hg, ST)
Perchloroethene	165.8	2.97	2.03	206	17.8
Trichloroethylene	131.4	2.47	1.83	1280	72.5
cis-Dichloroethene	96.94	1.98	1.64	4520	254
Vinyl Chloride	62.50	1.62	1.38	5631	2980
Dichloroethyne	94.94	1.12	1.64	13460	571

Chlorinated solvents are detrimental to human health through different exposure pathways. PCE, for example, can affect the central nervous system, eyes, kidney, liver, lungs, mucous membranes, and skin; the most frequently reported effect of PCE relates to the central nervous system (ATSDR, 2008). Many chlorinated solvents are known carcinogens, and many more are suspected carcinogens (CDC, 2012).

3.2 GROUNDWATER REMEDIATION TECHNOLOGIES

Many shallow groundwater remediation technologies are used to treat Cl-VOCs. These technologies include: air sparging, soil vapor extraction, permeable reactive barriers, phytoremediation, bioremediation, *in-situ* chemical oxidation, and pump and treat. Air sparging occurs when air is pumped underground to liberate contaminants from soil particles below the water table and allowing the contaminants to volatilize. For high vapor pressure (VP) compounds, this technology is often coupled with soil vapor extraction, which involves the use of a vacuum to remove contaminant vapors in the soil above the groundwater table (USEPA, 2012). Pump and treat is frequently used in groundwater remediation, but due to the cost and invasiveness of the process it is avoided when possible. Pump and treat technologies can require several decades to remove the contaminant to below permitted limits (USEPA, 2012). Any technology that removes groundwater to remediate the water above ground is classified as pump and treat. All sites have unique combinations of contamination, geology, hydrology, biology and human impact, among other factors that limit which technologies are applicable for a site.

3.2.1 Permeable Reactive Barriers. Permeable reactive barriers (PRBs) are used as a passive remedial technology. PRBs are typically used for sites below the groundwater level and are implemented down gradient of the contaminant source zone. The contaminated groundwater flows through the PRB, which allows the media in the barrier to react with, and degrade, the contaminant. A schematic of a typical PRB design can be found in Figure 3.2

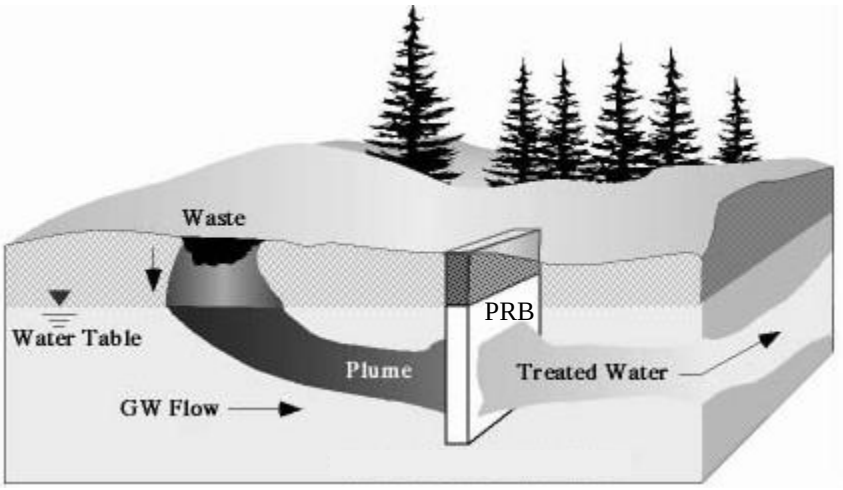

Figure 3.2 - Schematic of a Typical Permeable Reactive Barrier (PRB) Setup. Adapted from (USEPA, 1998)

The use of PRBs is favored, instead of technologies such as pump and treat, due to their low operational and maintenance cost (Gavaskar *et al.*, 2000). Zerovalent iron (ZVI) is often used in PRBs because the reduction potential of the iron can reduce many organic contaminants. Other metals such as copper and zinc are also used in PRBs, but iron is often preferred due to the lower cost and lower redox potential.

Chlorinated solvents can degrade through four pathways in the presence of ZVI. Chlorinated ethene reduction can happen via hydrogenolysis, hydrogenation, α-elimination, or β-elimination depending on the chemical species and environment (Lee and Batchelor, 2002). In most degradation mechanisms PCE reduces to TCE then cis-DCE, but PCE has been demonstrated to degrade through a different pathway in the presence of ZVI. PCE will predominately reduce into TCE and then quickly into dichloroethyne instead of cis-DCE in the presence of ZVI (Lim and Lastoskie, 2009). Abiotic exposure to ZVI predominantly favored β-elimination. Remediation of chlorinated solvents generally favors β-elimination because more intermediates are produced and the process is faster in comparison to hydrogenolysis (Gavaskar *et al.*, 2000). In one study, the β-elimination pathway accounted for 87% of PCE degradation resulting in dichloroethyne (Arnold and Roberts, 2000). Figure 3.3 shows the preferential pathway of PCE degradation under abiotic conditions from ZVI and β-elimination.

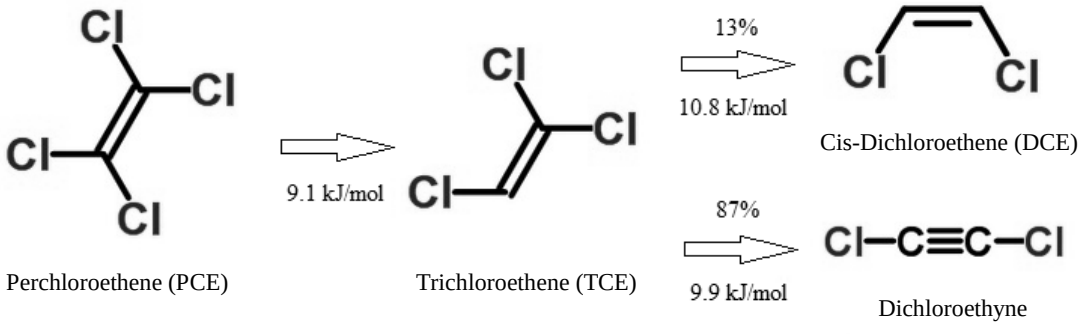

Figure 3.3 - β-elimination of PCE under Abiotic ZVI Conditions. Adapted from (Lim and Lastoskie, 2009)

The process of chlorinated solvent degradation with ZVI affects both the contaminant and the iron. As the iron reduces contaminants it becomes more oxidized. The chemical equation for the reaction between ZVI and chlorinated solvents is expressed below.

$$2Fe^0 + 3H_2O + (X - Cl) \rightarrow 2Fe^{2+} + 3OH^- + H_2 + (X - H) + Cl^-$$

Water and the chlorinated solvent are both reduced, while the ZVI is oxidized. Once the ZVI has been oxidized it no longer has the same reactivity with the contaminants. Visual recognition of the oxidation of iron is noted by the presence of rust formation and dissolution of iron (II). Exposure to natural weather systems can also cause rusting in the iron; therefore rust cannot be solely attributed to the occurrence of reductive dechlorination. The half-life of PCE in the presence of ZVI was first demonstrated to be 3.6 hr (Gillham and Ohannesin, 1994). The abiotic degradation rate of PCE with no added reducing agent was first reported to be 8.7×10^{10} hr^{-1} (Vogel *et al.*, 1987). The reaction rate of reductive dechlorination was determined to be pseudo-first order with respect to the compound (Gillham and Ohannesin, 1994). This pseudo-first order relationship can be represented by the following equation, which describes the concentration at a given time as equal to the initial concentration (C_0) multiplied by *e* to the negative normalized surface area rate constant (k_{sa}) multiplied by time (t) (Scherer *et al.*, 2000). The rate constant k_{sa} accounts for the observed reaction rate constant (k_{obs}) and the surface area of the ZVI (ρ_a).

$$C = C_0 e^{-ksa*t}$$

The specific ZVI parameters and the amount of ZVI present greatly impacts the effectiveness of the remediation effort. One of the most important parameters for ZVI is the Brunauer, Emmett, and Teller (BET) surface area due to the reactions occurring between the surface of the iron and the contaminated water. ZVI exposed to stagnant water results in a reduction in kinetics; the transfer rate becomes diffusion controlled (Yu *et al.*, 2006). When continuous mixing of the ZVI and contaminant solution is introduced, higher reaction rates occur due to the increased surface area with which the contaminant can react (Gillham and Ohannesin, 1994). The kinetics of TCE exposed to ZVI under continuous and intermittent mixing is shown in Figure 3.4.

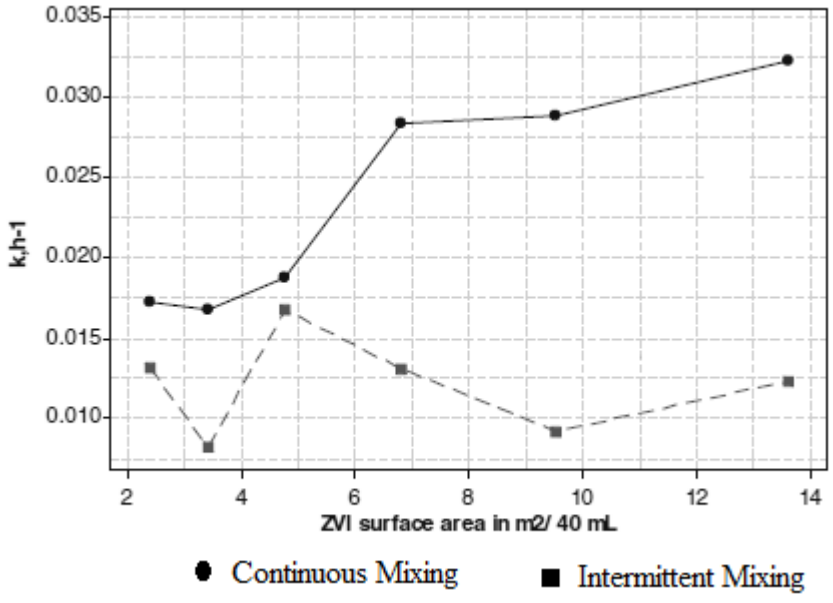

Figure 3.4 - Kinetics of TCE Exposed to ZVI under Continuous and Intermittent Mixing. Adapted from (Thangavadivel *et al.*, 2013)

Figure 3.4 shows continuous mixing with circles and intermittent mixing with squares. The rate constants in Figure 3.4 for continuous mixing and intermittent mixing range from 0.017 to 0.032 hr^{-1} and 0.008 to 0.017 hr^{-1}, respectively. Continuous mixing helps degrade TCE in less time than intermittent and no mixing; this results in a higher rate constant (Thangavadivel *et al.*, 2013). Many factors affect the degradation of chlorinated solvents by ZVI, but the most important factors seems to be the surface area of the ZVI, concentration of ZVI, and mixing of ZVI and solution.

3.2.2 Bioremediation. Bioremediation is the use of microorganisms to reduce or stabilize compounds in the subsurface. Biodegradation of some compounds is not possible. Microorganisms are very diverse; microorganisms can use different sources to derive energy and obtain carbon. Many pollutants can act as electron donors, and this eventually leads to the proliferation of the degrading organisms. In the degradation of hydrocarbons, the limiting factor is often the availability of the appropriate electron acceptors, such as oxygen or nitrate. Whereas for chlorinated solvents, the degradation process often involves using the chlorinated solvent as an electron acceptor (McCarty and Semprini, 1994).

In nature most compounds can be degraded by microbiota that is already present in the natural system. Microorganisms can be easily isolated from sites where they are already present to introduce to a different site that may not contain the necessary microorganisms to degrade the contaminant of concern (Bhatt *et al.*, 2007). Microorganisms can use chlorinated solvents as a terminal electron acceptor, similar to the human use of oxygen. Several studies have noted that the metabolism of chlorinated solvent degrading microorganisms is correlated to reductive dehalogenation (Wohlfarth

and Diekert, 1997). Acetate, a common electron donor for laboratory isolation and culturing of dechlorinating bacteria, can be applied to act as a carbon source for *Dehalococcoides sp.* (DHC) (Cole *et al.*, 1994; Wen *et al.*, 2015). DHC was the first reported microorganism with the ability to fully degrade PCE into ethane, and is now the most studied reductive dehalogenating bacterium (Maymó-Gatell *et al.*, 1997; Aulenta *et al.*, 2006).

Dechlorination of some chlorinated solvents, such as PCE, has been coupled with growth in some microbial cultures (Holliger *et al.*, 1993). Figure 3.5 shows the rate of dechlorination and intermediate transformation over time by DHC.

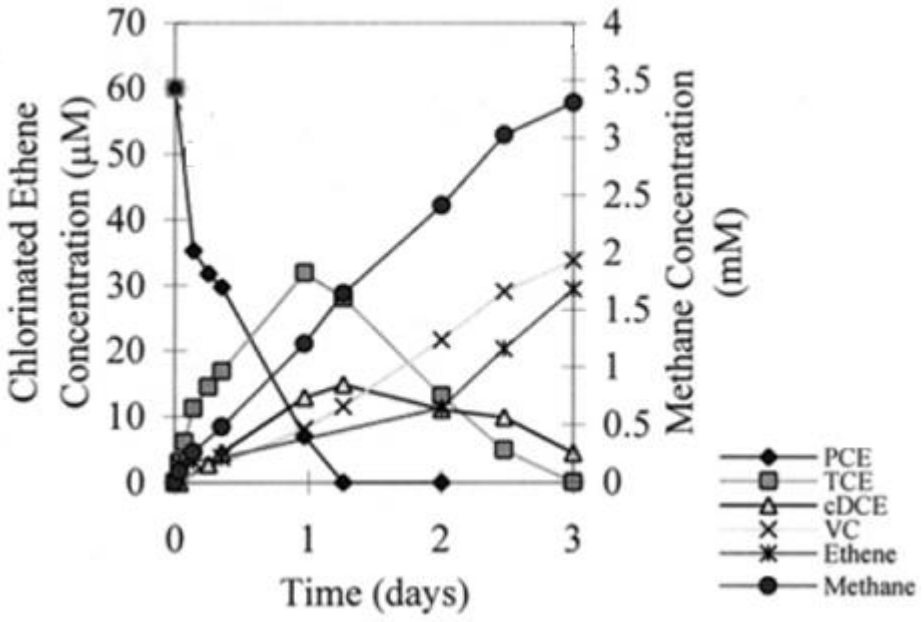

Figure 3.5 - Reduction of PCE and Transformation of Intermediate Chlorinated Compounds over Time by *Dehalococcoides ethenogenes.* Adapted from (Adamson and Parkin, 2001)

The reduction of PCE can result in the ultimate production of methane or carbon dioxide (Adamson and Parkin, 2001). The final pathway is dependent on the species of microbe utilizing the PCE. The final product differs in the breakdown of VC, but the beginning breakdown consistently follows PCE to TCE to DCE to VC during hydrogenolysis as shown below in Figure 3.6.

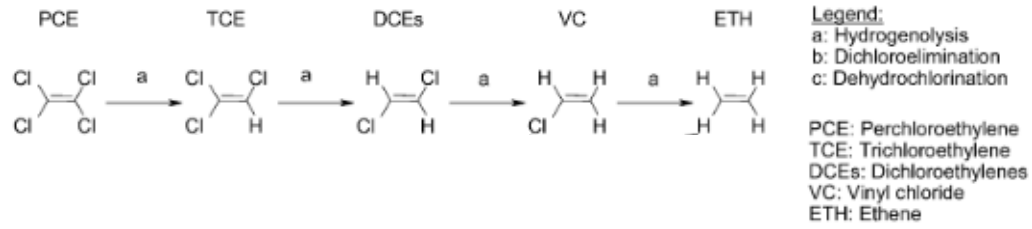

Figure 3.6 - Degradation Pathways of PCE through Hydrogenolysis, Dichloroelimination, and Dehydrochlorination. Adapted from (Aulenta *et al.*, 2006)

The ability and extent to which a microorganism can degrade a chlorinated compound is related to the structure of the compound, the position of the chlorine in the molecules, and the degree of chlorination (Bhatt *et al.*, 2007). Most chlorinated solvents are degraded through reduction, but some oxidized during their degradation instead (Seol and Schwartz, 2000). Chlorinated compounds are most frequently degraded by anaerobic processes, but aerobic processes also occur. Studies have shown that DCE and TCE are able to be degraded under aerobic conditions (Hopkins and McCarty, 1995; Lee *et al.*, 2000). PCE is commonly degraded anaerobically implying it can be used as a terminal electron acceptor (Vogel and McCarty, 1987). Under methanogenic conditions

reductive dechlorination is a common degradation pathway (Semprini, 1997). *Dehalococcoides ethenogenes* is capable of completely dechlorinating PCE, and all of its intermediates, under anaerobic conditions (Maymó-Gatell *et al.*, 1997; He *et al.*, 2003). *Desulfomonas michiganensis*, another microorganism, was identified with the ability to use PCE as an electron acceptor and acetate as an electron donor (Sung *et al.*, 2003). This species is not able to fully degrade PCE to ethane using the mechanisms that *Dehalococcoides sp.* uses. Many other DHC species have been found over time and have been placed in their own class, Dehalococcodia, and phylum, Chloroflexi, to accommodate their unique characteristics (Löffler *et al.*, 2013).

Chemical spills that require environmental remediation often leave a habitat unsuitable for microbial degradation without human interaction. In situations where natural microbial degradation is unsuitable, biostimulation is often required. Biostimulation includes adding nutrients or carbon sources into the environment to act as electron donors for microbial degradation. Many electron donors can be used in chlorinated solvent degradation. Many substrates have been found to act as electron donors in reductive dehalogenation. Methanol, glucose, acetone, and acetate, listed in order from greatest to least energy potential, are substrates that act as electron donors for many reductive dehalogenating bacteria (Nies and Vogel, 1990). Molasses has been shown to be a successful carbon source for bioremediation (Liu *et al.*, 2015). Molasses has the added benefit of being a viscous byproduct of sugarcane refinement and is not rapidly degraded. This allows for microbial communities to use this carbon source for a longer period of time. Molasses was found to have several essential trace elements for bacteria that activate enzymes that aid in degradation processes (Link *et al.*, 2013). As

molasses ferments the pH of the water decreases and a reduction of flow in groundwater flow can occur (Dyer *et al.*, 2000). Since molasses and carbon dioxide are weak acids, the pH increase is likely due to microbial activity and the groundwater flow reduction is likely caused by increased biomass of the microbial community. Proprietary products have also been developed for electron donors. Hydrogen release compounds were developed to have varying release profiles for the needs of specific projects (REGENESIS, 2015).

Bioremediation is a well-developed field and has the potential to be a strong supporting technique in integrated systems. Many microorganisms have a documented ability to degrade PCE, and the intermediates differ from the degradation of PCE in the presence of ZVI. Overall, this technology is effective in many environmental remediation situations.

3.2.3 Phytoremediation. The use of vegetation to mitigate environmental pollutants to protect human health is called phytoremediation. Phytoremediation can be used to remediate air, soil, and groundwater contamination of some compounds. Phytoremediation can be used in groundwater remediation for a number of groundwater contaminants, including chlorinated solvents (Ali *et al.*, 2013; Truu *et al.*, 2015). Some of the mechanisms by which phytoremediation works are rhizodegradation, phytovolatilization, phytoextraction, and phytodegradation (Arthur *et al.*, 2005). These mechanisms are based on chemical and plant properties, and can be designed to promote one mechanism over another.

Rhizodegradation is the degradation of contaminants in the rhizosphere of a plant by microorganisms that use the roots for energy (Yifru and Nzengung, 2008).

Phytoextraction is the use of plants to uptake inorganic contaminants, retaining the compounds inside the vegetation (Arthur *et al.*, 2005). Plants with extracted contaminants can then be disposed of safely or incinerated to reduce the mass and volume on contaminant to be handled. Phytodegradation occurs when a plant takes up a contaminant and enzymes within the plant help to break down the contaminant into more bioavailable forms (Arthur *et al.*, 2005). Multiple methods of phytoremediation can work simultaneously to remediate contaminated soil and groundwater, and systems can be designed to focus on specific pollutants or specific polluted media. These mechanisms are shown in Figure 3.7.

For Cl-VOCs phytoremediation is a low cost method that passively removes the contaminated groundwater and relocates the contaminants within the vegetation or into the atmosphere (Ma and Burken, 2003). The loss of contaminants to the atmosphere is a potential concern with chlorinated solvents. Many contaminants are rapidly degraded in the atmosphere, while others persist (Ma and Burken, 2002). The impact on the food chain due to chlorinated solvent concentrations in consumable parts of plants is also a concern (Doucette *et al.*, 2007). The uptake of chlorinated solvents and other readily translocated pollutants by trees is not advantageous to the tree. The higher the degree of chlorination of a compound the more phytotoxic the compound (Dietz and Schnoor, 2001).

Although phytoremediation potentially can reduce the costs associated with remediation, it has many limitations. As biological organisms, plants are susceptible to detrimental impacts of phytotoxicity and environmental factors such as climate and

pestilence. Long term remediation times, potential plant mortality, and uncertain degradation rates have detracted the widespread application of phytoremediation.

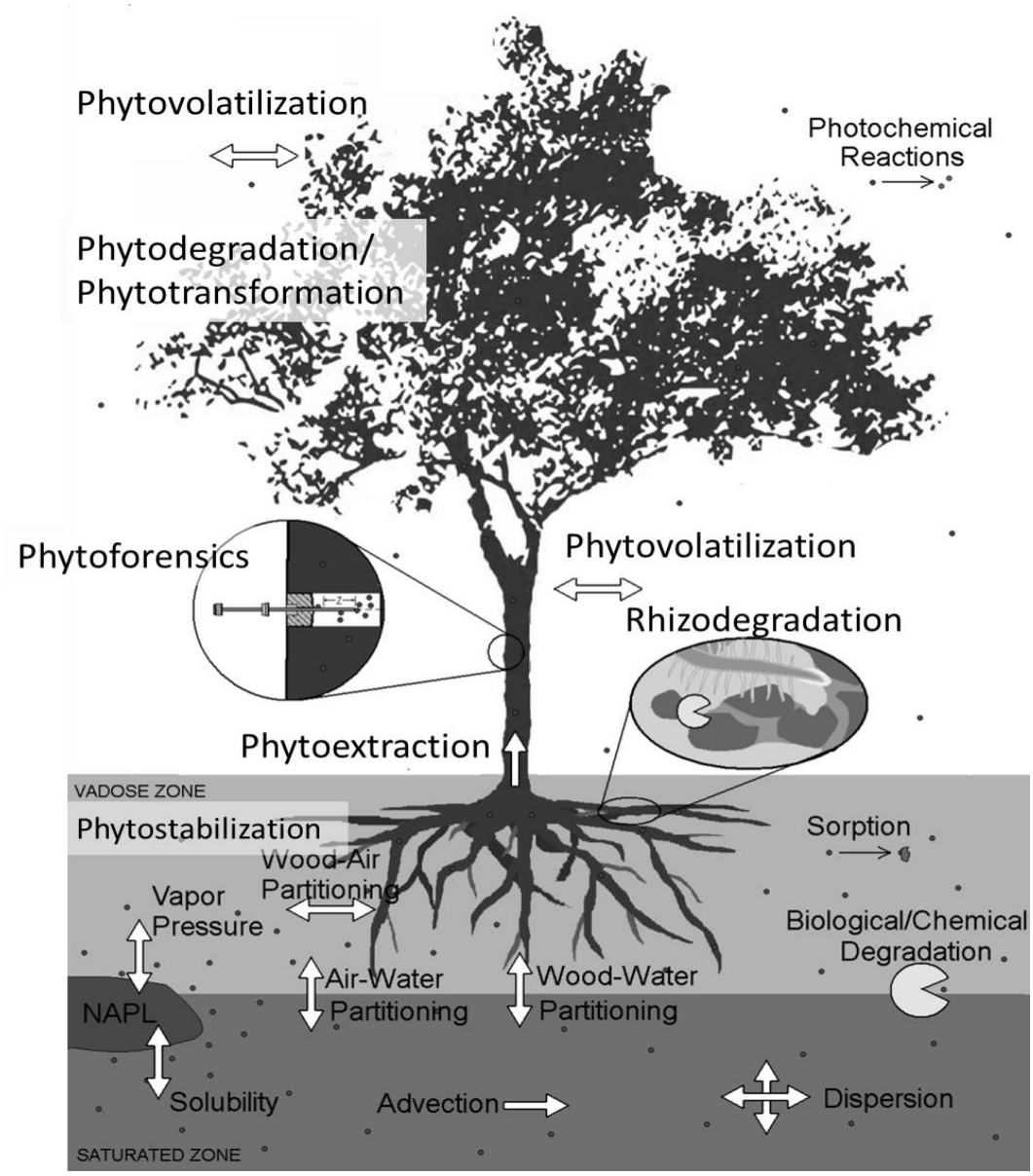

Figure 3.7 – Phytoremediation Model Showing Various Remediation Pathways. Adapted from (Limmer, 2014).

3.2.4 Integrated Technology Systems. For the most part, remedial efforts focus on a single technology for the cleanup of contaminated groundwater, but other factors are often also important. Monitored natural attenuation (MNA) is the degradation of contaminants completely by the natural biota in the system. The potential of MNA is difficult to gauge for a site, but in most situations, MNA aids the chosen technology in a remedial effort. Several studies have investigated the combination of two or more remedial technologies to determine the overall effect.

PRBs and bioremediation have been coupled in some sites to increase the reduction of PCE. When ZVI reduces PCE, hydrogen and hydroxide ions are released from the reduction of water. Microorganisms that are present in the PRB can utilize the hydrogen as an electron donor to increase degradation of chlorinated solvents using the solvents as electron acceptors (Link *et al.*, 2013). Bacteria contribute significantly to the degradation of chlorinated solvents in PRBs and increases the capacity of a PRB by slowing the rate of oxidation in the reactive zone (Burmeier *et al.*, 2006). The hydrogen released from the reaction between Cl-VOCs and ZVI may be used by microorganisms as an electron donor for reductive dechlorination. The major pathway in an integrated ZVI and DHC system is different than in other systems; 1,1-DCE is formed in place of cis-DCE in a ZVI and DHC system (Wu and Ma, 2011). The pathways for PCE dechlorination by separate and integrated systems of ZVI and DHC mixed cultures are shown in Figure 3.8. The reaction rates, in units of day^{-1}, for the dechlorination of PCE and its intermediates using ZVI, DHC, and a ZVI-DHC integrated system are shown in Table 3.2. In a ZVI system reaction rates for PCE reduction to TCE was greater than the reaction rate from PCE to dichloroacetylene, shown in Figure 3.2 (Wu and Ma, 2011).

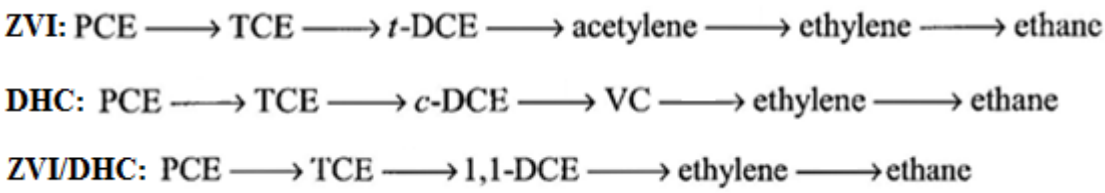

Figure 3.8 - Reaction Pathways for PCE in ZVI, DHC, and ZVI-DHC Systems. Adapted from (Wu and Ma, 2011)

Table 3.2 - Reaction Rates (1/day) for PCE and Metabolites in ZVI, MB, and ZVI-MB Systems. Adapted from (Wu and Ma, 2011)

Parent compound	*Products*	*Degradation system*		
		ZVI	*DHC*	*ZVI/DHC*
PCE	TCE	$1.07(\pm0.11) \times 10^{-1}$	$2.30(\pm0.16) \times 10^{-2}$	$1.70(\pm0.16) \times 10^{-1}$
PCE	dichloroacetylene	$4.66(\pm1.22) \times 10^{-3}$	0	$7.45(\pm0.18) \times 10^{-3}$
TCE	t-DCE	$2.62(\pm1.54) \times 10^{-2}$	$7.14(\pm4.94) \times 10^{-3}$	$2.19(\pm0.36) \times 10^{-2}$
TCE	c-DCE	$1.23(\pm0.88) \times 10^{-2}$	$2.02(\pm0.70) \times 10^{-2}$	$4.36(\pm2.96) \times 10^{-2}$
TCE	1,1-DCE	$6.02(\pm5.75) \times 10^{-3}$	$1.90(\pm0.49) \times 10^{-3}$	$9.65(\pm2.07) \times 10^{-2}$
t-DCE	acetylene	$9.50(\pm6.28) \times 10^{-2}$	0	$3.74(\pm0.83) \times 10^{-1}$
c-DCE	VC	$2.19(\pm0.98) \times 10^{-1}$	$3.59(\pm0.48) \times 10^{-2}$	$7.55(\pm6.44) \times 10^{-2}$
1,1-DCE	ethylene	$9.96(\pm2.11) \times 10^{-2}$	0	$5.85(\pm2.46) \times 10^{-2}$
VC	ethylene	$1.02(\pm1.03) \times 10^{-1}$	$2.51(\pm0.18) \times 10^{-2}$	$3.87(\pm1.18) \times 10^{-2}$
acetylene	ethylene	$9.96(\pm0.77) \times 10^{-2}$	0	$2.77(\pm1.56) \times 10^{-2}$
acetylene	ethane	$1.02(\pm0.23) \times 10^{-1}$	0	$1.09(\pm0.12) \times 10^{-1}$
ethylene	ethane	$1.51(\pm0.15) \times 10^{-2}$	0	$5.22(\pm7.65) \times 10^{-2}$

Contrary to these results Arnold and Roberts found that a majority of their PCE was reduced to dichloroacetylene instead of TCE (2000). A ZVI-DHC integrated system had a PCE reduction of 99.9% and 24.0% of the remaining organics were in the

form of VC (Wu and Ma, 2011). The PCE reduction rate of the separate ZVI and DHC systems were 76.9% and 54.0%, respectively; both sets had a majority of their remaining organics as PCE and TCE (Wu and Ma, 2011). Integrated ZVI-DHC systems may reduce PCE more effectively because microorganisms may help dissolve the iron oxides on the ZVI surface, which enables ZVI to maintain a greater reactive surface area (Wu and Ma, 2011).

Biostimulation with Vitamin-B_{12} is another example of an integrated remedial technology. Vitamin-B_{12} is shown to increase the removal rate of PCE by a mixed culture of *Methanosaeta concilii* and other *Methanosaeta sp*. (Chiu *et al.*, 1999). The addition of ZVI powder to the system decreased the chlorinated intermediates formed (Chiu *et al.*, 1999). Table 3.3 shows the degradation rate constants for DHC, ZVI, DHC-ZVI, and a DHC-ZVI integrated system biostimulated with Vitamin-B_{12}.

Table 3.3 - Degradation Rates of Integrated Systems of ZVI, Mixed Microbial Communities, and Vitamin-B_{12}. Adapted from (Chiu *et al.*, 1999)

Reactive Addition	**Pseudo-first-order Reaction Constant (1/hr)**
DMC	0.11
5 g ZVI/L	0.43
5 g ZVI/L and DMC	0.44
5 g ZVI/L, DMC, and 240nM Vitamin B12	0.49

ZVI and DHC was shown to work well together to ultimately remove PCE. The Vitamin-B_{12} increased the initial removal rate of PCE but did not significantly impact

the system otherwise (Chiu *et al.*, 1999). Integrated systems including vitamin-B_{12} augmentation are not feasible if a significant benefit is not found.

Integrated remediation technologies tend to have a more significant chlorinated solvent reduction than either component of the system alone. Incorporating phytoremediation into integrated systems could be advantageous to groundwater treatment because of the groundwater uptake by plants. ZVI and DHC systems have been researched heavily, but there is little research on systems with more than two remediation technologies.

3.3 PHYTOFORENSICS

Phytoforensics, or phytoscreening, is the emerging field of detecting groundwater contaminants by sampling vegetation on a site instead of drilling monitoring wells. This method does not eliminate the need for monitoring wells, but acts as a guide to help place monitoring wells more effectively and provide greater spatial data. Drilling monitoring wells is a costly technique to locate groundwater contamination. Installing a single monitoring well can cost over $2000, and often multiple monitoring wells are required (USEPA, 1997). Monitoring is a large percentage of the national budget. Long term monitoring (LTM) for all the Department of Defense's monitoring programs, alone, costs over $100 million yearly (SERDP-ESTCP, 2015). Phytoforensics, on the other hand, costs significantly less. A rough estimate of using phytoscreening on a site of 120 trees with two samplers would cost under $9,000 (Rein *et al.*, 2011). Phytoforensics can be employed on sites where using heavy equipment necessary to drill wells would be

complicated, such as swampy (Holm *et al.*, 2011). Phytoforensics uses the natural ability of trees to withdraw contaminated groundwater into the xylem of the tree. Inside the xylem, the contaminants reach equilibrium with the surrounding tree tissues or other matrices; phytoforensics involves the removal and analysis of the equilibrated matrices to deduce the contaminant-groundwater profile. The employment of this method to determine areas with the highest levels of contamination allows for the placement of monitoring wells to provide more accurate data. Phytoforensics can reduce the cost associated with the remediation of a site with significantly disturbances on the environment, while providing better site assessment.

Phytoforensics has inherent limitations, such as chemicals with high vapor pressures may be lost to the atmosphere during sampling and the qualitative nature of the data obtained from tree core analysis (Sorek *et al.*, 2008). Tree species and depth to groundwater both impact the concentration of chlorinated solvents in tree samples (Vroblesky *et al.*, 2004). Diameter of tree trunks also affects the contaminant concentration in the sample. Higher concentrations of PCE and TCE can be found in tree samples taken from further into the tree trunk a sample than samples taken near the bark (Limmer *et al.*, 2014). Seasonal variations were also found to affect chlorinated contaminant concentrations within the trees (Limmer *et al.*, 2014). Figure 3.9 shows the variation between contaminant concentrations in one tree over four years.

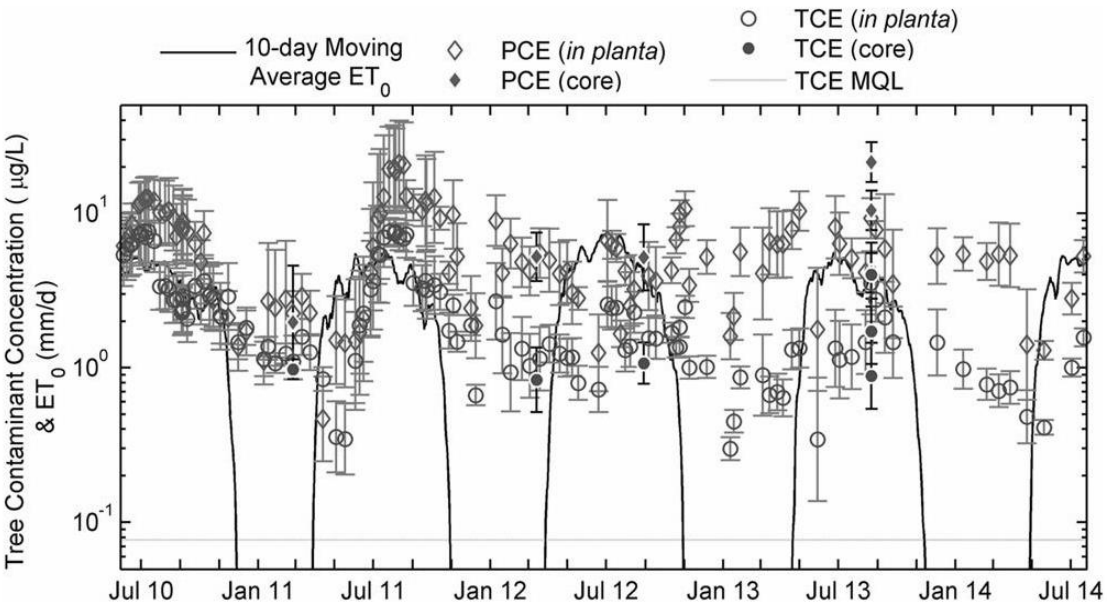

Figure 3.9 - Seasonal Variation of PCE in a Single Tree over the Span of Four Years. Adapted from (Limmer *et al.*, 2014)

The main causes of the seasonal variation in trees are temperature and precipitation; winters tend to produce lower concentrations and rain events dilute the contaminant in the tree (Sorek *et al.*, 2008; Limmer *et al.*, 2014). Other studies have noted winter to provide higher sample concentrations. This is reasonable because during the summer, the higher temperatures result in a reduced concentration detection due to compound volatilization (Vroblesky, 2008; Holm and Rotard, 2011). Vegetation species may also affect the observed differences in winter and summer tree core contaminant concentrations. Phytoforensics is a complicated technology due to species and environmental influences, but has proven to be useful in locating areas of high contaminant concentration while having a minimal impact to the environment.

3.4 PHYTOSCREENING SAMPLING METHODS

Another variation in tree core measurements is in the location from which the sample is taken. Concentrations of contaminants in xylem tissues tend to be the highest because of tree flow regimes (Wullschleger *et al.,* 1998). Directionality of water uptake can impact the distribution of chlorinated solvent concentration within the trees (Holm and Rotard, 2011; Limmer *et al.*, 2013). Directional uptake pattern is helpful to pinpoint the plume location. If one side of the tree has a higher concentration than the other sides, details on the plume location can be deduced. The use of tree rings can also aid in mapping the history of a plume, since tree rings can be stunted in environments of high chlorinated solvent concentrations (Rein *et al.*, 2015). The contaminant concentration within tree rings may provide insight to the hydraulic conductivity of the soil through the history of the plume and tree ring growth. Figure 3.10, below, shows the use of phytoscreening on a rural site to delineate a contaminant plume.

The placement of a limited number of monitoring wells is not likely to pinpoint source areas on a site. Using phytoforensics, more data can be collected more cost effectively and quicker to better delineate contamination on the site. Figure 3.10 shows the same map with a plume derived from (a) monitoring wells and (b) phytoscreening. The monitoring wells did not spatially identify the highest concentrations whereas the phytoscreening located four area of elevated concentration sources that were all attributed to a single source prior to the screening (Limmer *et al.*, 2011). The use of phytotechnologies can map current and past contaminant plumes and has the potential to provide information on the hydraulic conductivity of the subsurface for less time and

money than conventional methods. Phytotechnologies also reduce the need for heavy equipment and environmental disruption.

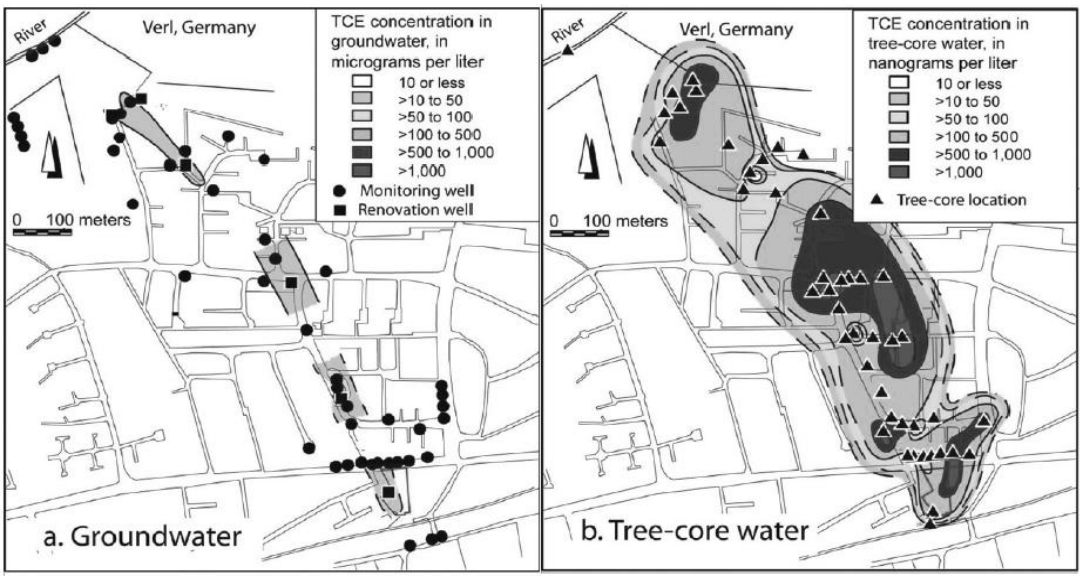

Figure 3.10 - Comparison of Plume Delineation by Phytoscreening and Monitoring Wells. Adapted from (Limmer *et al.*, 2011)

3.4.1 Solid Phase Microextraction Analysis. Initially, headspace injection was the method to detect Cl-VOCs in tree samples (Vroblesky, 2008). Solid Phase Microextraction (SPME) has been often applied for sampling plant biomass and introduction to gas chromatography (GC). The minimum detection limit (MDL) of PCE that could be detected using headspace injection was 6.7 ppt, but with SPME sampling PCE can be detected as low as 0.5 ppt (Limmer *et al.*, 2011). SPME extracts compounds from a matrix without using solvents (Zhang and Pawliszyn, 1993). The SPME method adsorbs compounds to a thin fiber coating and then desorbs those compounds into a GC.

SPME fibers have different coatings that are more applicable to the sensitive analysis of different compounds. For example, organic compounds can be adsorbed to polyacrylate (PA) and polydimethylsiloxane (PDMS) fiber coatings (Aguilar *et al.*, 1998; Guimarães *et al.*, 2008). PA fibers reach equilibrium more slowly than PDMS fibers for PCE. PDMS fibers can reach equilibrium with many chlorinated solvents in under four minutes (Meng and Pawliszyn, 1995). Both PA and PDMS fibers have a fused silica fiber core, but their coating is compatible with different types of compounds. PA fibers are compatible with polar semi-volatiles, while PDMS fibers are compatible with volatile compounds and often have lower MDLs. Since PCE is non polar, PA fibers have lower partition coefficients for PCE than PDMS fibers. This allows PA fibers to be used to quantify high PCE concentrations without reaching equilibrium.

The fiber-gas partitioning (K_{fg}) is highly temperature dependent. At 22˚C the K_{fg} for PCE is 2,025, but if the temperature is reduced to 10˚C the K_{fg} for PCE increases to 8,685 (Avila and Breiter, 2007). Humidity also affects the K_{fg} of fibers by about 10% at humidity levels about 90% (Meng and Pawliszyn, 1995).

Tree core sample methods have different analysis due to differences in partitioning coefficients. SPME sampling in tree ports provides a higher sensitivity than the wood from the original tree boring. Table 3.4 shows the difference between tree core and SPME concentrations of TCE and PCE. The response on a GC between a SPME sample and a tree core sample is often about two orders of magnitude, with SPME having the higher response (Burken *et al.*, 2009). The large difference in response is due to the higher partitioning coefficient of a SPME fiber to PCE and TCE than that of tree tissues.

Table 3.4 shows the difference between tree core sample and SPME sample concentrations of TCE and PCE.

Table 3.4 - Comparison of Chlorinated Solvent Peak Areas (Hz*s) from Tree Core and SPME Analysis. Adapted from (Burken *et al.*, 2009)

Tree #	Cores-TCE	Cores-PCE	SPME-TCE	SPME-PCE
Tree 1	3.8×10^2	2.1×10^4	5.8×10^3	1.2×10^6
Tree 2	6.1×10^2	1.9×10^4	1.7×10^4	4.4×10^6
Tree 3	9.4×10^1	5.2×10^2	5.8×10^2	2.5×10^3
Tree 4a	5.3×10^1	2.8×10^3	3.7×10^2	3.3×10^4
Tree 4b	3.6×10^2	6.2×10^3	4.3×10^3	7.1×10^4
Tree 5	ND	1.4×10^2	ND	7.2×10^3

The use of SPME analysis allows more sensitive sampling on sites and *in-planta* SPME increases ability to monitor on sites. Traditional phytoscreening technologies included removing a small section of tree sapwood for every sample. Tree core sampling of the same tree over time could severely injure a tree. *In-planta* SPME was found to prevent repeated damage to a tree from multiple traditional phytoscreening events (Limmer *et al.*, 2014). Despite the K_{fg} fluctuations due to humidity and temperature, SPME analysis provides quick, easy, and economical quantifications of chlorinated solvents, so as long as the fiber being used for adsorbing the contaminants is suitable for the application.

3.4.2 Solid Phase Sampling. Solid phase samplers (SPSs) detect the concentration of contaminants in the groundwater. SPSs are a method of using a polymer phase placed in the plant tissue as a passive sampling device. The samplers absorb

organic compounds via diffusion until equilibrium with the surroundings is obtained. SPSs used in one study were made from Tygon® Tubing (R-3603) with stainless steel wire to maintain the placement of the SPSs for easy removal (Limmer *et al.*, 2013). SPSs can be used in trees or in the subsurface. Multiple types of polymer media have been tested for chlorinated solvent SPS application. Polydimethylsiloxane (PDMS), low-density polyethylene (LDPE), linear low-density polyethylene (LLDPE), and polyoxymethylene (POM) are used as SPSs with chlorinated (Shetty *et al.*, 2014).The range of material-air partitioning coefficient (K_{ma}) of these five media and with four chlorinated solvent species are shown in Figure 3.11.

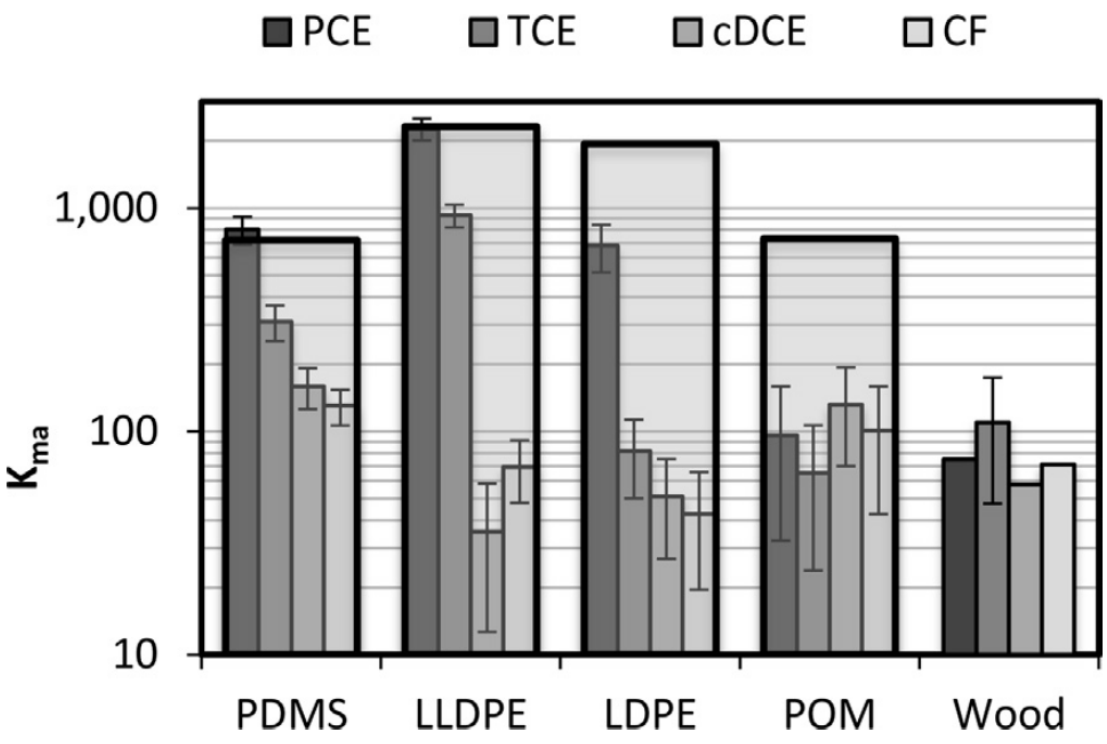

Figure 3.11 - Material-air Partitioning Coefficients (K_{ma}) for Materials Tested as SPS Materials. Adapted from (Shetty *et al.*, 2014)

The shaded boxes represent the coefficient necessary in order to achieve a negligible depletion of the contaminant with the chosen sampler mass. PDMS and LLDPE were the only appropriate materials to be used as SPSs; PDMS is more useful due to the faster equilibration time (Shetty *et al.*, 2014). The partitioning for PDMS SPSs is not sensitive to changes in temperature (Avila and Breiter, 2007). SPS concentrations are higher for PCE compared to tree core concentrations, but are lower for TCE (Burken *et al.*, 2009). SPSs can help delineate contaminant plumes while reducing environmental impact of sampling. SPSs can be implanted into trees through an increment bore hole. Implanted SPSs can be sampled instead of tree samples to reduce the stress on the trees being sampled during repeat analysis. Phytoscreening has low environmental impact compared to traditional plume delineation technologies, and employing SPSs to equilibrate with the contaminant concentrations in trees further reduces the environmental impact.

3.5 ECOSYSTEM SERVICES

The main application of phytoforensics is for urban and residential areas with current trees. Phytoforensics is a possibility when property owners are reluctant to disturb the wood causing drilling wells to become difficult (Sorek *et al.*, 2008; Limmer *et al.*, 2011). Trees will naturally withdraw contaminated water without any phytoremediation planning or monitoring. Phytoforensics can be integrated with natural phytoremediation for useful monitoring of remediation impacts over long periods. In areas that do not have existing trees, other vegetation can be planted to serve multiple benefits. The minimal

upfront and maintenance cost of planting can move large volumes of groundwater through evapotranspiration (Burken and Ma, 2002). New plants act as additional natural pumps that utilize solar and wind power to drive evapotranspiration and enhance groundwater treatment rates. New plants add to ecosystem services including: biodiversity, carbon sequestration, and water purification (Holzman, 2012). Norway and Vietnam both are noted in investing capital to protecting rainforest or adding new growth for environmental services (TEEB, 2010; NORAD, 2014).

These ecosystem services are beneficial for the environment as well as being financially beneficial. The ecosystem services provided by the U.S. National Wildlife Refuge Service is estimated at over $25 billion/year (Ingraham and Foster, 2008). The environmental and financial benefits of these services is dependent on the ecosystem. The global value of ecosystem services ranges from $490/year for an average hectare of open ocean to $350,000/year for an average hectare of coral reef (de Groot *et al.*, 2012). Impacts are also substantial in urban areas. Property values next to vegetated areas are on average greater than those with no vegetation (Escobedo *et al.*, 2015). Having trees incorporated into a yard increases the shade to reduce cooling costs, increases biodiversity, and improves character of the property. Impacts due to increased biodiversity are not well defined in the full value of ecosystem services, but will increase the overall value (Carrasco *et al.*, 2014). Future studies will undoubtedly further increase the economic understanding of ecosystem services. An increase in property value after a remedial action can also help to offset the cost of the project on top of providing ecosystem services to the community near the site. Quantifying the comprehensive value of natural treatment systems is difficult and requires more in-depth investigation.

PAPER

INTEGRATING PHYTOFORENSICS WITH BIOREMEDIATION AND ZEROVALENT IRON IN GROUNDWATER REMEDIATION

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ABSTRACT

Subsurface pollution is widespread from decades of mismanaged pollutants entering groundwater. Chlorinated solvents are the most common groundwater contaminants that persist in aquifers, and remediation of these wide-spread plumes is difficult. Remediation of chlorinated solvents in the environment is necessary due to the toxic and carcinogenic characteristics of these compounds. Bioremediation, permeable reactive barriers, and phytoremediation are three remedial strategies that have been developed and applied to treat chlorinated solvents in groundwater systems. This study integrates these three remedial technologies in different combinations to provide a proof of concept for the remediation potential of this integrated approach. Previous studies have assessed chlorinated solvent degradation rates in integrated systems, but phytoforensics has not been incorporated to assess groundwater treatment. Bioaugmentation of a dehalogenation community, *Dehalococcoides sp.* (DHC), and zerovalent iron (ZVI) were applied separately and in combination to phytoremediation reactors for reduction of

perchloroethene (PCE). Laurel leaf willows, *Salix pentandra*, were planted in reactors and concurrently served as monitoring tools. Characteristics studied between reactor combinations included plant health, contaminant degradation rates, and water uptake. By creating an area of lower water potential, trees direct groundwater flow through the reactive zone and uptake the contaminated groundwater after contaminant degradation. Alone, ZVI and DHC showed degradation of up to 92.0% and 99.3% reduction of PCE, respectively. Combined, ZVI and DHC reduced PCE concentrations by 99.7%. Dichloroethene (DCE) was only found in all reactors containing DHC, but in no reactors without DHC. Translation of wind and solar power energy into groundwater removal by plants has been shown to allow a higher volume of contaminated water to be treated by integrated systems. Alone, phytoremediation would release PCE into the atmosphere to be photodegraded, but integrated ZVI, DHC, and phytoremediation systems release reduced, less toxic, PCE byproducts into the atmosphere to be photodegraded. Plant sampling was shown to reveal degradation profiles and offer a low impact, low cost approach to monitoring PCE degradation processes in the subsurface.

INTRODUCTION

Since the 1980s, chlorinated solvents have been identified as the most prevalent groundwater contaminants and have contributed to negative health effects in humans. PCE, trichloroethene (TCE), and vinyl chloride (VC) are known carcinogens, and DCE and dichloroethyne are suspected carcinogens (CDC, 2012). Despite carcinogenicity, all chlorinated solvents are toxic to humans and can affect the central nervous system, eyes, kidney, liver, lungs, mucous membranes, and skin (ATSDR, 2008).

Many technologies have been developed to remediate chlorinated solvents in groundwater. All groundwater cleanup technologies have limitations, particularly *in-situ* technologies. Permeable reactive barriers, bioremediation, and phytoremediation are examples of *in-situ* technologies that have been approved for groundwater cleanup sites by the U. S. EPA (USEPA, 1993; USEPA, 1998; USEPA, 1999). The use of integrated systems of multiple remediation technologies, such as permeable reactive barriers (PRBs) coupled with bioremediation, can increase the rate and efficacy of environmental cleanup (Wu and Ma, 2011).

Many technologies act to enhance abiotic processes. An example of an abiotic technology for pollutant degradation is zerovalent iron (ZVI) acting as a PRB. Permeable reactive barriers provide a lower water potential than the surrounding subsurface, which promotes contaminated groundwater to contact media particles within the barrier. The particles act as reaction sites and reduce the contaminant in the groundwater to a lower oxidation level, which has been effective in degrading a variety of chlorinated solvents Many metals are effective at reducing oxidized organic compounds; iron is the most

widely used because ZVI is readily available, is more cost effective, and has been more thoroughly researched. As a groundwater treatment technology, ZVI is advantageous because the system is abiotic and passive with respect to energy and maintenance required, but ZVI can be disadvantageous due to the low reactivity caused by the passive layer, the small range of permissible pH, and the precipitation of metal oxides (Guan *et al.*, 2015). PRBs degrade with time and can lose functionality and reactivity with the contaminant of concern.

In most situations a single technology is administered to a site, but advantageous natural biodegradation usually occurs simultaneously (Löffler *et al.*, 2005). Natural attenuation occurs when the native microbial communities are shown to degrade the contaminant of concern at a sufficient rate to reach permissible contaminant levels in the required timeframe. If the natural microbial community is not sufficient degrade the contaminants, bioaugmentation or biostimulation can be integrated to enhance the rates and degradation processes. Bioaugmentation is the addition of microbes to the system that do not occur naturally at a location. Biostimulation is the addition of nutrients to the system to encourage the growth and activity of the microbes desired for the site. Bioremediation, which includes bioaugmentation, biostimulation, and natural attenuation, has the advantage of being relatively inexpensive with little energy expenditure. Low bioavailability of the contaminant and other present toxic compounds can cause bioremediation rates to languish (Dua *et al.*, 2002). The use of *Dehalococcoides sp.* (DHC) for bioremediation of chlorinated solvents has proven advantageous for fully reducing PCE to ethane (Maymó-Gatell *et al.*, 1997; Aulenta *et al.*, 2006).

Bioremediation is an acceptable choice for some sites, but subsurface toxicological and permeability data for the site is needed to successfully complete a bioremediation project.

Phytoremediation is another biotic groundwater remediation technology. Vegetation acts as a natural pump by creating a lower water potential zone than their surroundings and withdrawing contaminated water through evapotranspiration (ET). Roots interact with subsurface pollutants in soil vapors and groundwater. Trees have deep roots that can tap into groundwater sources and access subsurface pollutants that most grasses and shrubs cannot reach. If the roots cannot reach the contaminated groundwater, phytoremediation is not useful for groundwater remediation. Also, phytoremediation alone may not be able to take-up the contaminant encountered due to chemical property limitations which may not allow the contaminant to translocate across root membranes; this is often true of heavy metals. Other contaminants can be fatal to plants. Contaminated groundwater can be managed with phytoremediation, but the fate of pollutants must be considered. For example, volatile organic compounds (VOCs) can be released from the plant through diffused ET to the atmosphere where many pollutants are rapidly degraded by photodegradation, but some may persist (Ma and Burken, 2002). Phytoremediation is advantageous for some sites by having inexpensive installation and maintenance costs while providing ecosystem services such as wildlife habitat and carbon sequestration, but can fail due to contaminant toxicity or environmental factors (Trapp and Karlson, 2001). The use of phytoremediation serves many benefits, but long term remediation times, potential plant mortality, and uncertain degradation rates detract from widespread application.

In addition to phytoremediation, plants can be used for long term monitoring (LTM) and to assess dispersed contaminant plumes through phytoforensics. LTM is a large percentage of the national budget. The Department of Defense's monitoring programs, alone, costs over $100 million yearly (SERDP-ESTCP, 2015). Phytoforensics, on the other hand, costs significantly less. Using phytoforensics on a site of 120 trees with two samplers could cost under $9,000 (Rein *et al.*, 2011).

The field of phytoforensics uses trees instead of wells to gather data about contaminants in the subsurface. Tree tissue samples can be taken from a tree over a plume for a fraction of the price, time, and environmental disturbance of drilling and monitoring a well. Phytoforensics has been shown to inexpensively provide detail on the relative concentration of groundwater contaminants. Although phytoforensics will not give the exact concentration in the subsurface, a qualitative gradient map can be deduced on the relative contamination response of tree cores sampled at multiple locations (Shetty *et al.*, 2014). Instead of placing multiple monitoring wells to locate the source of a contaminant plume, phytoforensics can be used to delineate the plume and determine the more significant locations to drill wells, saving both time and money through a less environmentally invasive process. Phytoforensics can also provide a monitoring option for long term remediation with minimal impact on the remedial action or environment (Limmer *et al.*, 2014). This approach can be integrated with options such as permeable reactive barriers, bioremediation, and phytoremediation for increased contaminant reduction and groundwater transport with a significant reduction of cost and time.

Permeable reactive barriers, bioremediation, and phytoremediation all have some limitations. Among these limitations is the LTM of impacts of the treatment, as these

technologies are slow to degrade contaminants. By integrating these three technologies, higher degradation rates and specific degradation profiles can be achieved to reduce the length of LTM and maintenance costs. Limitations of integrating these systems are not well understood because the relationships between plants and other remediation technologies have not been studied. Iron and microbiota are beneficial for plants in small concentrations, but the low redox conditions that these remediation technologies create could prove toxic for many plants. Despite the lack of current understanding of the relationships between remediation technologies and plants, each technology can synergistically address groundwater and soil contamination.

This study was broken into three objectives. The first objective was to determine if plant sampling can be used as a surrogate for groundwater monitoring of chlorinated solvents downstream of other reactive technologies. The second objective was to determine if metabolite profiles of chlorinated solvents can be sampled in plant tissues to give details to degradation mechanisms occurring in the subsurface. The third objective was to promote a proof of concept for integrated *in-situ* degradation mechanisms followed with phytoremediation for enhanced groundwater treatment rate.

EXPERIMENTAL PROTOCOLS

EXPERIMENTAL DESIGN

Each reactor was constructed in a 2-liter clear glass jars containing a series of four solid phase samplers (SPSs), three separate layers of media, a 1.5 mm ID polytetrafluoroethylene (PTFE) tubing siphon, a 250 mL amber glass bottle, and a laurel leaf willow cutting (*Salix pentandra*). The reactor configuration can be found in Figure 1.

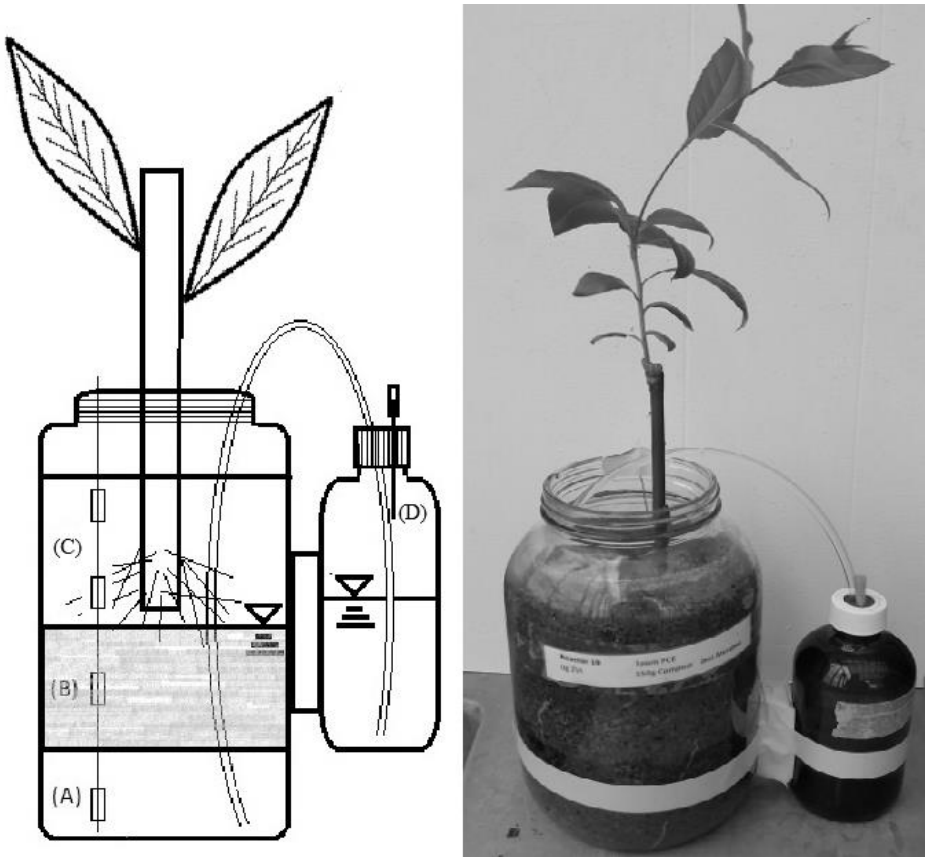

Figure 1 - Reactor Configuration Showing the Distribution Layer (A), Reactive Layer (B), and Rooting Zone (C). Dosing Bottle (D) was Connected via Siphon to the Reactor

The three layers of media were (A) a sand layer at the bottom of the reactor for flow distribution, (B) a reactive layer consisting of sand, ZVI, and/or compost, and (C) a sand layer above the reactive layer for plant rooting. The reactive layer varied on the 9 reactor series and is documented in Table 1. All reactor series had 350 mL of sand in layer (A) and 950 mL of sand in the rooting layer (C).

Table 1 - Reactor Series and Configuration

		Reactive Layer Composition (mL)			*DHC Stock Innoculum*	
Reactors	*Reactor Series*	*Vol. Comp*	*Vol. ZVI*	*Vol. Sand*	*Conc. Stock (DHC/L)*	*Vol. Stock Added (mL)*
1-8 and 33	No ZVI	0	0	340	0	0
9-12	20 g ZVI	0	6	330	0	0
13-16	100 g ZVI	0	28	310	0	0
17-20	Compost	100	0	240	0	0
21-24	Compost and DHC	100	0	240	$4.1x10^{11}$ *	2
25-28	20 g ZVI, Compost, and	100	6	230	$4.1x10^{11}$ *	2
28-32	100 g ZVI, Compost, and	100	28	210	$4.1x10^{11}$ *	2

* As provided by Terra Systems Inc., and made possible by Glen Ulrich, Parsons

The sand was a quartz silica sand; no testing was done to determine the grain size distribution or composition of the sand. H_2Omet 58 ZVI was obtained from RioTinto Metal Powders and had a Brunauer, Emmett, and Teller (BET) specific surface area of 0.03-0.04 m^2/g (RioTinto, 2016). Compost was obtained from Missouri S&T physical facilities; no testing was done of the composition of the compost. A 40 cm, 1.5 mm ID, PTFE tubing section connected the 1-liter jar with the dosing bottle to create a siphon to transfer water from the dosing container to the bottom of the 1-liter jar. The dosing siphon was used to simulate groundwater elevation fluctuations. Willow cuttings were obtained from Schumann Park in Rolla, MO from a well-researched phytoplot (Limmer, 2014). The cuttings were trimmed to 30 cm and kept in Hoagland's solution until new leaf and root emergence occurred (Hoagland and Arnon, 1950). Upon established root and leaf growth occurrence the cuttings were transplanted into layer (C) of the reactors. SPSs were constructed out of polydimethylsiloxane (PDMS) and cut into 1 cm pieces; the segments were connected with galvanized steel wire where one SPS was in layer (A), one

in layer (B), one just above layer (B), and one just below ground surface in layer (C) for removal of SPSs without reactor destruction.

Reactors were dosed with a 1 ppm PCE solution three times per week over the 17-week duration of the experiment. Five control reactors were kept uncontaminated. One of the controls did not have a willow cutting and was used as an evaporation control; the other four controls received a willow cutting and were used as tap water controls for ET. After eight weeks, reactors containing DHC were dosed with an addition of 5 mL/L Brer Rabbit Molasses® in the PCE dosing solution. An injection of 2 mL stock dehalogenation culture, based on a concentration of $4.1x10^{11}$ *Dehalococcoides sp.* (DHC)/L listed on the Terra Systems, Inc. supplied culture, was added to layer (B) of the reactor series listed as containing DHC in Table 1 (Lee, 2015). The molasses solution was deoxygenated for thirty minutes by bubbling nitrogen gas through the solution. PCE was added to the molasses solution after the nitrogen bubbling ceased to create the 1 ppm PCE anaerobic solution. Molasses was used to increase the biological oxygen demand (BOD) of the reactor and to act as an additional electron donor and carbon source for the DHC. In this experiment ET is considered equivalent to the volume of solution added to the reactor at each dosing and was recorded three times per week for the duration of the experiment.

SAMPLING METHODS

Leaf area of the willow cuttings was taken at harvest and quantified using Easy Leaf Area™ software (Easlon and Bloom, 2014). Soil samples were taken following EPA Method 9045D from layer (C) of the reactor at the time of the harvest (USEPA, 2004). Tree tissue samples were the bottom 5 cm of the above-ground portion of tree; tree samples were taken in duplicate by quartering the sample vertically, each sample

remaining 5 cm long, and opposite corners were placed together in 20 mL MicroLiter screw top headspace vials. The SPS series was removed altogether, rinsed with distilled water to remove ZVI particles, disassembled, and individual SPSs were placed in separate headspace vials. The tree samples and SPSs were analyzed for chlorinated solvent concentrations using a 7890 Agilent Gas Chromatograph (GC) equipped with a VOCOL® capillary GC column and electron capture detector (μECD) and 85-μm Polyacrylate (PA) SPME fiber (Limmer, 2011). The PA fiber extraction of the headspace was 5 minutes, with a time desorption of 3 minutes at 230°C in the μECD inlet. The oven temperature started at 40°C for 0.75 min then had a ramp of 20°C/min from 40°C to 160°C, resulting in a 6.750-minute run time.

RESULTS AND DISCUSSION

PHYTOSCREENING VIABILITY

The subsurface conditions in each reactor varied with the reactor series, which could have had an effect on plant growth due to the reductive and anoxic conditions present. To fulfill the first objective, the health of the plants under these reactive conditions needed to be assessed. Cumulative ET of solution was used to determine the change in health of the reactors over time and to assess if subsurface conditions affected the solution uptake. ET was measured by equating the solution added to the dosing bottle to the solution lost to the atmosphere via ET. No visible leaks were found during the experiment which suggested ET as the only pathway for solution to leave the reactor. Figure 2 shows the cumulative ET rate of the reactor series (represented by circles), with a 90% confidence interval, compared to the evaporation control (represented by squares).

The evaporation control reactor had a statistically significant lower cumulative ET than the reactors with trees. A higher ET in planted reactors supports that the cuttings were acting as natural pumps to remove the contaminated groundwater, and increases the flow through the permeable reactive zones. The tap water control reactor series had an average cumulative ET of 3200 mL, which was located in the middle of the range of ET values for the different series, and there was no significant difference between the tap water control series and the remaining reactor series. The lack of statistical difference in cumulative ET between the reactor series with different reactive zones gives partial satisfaction to the first objective supporting that plants are able to survive in conditions formed from degradation mechanisms.

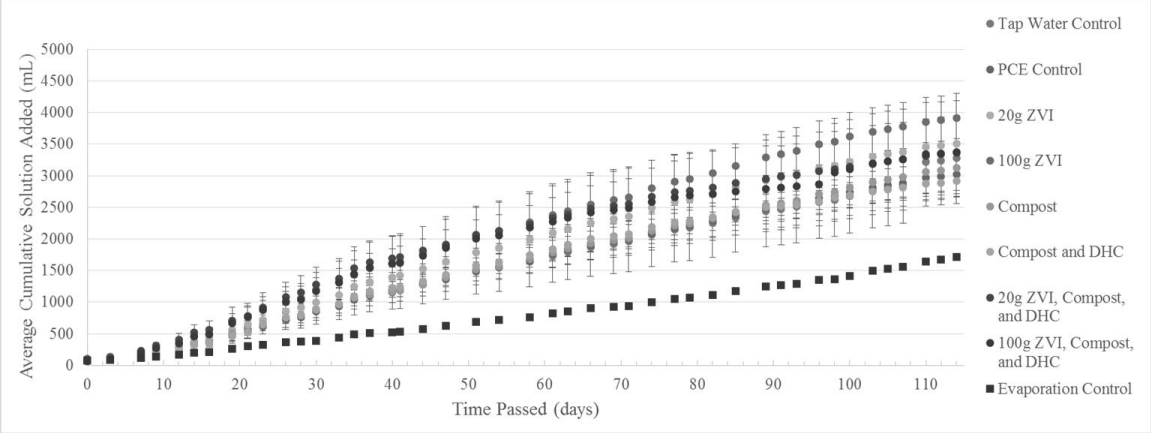

Figure 2 - Average Cumulative Evapotranspiration for Reactor Variations

The reactors that contained DHC were fed 5 mL/L molasses with the PCE solution to provide an additional carbon source for the DHC and to increase the BOD of

the reactor in order to enhance the anaerobic conditions. The depth of the media in the reactors varied due to the density of the reactive layer in each series. Figure 3 depicts the cumulative ET compared to the depth of the media for all the reactors. The differences in ET rates could be due to the depths of the reactors because higher ET rates would occur in reactors with shallower media. However, no correlation was observed between depth of the reactors and ET, supporting that plants are acting as pumps increasing the volumetric treatment rate of the groundwater, instead of evaporation being the primary water loss mechanism.

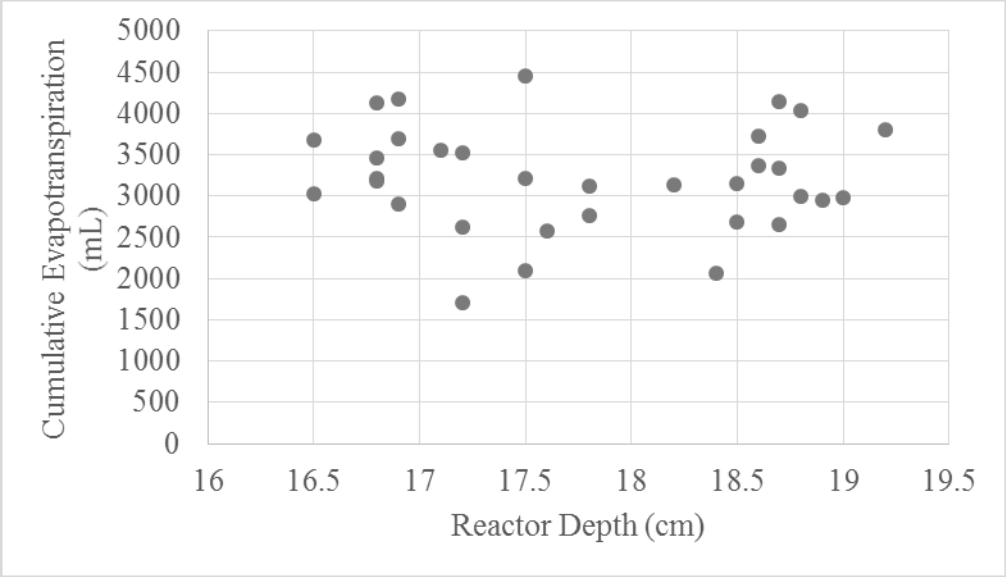

Figure 3 - Cumulative Evapotranspiration Compared to Reactor Depth

At week 12 of the experiment the reactors containing molasses and DHC began exhibiting slightly lower ET rates than the reactors without molasses, as shown in Figure

2. This could be attributed to the molasses stimulating the microbial growth; the increased biomass could have reduced the permeability of the sand or suggest that the microbial activity led to greater reducing conditions impacting plant health. During molasses fermentation in bioremediation sites, the pH of the groundwater increases and groundwater flow has been shown to be reduced (Dyer *et al.*, 2000). The reduction in groundwater flow is likely due to molasses being broken down by microorganisms and increasing microbial biomass. The average soil pH of the reactors is shown in Figure 4 with a 90% CI. The pH was significantly more basic in two of the three reactor series containing molasses than the reactors without molasses. Several of the reactors containing DHC and molasses also expressed a red biofilm with bubble formation, although the makeup of the biofilm was not analyzed. The increased pH and biofilm production may have been associated with the reduced groundwater flow. The reduced ET in the reactors containing DHC and molasses supports the idea that those conditions were negatively affecting the water uptake and health of those trees. The reduction of ET in the reactors containing DHC provides fundamental knowledge for the treatment rate of contaminated groundwater in integrated systems, which partially satisfies the second objective.

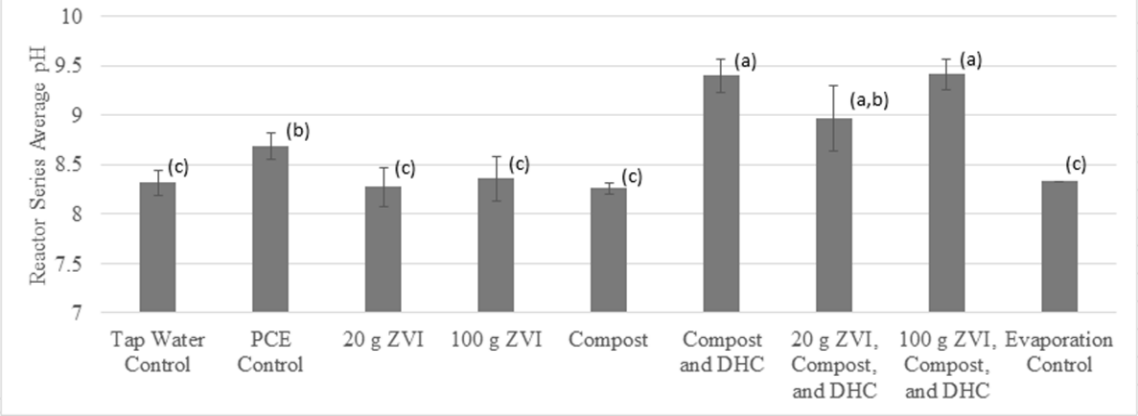

Figure 4 - Reactor Series Average pH

Several factors above ground may have affected leaf growth in addition to subsurface conditions. Spider mites began to affect all willow cuttings during week 6. The pesticide, Safer™ Insect Killing Soap, used to control spider mites also appeared to negatively impact plant health. Reactors lost leaves at an accelerated rate after the pesticide application. At the conclusion of the experiment, all reactors a smaller leaf area than at the beginning of the experiment, including the control reactors. Only one reactor was fatally affected by the combined system factors and was not included in analysis; this reactor was in the series with the most ZVI, Compost, and DHC. Compared to the other reactor series, reactors with DHC on average had less leaf area at the end of the experiment. Reactors with iron tended to have the highest average leaf area. Low doses of iron appeared to be beneficial for plant growth as reactors; reactors with 20 g ZVI exhibited the highest leaf surface area and ET rates, but the observation was not studied. DHC appeared to negatively affect plant growth, due to the reduced leaf area in the reactors containing DHC. This observation satisfies the first objective. Despite the

harmful properties associated with the integrated systems, all but one tree survived satisfying objective two; implementation of phytoforensics on integrated systems of multiple degradation mechanisms is feasible. Leaf surface area was collected and shown in Figure 5. The letters in Figure 5 represent statistical significance of each reactor where reactors containing a letter are not statistically different from other reactor series with the same letter, but are statistically different than reactor series with a different letter.

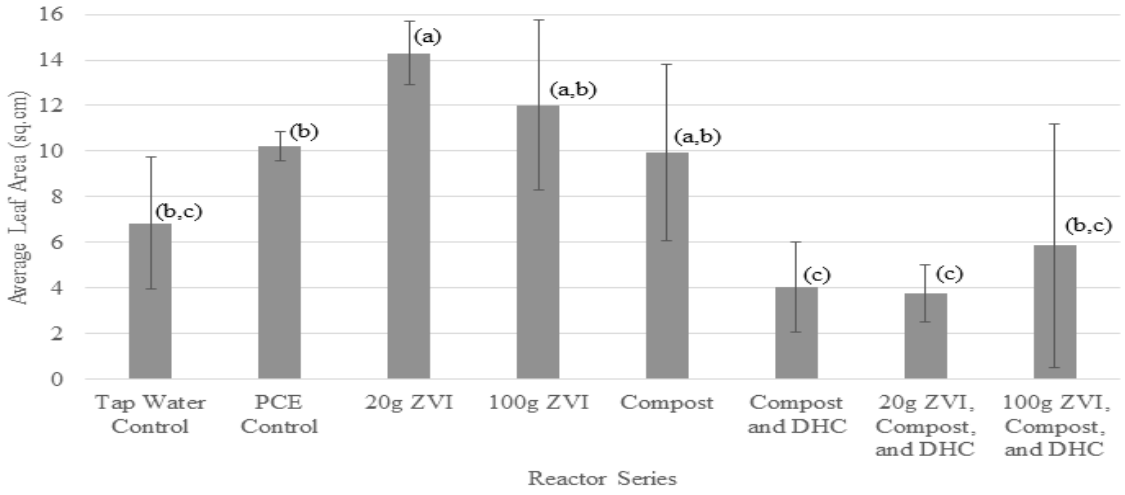

Figure 5 - Average Leaf Area per Reactor Series

PHYTOFORENSIC ANALYSIS

Tree samples provide insight on the specific subsurface reactions by extracting groundwater that has already undergone those specific reactions. Table 2 shows the average concentration of PCE found in tree samples of each reactor series and the percent reduction in that reactor series compared to the PCE control. In all reactors that contained ZVI and/or DHC, the PCE was reduced below that of the PCE control. Neither ZVI nor

DHC were tested in reactors without willows for two reasons. (1) The focus of this study was on the relationship between trees and *in-situ* degradation mechanisms, and (2) the degradation of chlorinated solvents by DHC and ZVI has been shown in multiple studies and is widely used in the field.

Reduction of PCE was shown to occur with the increased dose of ZVI. Integrated ZVI and DHC systems showed the greatest reduction, with more than 99.6% PCE degradation compared to systems without DHC. The introduction of compost was not shown to reduce the concentration of PCE, and was omitted from Table 2. The concentration of PCE in the compost reactor series had a higher average PCE concentration than the PCE in the PCE control series; this higher average was not statistically significant, and was likely due to biological variability in the willows leading to different contaminant uptake rates and capacity. However, compost combined with DHC showed a 99.3% reduction of PCE indicating DHC can reduce PCE concentrations. Average PCE concentrations in each reactor series are shown in Figure 6 with a 90% confidence interval. To fulfill the third objective, the reduction of PCE by integrated systems needed to be detected through phytoforensics. The third objective was satisfied due to highest PCE percent reduction in the reactors with integrated degradation mechanisms.

Table 2 - PCE Percent Reduction from PCE Control

Reactor Series	Avg. PCE Conc. (ppt)	Percent Reduction
PCE Control	55000	0.0%
20 g ZVI	15100	72.5%
100 g ZVI	4410	92.0%
Compost and DHC	365	99.3%
20 g, ZVI, Compost, and DHC	148	99.7%
100 g ZVI, Compost, and DHC	243	99.6%

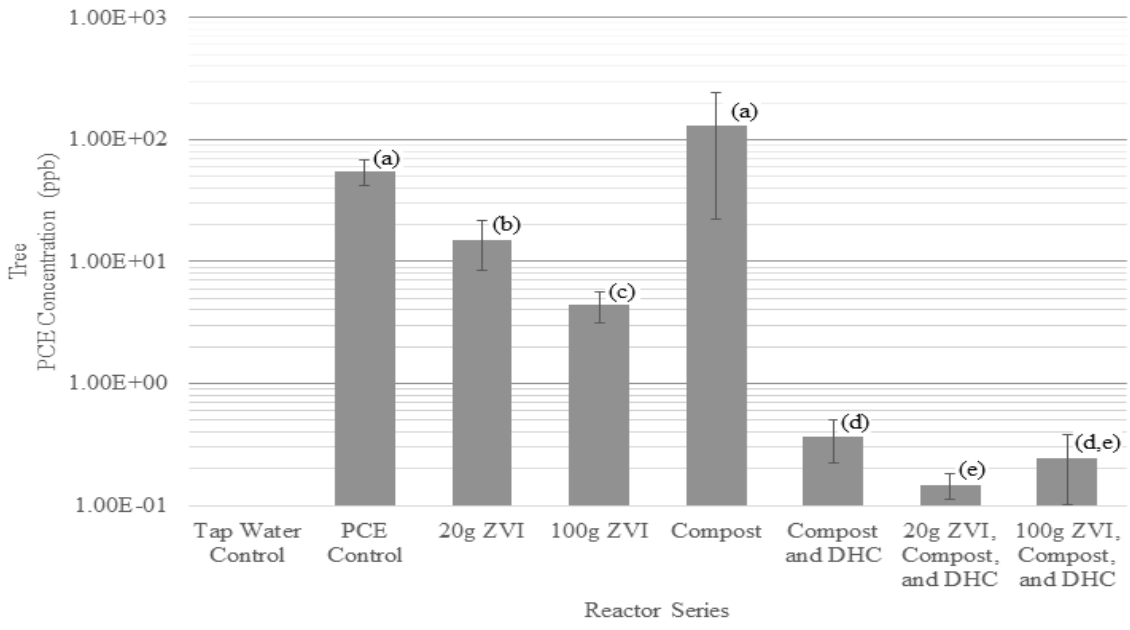

Figure 6 - Reactor Series Concentration of PCE

TCE was not quantified due to the co-elution of other compounds on the GC. Cis-DCE was only encountered in tree samples where the reactors that contained DHC; DCE was also found in all reactors that contained DHC. The tree sample concentrations of DCE are shown in Figure 7 with a 90% confidence interval. Presence of cis-DCE was not expected in the samples with no DHC because the degradation pathway of PCE in the presence of ZVI results in dichloroethyne instead of cis-DCE (Lim and Lastoskie, 2009). Dichloroethyne and VC concentration were not detected in any tree samples. This may be due to the high vapor pressure of these chlorinated solvents allowing them to volatilize away during the extraction and sample preparation processes before detection could occur (Sorek *et al.*, 2008).

The use of willow cuttings is effective for identifying the subsurface conditions below the tree by giving a semi-quantification of chlorinated solvents in the subsurface, and ascertaining the presence of metabolites. The specific metabolite profile indicates the phytoforensic methods can show degradation is occurring, and also give insight to the specific degradation processes. The metabolite profiles in tree samples partially satisfies the second objective of this study.

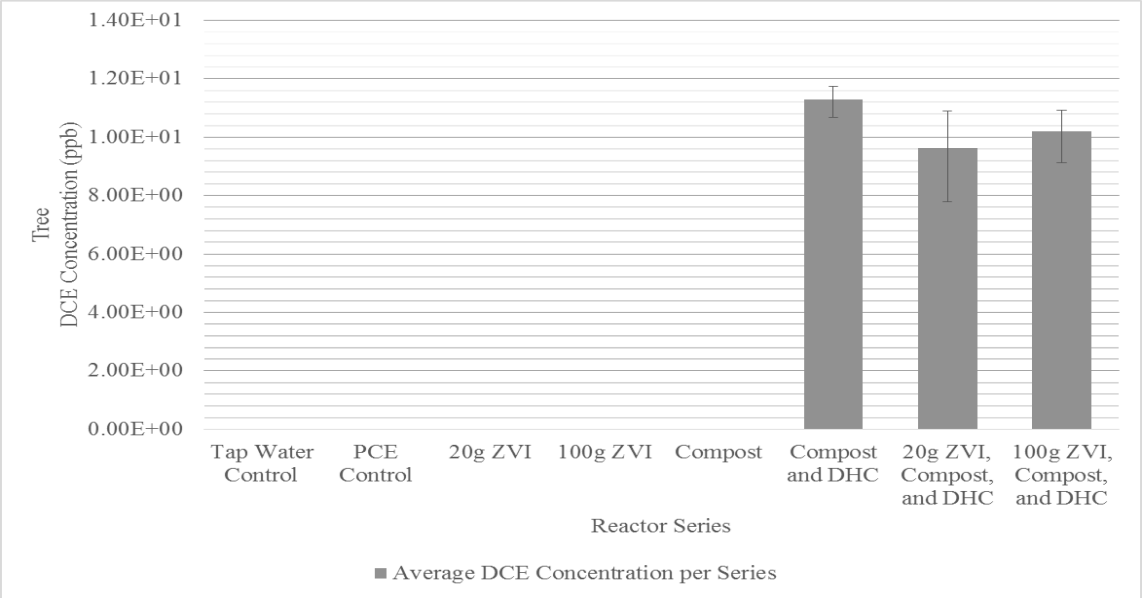

Figure 7 - Reactor Concentrations of DCE

SPSs used in the experiment provided similar results as the willow cuttings. The reactors containing DHC showed statistically significant degradation of PCE compared to those without DHC. Figure 8 shows the PCE concentration in each set of SPSs in the different reactor series. The concentration of PCE in the SPSs in layers (A) and (B) were often less than the SPSs above the reactive layer. This could be attributed to the flux of dosing solution in the reactor causing the SPSs in layer (C) to be exposed to the air more frequently and having better mixing rates not restricted to diffusion. The SPS in layer (A) did not show a concentration comparable to the dosing concentration; the lower concentration found in this SPS may be due to diffusion mass transfer limited conditions to the SPS in the bottom of the reactor. TCE was found in the reactor series containing 100 g ZVI and all the reactor series containing compost. TCE and DCE were both encountered in the SPSs containing DHC. The concentrations of TCE and DCE in the

SPS layers are shown in Figure 9 and Figure 10, respectively. The presence of TCE in the reactors containing only compost suggests there may have been a native dehalogenation microorganism community or reducing agent in the compost prior to the experiment. As in the trees samples, dichloroethyne and VC concentration were not detected in any SPSs. The detection of cis-DCE from the reactors containing DHC in the SPSs and in the tree samples supports plant sampling can provide metabolite profiles and give evidence of degradation mechanisms occurring in the subsurface. The metabolite profiles relationship to the degradation mechanisms satisfies the second objective of this study.

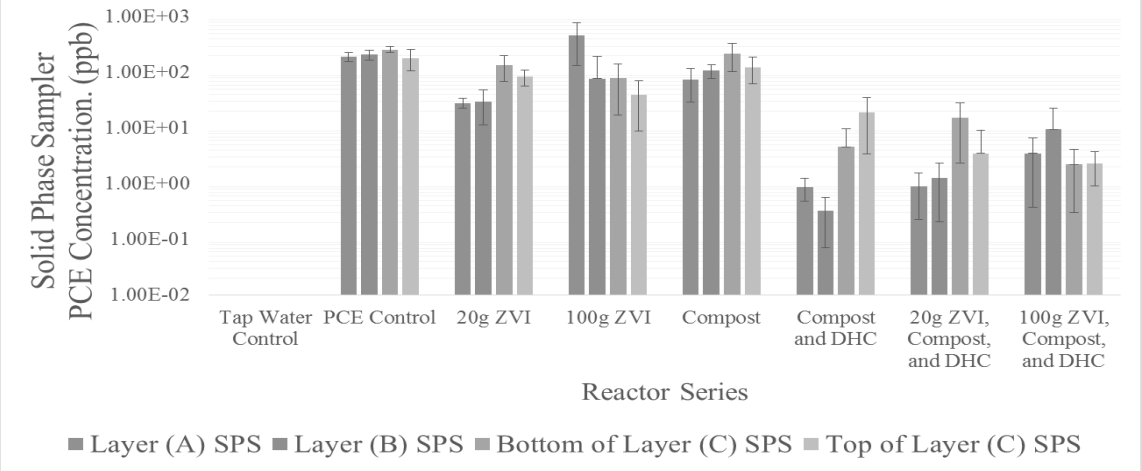

Figure 8 - Reactor Series PCE Concentration in SPSs in Different Layers

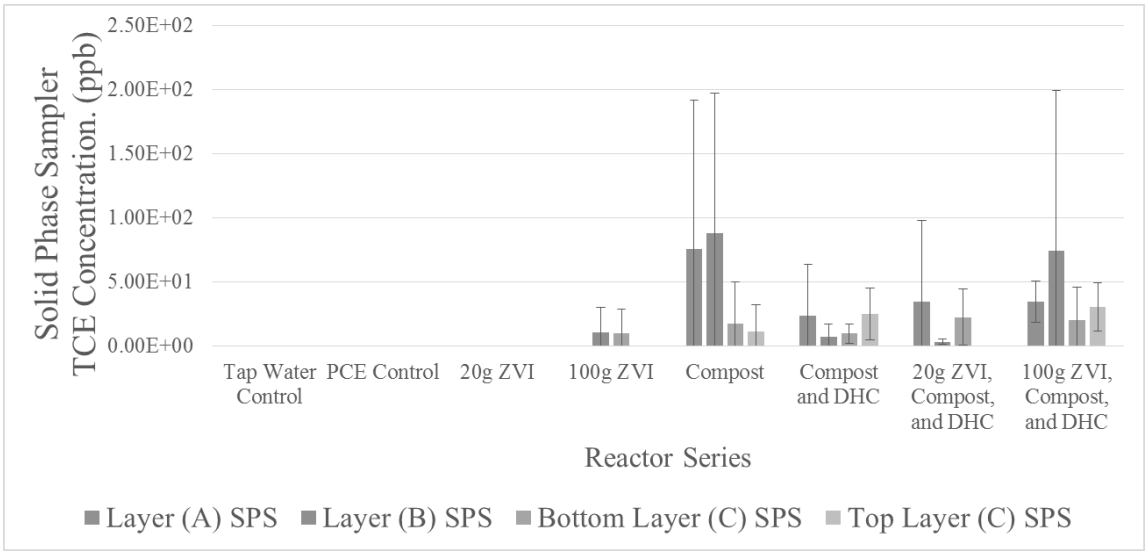

Figure 9 - Reactor Series TCE Concentration in SPSs in Different Layers

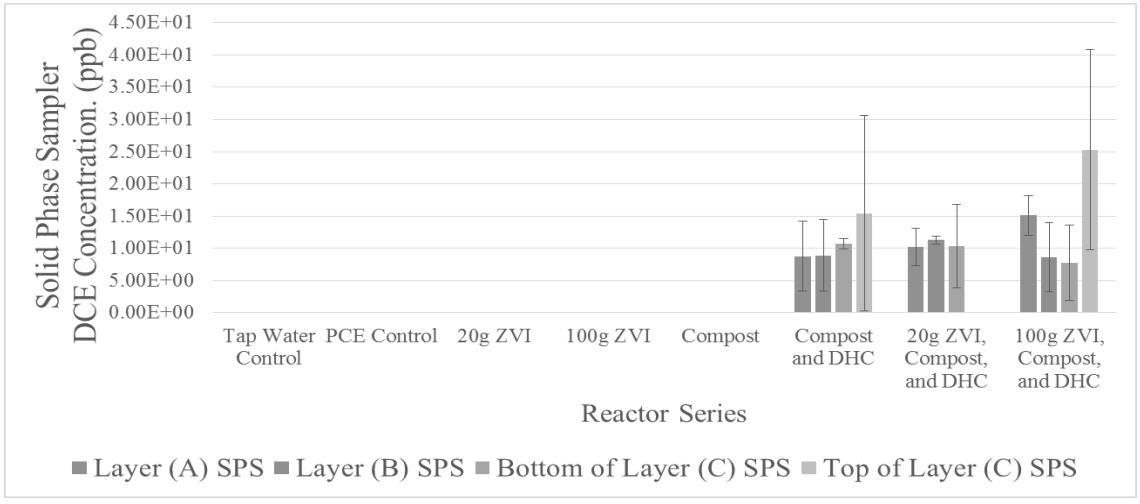

Figure 10 - Reactor Series DCE Concentration in SPSs in Different Layers

SUMMARY

Phytoforensic methods of sampling willows were used to detect chlorinated solvents in the subsurface, and show pollution degradation. Planted willows acted as natural pumps to increase the flow of contaminated water through the reactive zone. Although the flowrate was increased through reactive zones, which reduced the contact time with reactive media, those zones are conservatively designed and are likely still able to fulfill their intended purpose. If the contact time is reduced beyond the conservative measures than an increase in reactive zone width would need to be implemented. Integrated systems of ZVI and DHC were shown to reduce the concentration of PCE in groundwater systems more effectively than either system alone. The degradation was speculated to be primarily due to the DHC in the presence of cis-DCE, which is not a frequent byproduct of PCE reduction due to ZVI. DHC was the most significant individual source of PCE degradation in the multiple tested degradation processes. DHC metabolites of PCE were predominately found in the integrated DHC and ZVI systems, suggesting that DHC reduction of PCE was more abundant than ZVI reduction of PCE. Sampling also indicated potential to differentiate between different degradation processes of ZVI and DHC in this study. Cis-DCE was found in all reactors containing DHC, but never in any reactor without DHC. Shown the chromatographs from a random reactor in this experiment the reactor series degradation mechanism could accurately be determined based on cis-DCE presence. Despite having the highest reduction in PCE concentrations, the DHC and ZVI combined systems were the most detrimental to the willows in terms of

leaf area and ET although these plants survived the stress of added pestilence during the experiment.

The three objectives outlined in the study were satisfied. First, plant sampling was successfully determined to be a viable surrogate for groundwater monitoring of chlorinated solvents; only one plant did not survive the duration of the experiment, despite the additional environmental stress caused by the spider mites and pesticide. Second, metabolite profiles were able to be determined from plant tissue samples indicating the degradation mechanism occurring in the subsurface for this study. Third, a proof of concept was established that promotes integrated *in-situ* degradation methods followed with phytoremediation for enhanced groundwater treatment rates and low cost sampling.

Overall, this study provided clear evidence of an integrated system to concurrently degrade pollutants more thoroughly and to treat groundwater at an elevated volumetric rate due to the active groundwater extraction rate of the trees. This can be projected for sites prior to planting phytoremediation systems by incorporating phytoremediation plots downgradient of *in-situ* remediation technologies. The increase in PCE reduction by integrating degradation mechanisms could reduce the time needed to complete a remediation project. Phytoforensics is a viable tool for detecting groundwater contamination of most chlorinated solvents and can be performed for low cost, minimal environmental impact, and quick sample processing. This study provided fundamental knowledge on metabolite profiles and integrated systems and can be expanded on with different contaminants and *in-situ* degradation mechanisms.

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DISCLOSURE

There were no competing financial interests in this study.

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SECTION

4. INTRO COURSE PHYTOFORENSICS EXPERIMENTAL LEARNING

A pilot study was administered through classroom learning for the purpose of teaching phytoforensics in the introductory course for environmental engineering. Students were able to experimentally determine what the subsurface conditions were in different reactors based on the compounds they found through the harvest and analysis of tree tissue samples. This experiment was initiated a month prior to the classroom learning to allow the plants to reach equilibrium with the contaminated water.

Reactors were designed in 1-liter clear glass jars containing a series of two solid phase samplers (SPSs), three separate layers of media, 1.5 mm ID polytetrafluoroethylene (PTFE) tubing siphons, 250 mL amber glass bottles, and two laurel leaf willow cuttings. The three layers of media were (A) a sand layer at the bottom of the reactor, (B) a reactive layer consisting of sand or ZVI, and (C) a sand layer above the reactive layer. The reactive layer varied on the four reactor series and is documented in Table 4.1. All reactor series had 200 mL of sand in layer (A) and 500 mL of sand in the vadose layer (C). The PCE control reactors, 20 g ZVI reactors, and 100 g ZVI reactors were all made in replicates of seven. Reactors were dosed with a 5 ppm PCE and 5 ppm TCE solution three times weekly for the eight-week duration of the experiment. The six control reactors were dosed with tap water instead of PCE solution. The dosing instructions can be found in Appendix E.

Table 4.1 - Reactor Reactive Layers for Intro Lab Experiment

		Reactive Layer (mL)	
Reactors	*Reactor Series*	*Vol. ZVI*	*Vol. Sand*
1-4, 17, 21, 25	PCE Control	0	170
5-8, 18, 22, 26	20 g ZVI	6	165
9-12, 19, 23, 27	100 g ZVI	28	140
13-16, 20, 24	Tap Water Control	0	170

Quartz silica sand, obtained from the Missouri S&T Concrete Lab, was used in this experiment. H_2Omet 58 ZVI was obtained from RioTinto Metal Powders and had a BET of 0.03-0.04 m^2/g (RioTinto, 2016). A 40 cm PTFE tubing section connected the 1-liter jar with the dosing bottle to create a siphon to transfer water from the dosing bottle to the bottom of the 1-liter jar. The dosing siphon was used to simulate groundwater elevation fluctuations. Willow cuttings were obtained from Schumann Park in Rolla, MO from a well-researched planted phytoplot. The cuttings were trimmed to 30 cm and kept in Hoagland's solution until new leaf and root growth occurred; once root and leaf emergence occurred the cuttings were transplanted into layer (C) of the reactors. SPSs were constructed out of polydimethylsiloxane (PDMS) and cut into 1 cm pieces; the segments were connected with galvanized steel wire for removal of SPSs without reactor destruction. One SPS was below the reactive layer in layer (A) and the other above the reactive layer in layer (C). A diagram of the reactor setup can be found in Figure 4.1.

Four weeks into the experiment the reactors were used for the classroom experiment. Reactor jars were covered in aluminum foil to hide the visible reactive layer and contaminant information from the students. The students harvested and analyzed tree samples from 20 reactors at random. Letters were randomly assigned as the reactor names

in order to anonymously keep track of what reactor students were harvesting. The remaining trees were harvested and analyzed after eight weeks to add resolution to the data.

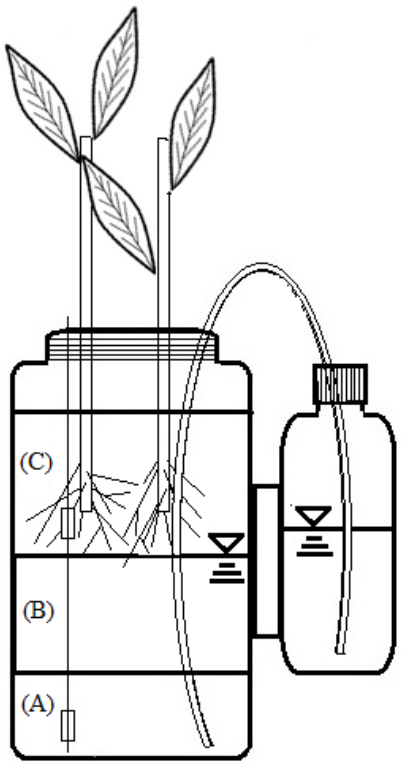


Figure 4.1- Reactor Configuration for Intro Class Learning

Leaf area of the willow cuttings was taken at harvest and quantified using Easy Leaf Area software (Easlon and Bloom, 2014). The Easy Leaf Area settings can be found in Appendix G. Tree tissue samples were the bottom 5 cm of the above-ground portion of tree; tree samples were taken in duplicate by quartering the sample vertically, each

sample remaining 5 cm long, and opposite corners were placed together in 20 mL MicroLiter screw top headspace vials. The SPS series was removed altogether, rinsed with distilled water to remove ZVI particles, disassembled, and individual SPSs were placed in separate headspace vials. The tree samples and SPSs were analyzed for chlorinated solvent concentrations using a 7890 Agilent Gas Chromatograph (GC) equipped with a VOCOL® capillary GC column and electron capture detector (μECD) and 85-μm Polyacrylate (PA) SPME fiber (Limmer, 2011). The PA fiber extraction of the headspace was 5 minutes, with a time desorption of 3 minutes at 230°C in the μECD inlet. The oven temperature started at 40°C for 0.75 min then had a ramp of 20°C/min from 40°C to 160°C, resulting in a 6.750-minute run time. The full GC method can be found in Appendix F.

The classroom experiment provided insight into subsurface reactions with chlorinated solvents and ZVI. A reduction of PCE and TCE was seen in both the 20 g ZVI and 100 g ZVI reactor series, with a greater reduction in the 100 g ZVI reactor. The classroom experiment observed higher concentrations of PCE and TCE in the tap water control. This may have been due to cross contamination between groups or improper labeling of vials leading to samples falsely being labelled as controls. The tap water controls done after the classroom experiment did not have as high concentrations of chlorinated solvents. A comparison between the classroom experiment and the full harvest four weeks later can be found in Figure 4.2, Figure 4.3, Figure 4.4, and Figure 4.5 with 90% confidence intervals. Figures 4.2 and 4.3 show PCE and TCE concentration in the tap water control reactor series; this is likely due to mislabeling of vials in the classroom experiment, because the tap water control reactor series had no detection of

PCE or TCE in the harvest performed four weeks after the classroom learning, as shown in Figures 4.4 and 4.5.

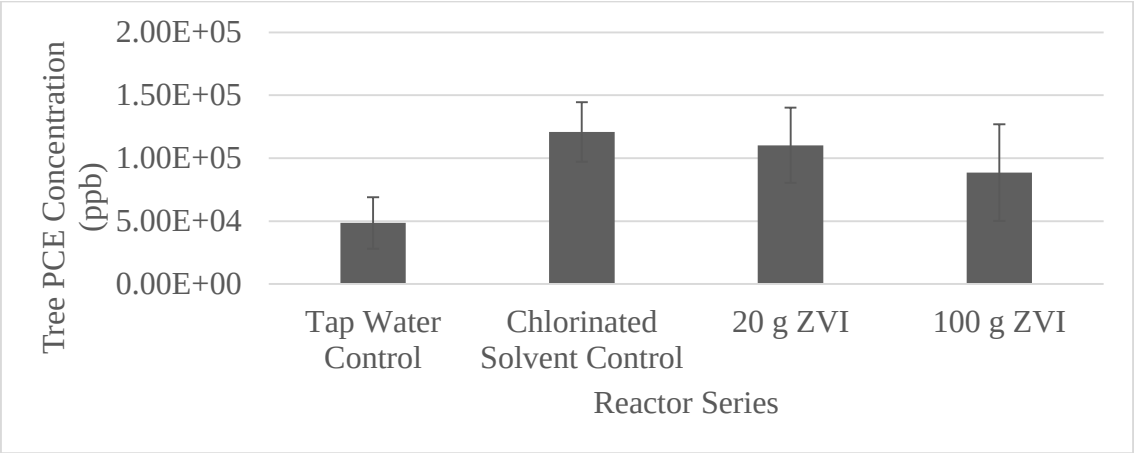


Figure 4.2 - Tree Concentrations of PCE in Intro Course Lab Experiment

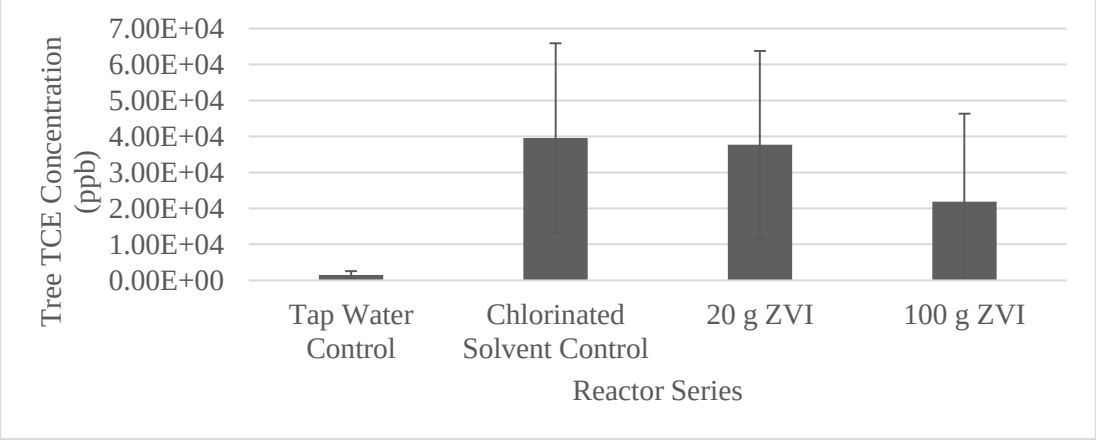


Figure 4.3 - Tree Concentrations of TCE in Intro Course Lab Experiment

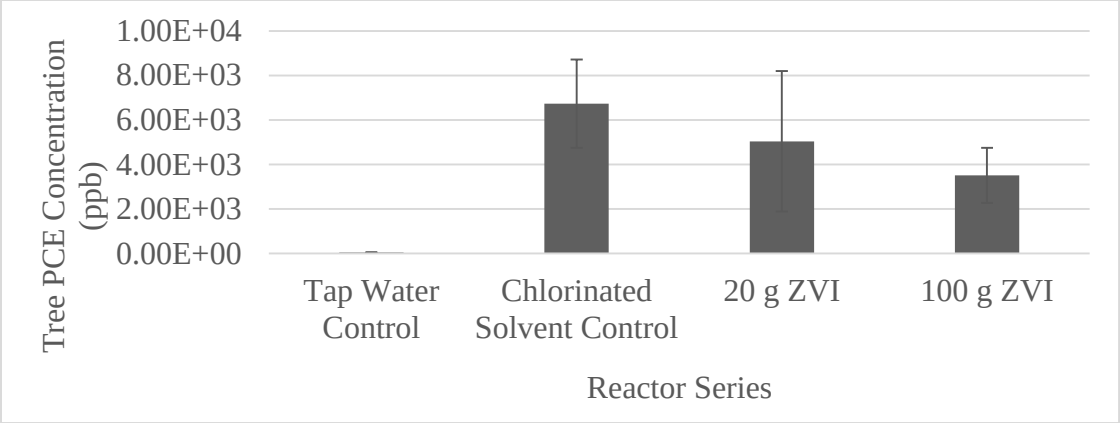


Figure 4.4 - Tree Concentrations of PCE Taken at End of Experiment

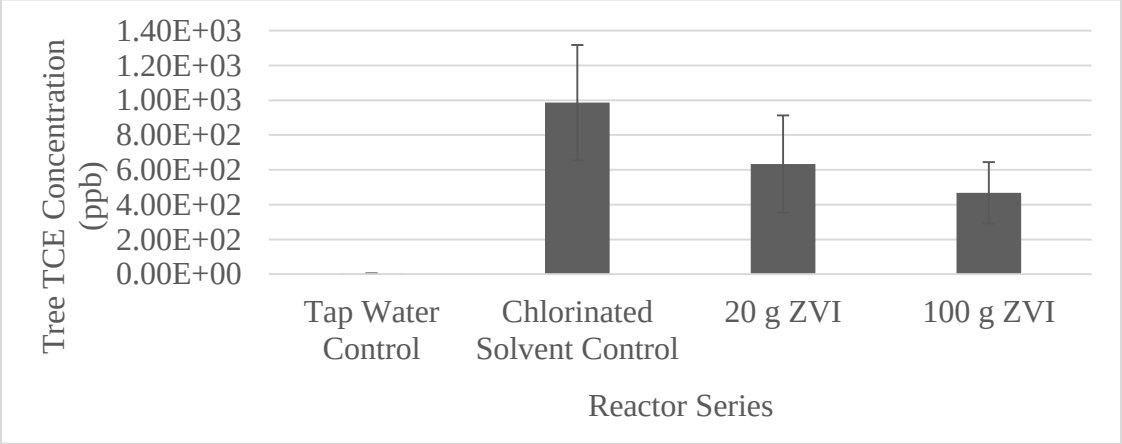


Figure 4.5 - Tree Concentrations of TCE Taken at End of Experiment

All reactors showed the same general reduction. PCE and TCE were both reduced in concentration to a greater degree in the 100 g ZVI reactors compared to the 20 g ZVI and control reactors. The SPSs provided similar information as the trees. The reactors

with more ZVI had greater reduction of PCE and TCE concentration. The concentration of PCE and TCE in the SPSs of each reactor series, with a 90% confidence interval, can be found in Figure 4.6 and Figure 4.7, respectively.

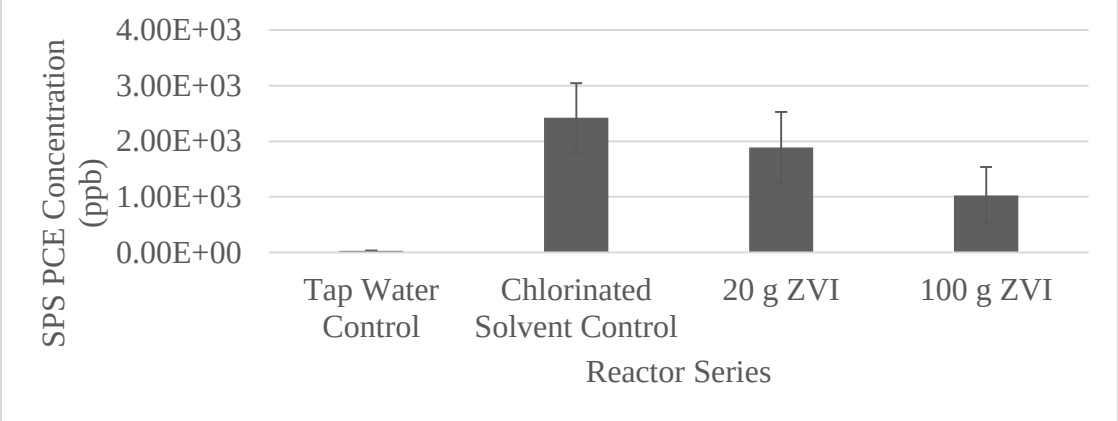


Figure 4.6 - SPS Concentrations of PCE in Intro Course Experiment

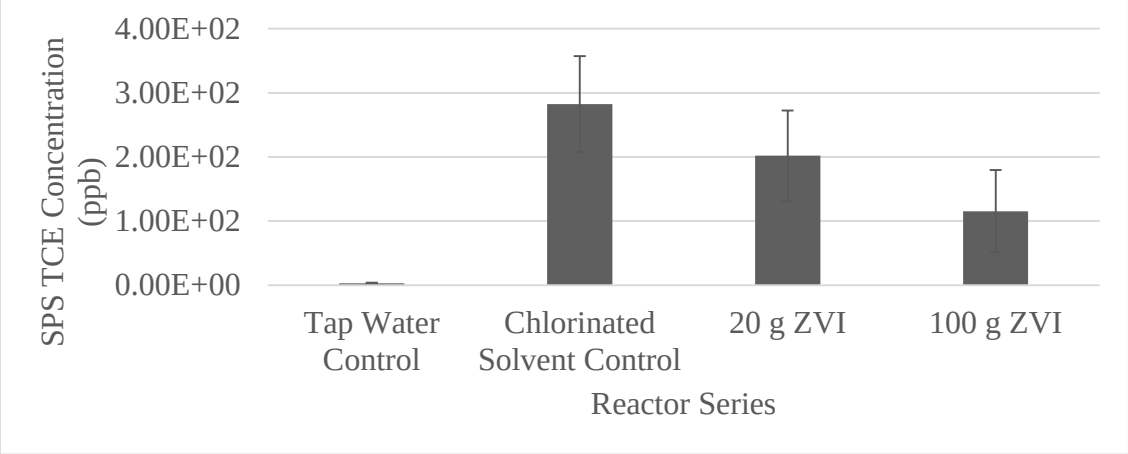


Figure 4.7 - SPS Concentrations of TCE in Intro Course Experiment

The leaf area provided information on the health of the trees at the end of the experiment. The leaf area of the reactor series were not significantly different from one another. Reactors from each reactor series survived and had a greater leaf area index at the end of the experiment than at the beginning, despite the toxicity from the iron and chlorinated solvents. Only two trees were lost during the experiment; one from the chlorinated solvent control series and the other from the 20 g ZVI series. A figure of the leaf area of each reactor series can be seen in Figure 4.8.

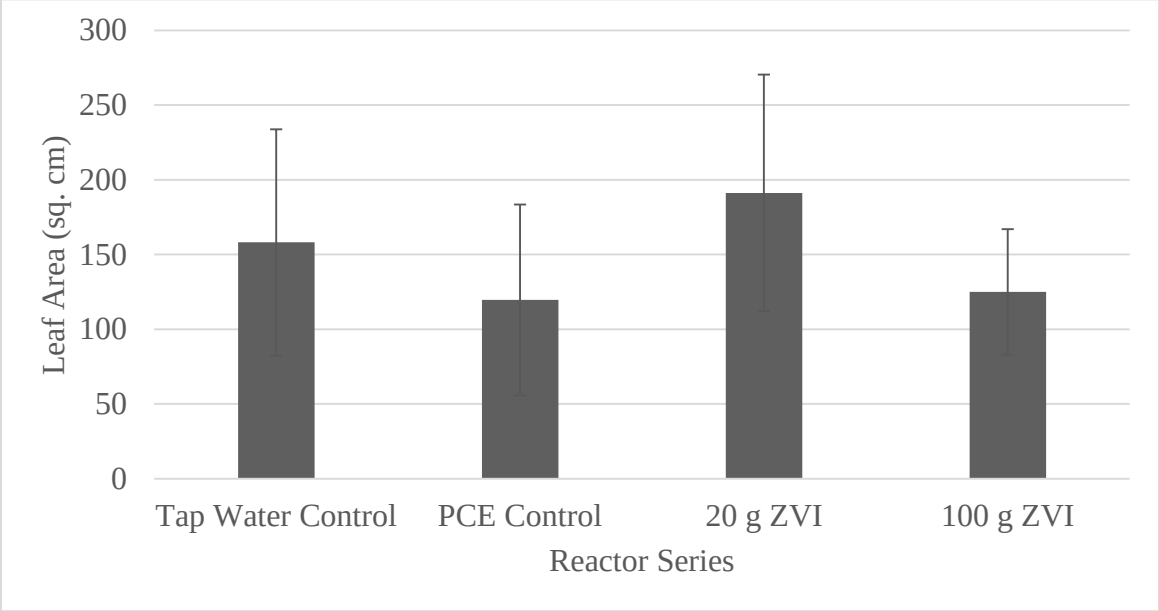


Figure 4.8 - Leaf Area from Intro Course Experiment

A reduction of PCE concentration in the subsurface was observed in the reactor SPSs. The greatest reduction in PCE and TCE concentrations were observed in the 100 g ZVI reactors, while the chlorinated solvent controls had the highest PCE and TCE

concentrations found in the SPSs. There was no significant difference in the health of the plants by the end of the experiment. The integration of phytoremediation and ZVI is a viable solution for environmental remediation.

5. CONCLUSIONS

The evidence of using above ground vegetation sampling for assessing specific contaminant degradation in the subsurface is a promising tool for future site assessments and monitoring contaminant plumes. In the both experiments, degradation profiles were shown for different degradation processes in the subsurface. Sampling of plants can be applied to observe natural degradation that may be ongoing in the subsurface. Plant sampling can project long term outcomes of monitored natural attenuation with greater spatial resolution and at much lower cost than traditional, invasive groundwater monitoring and assessment methods.

Leaf area, ET, and soil pH were all taken into account for the viability of using plants as surrogates. Plant sampling was successfully determined to be a viable surrogate for groundwater monitoring of chlorinated solvents. Only one plant did not survive the duration of the experiment, despite the additional environmental stress caused by the spider mites and pesticide. The high pH and low leaf area indicated higher stress on the reactors containing DHC, but they were still able to survive the conditions. Without the impact from the spider mite infestation and pesticide treatment, the one reactor that was lost may have survived.

Plants were shown to increase the evapotranspiration in reactors. The increase in water transport into trees also increases the flowrate of contaminated water through the *in-situ* reactive zones in reactors. The lower water potential created by the water uptake by plants has led to an increase in the groundwater treatment rates.

Plant sampling can also provide insight to which degradation mechanisms are taking place in the obscured subsurface. The presence of DHC resulted in a different

metabolite profile from the ZVI alone in PCE reduction; cis-DCE was never detected in reactors containing ZVI alone. Using this technology, a profile of which degradation mechanisms in a system are contributing to the groundwater treatment can be developed. The application of phytoscreening for long term monitoring of remedial technologies is likely to become a useful tool for quickly mapping changes in contaminant concentrations downgradient of reactive zones.

A proof of concept was established that promotes integrated *in-situ* degradation methods followed with phytoremediation for enhanced groundwater treatment rates and low cost sampling. This study has outlined a methodology that can be adapted to provide more insight to plant-contaminant interactions, and to be implemented in full site remediation projects. This proof of concept can also be applied to different contaminants and *in-situ* degradation mechanisms.

Overall, this study provided clear evidence of an integrated system to concurrently degrade pollutants more thoroughly and to treat groundwater at an elevated volumetric rate due to the active groundwater extraction rate of the trees. This can be projected for sites prior to planting phytoremediation systems by incorporating phytoremediation plots downgradient of *in-situ* remediation technologies. Phytoforensics is a viable tool for detecting groundwater contamination of most chlorinated solvents and can be performed for low cost, minimal environmental impact, and quick sample processing. This study provided fundamental knowledge on metabolite profiles and integrated systems and can be expanded on with different contaminants and *in-situ* degradation mechanisms.

6. RECOMMENDATIONS FOR FUTURE WORK

One further area of future work is to look at other remedial technologies in conjunction with phytoremediation. PRBs and bioremediation were used because they were cheap, easy, and passive. Other technologies, such as air sparging or pump and treat are important to examine due to the wide application in many fields despite the large associated cost.

Investigation of directional uptake by trees in conjunction with PRBs or bioremediation would be important to more fully understand. This study has shown that ZVI and DHC directly beneath the tree will provide degradation profiles in tree samples, but PRBs are not often incorporated under trees in the field. If a relationship between PRBs location and tree contaminant profiles exists, phytoforensics could more accurately describe subsurface conditions.

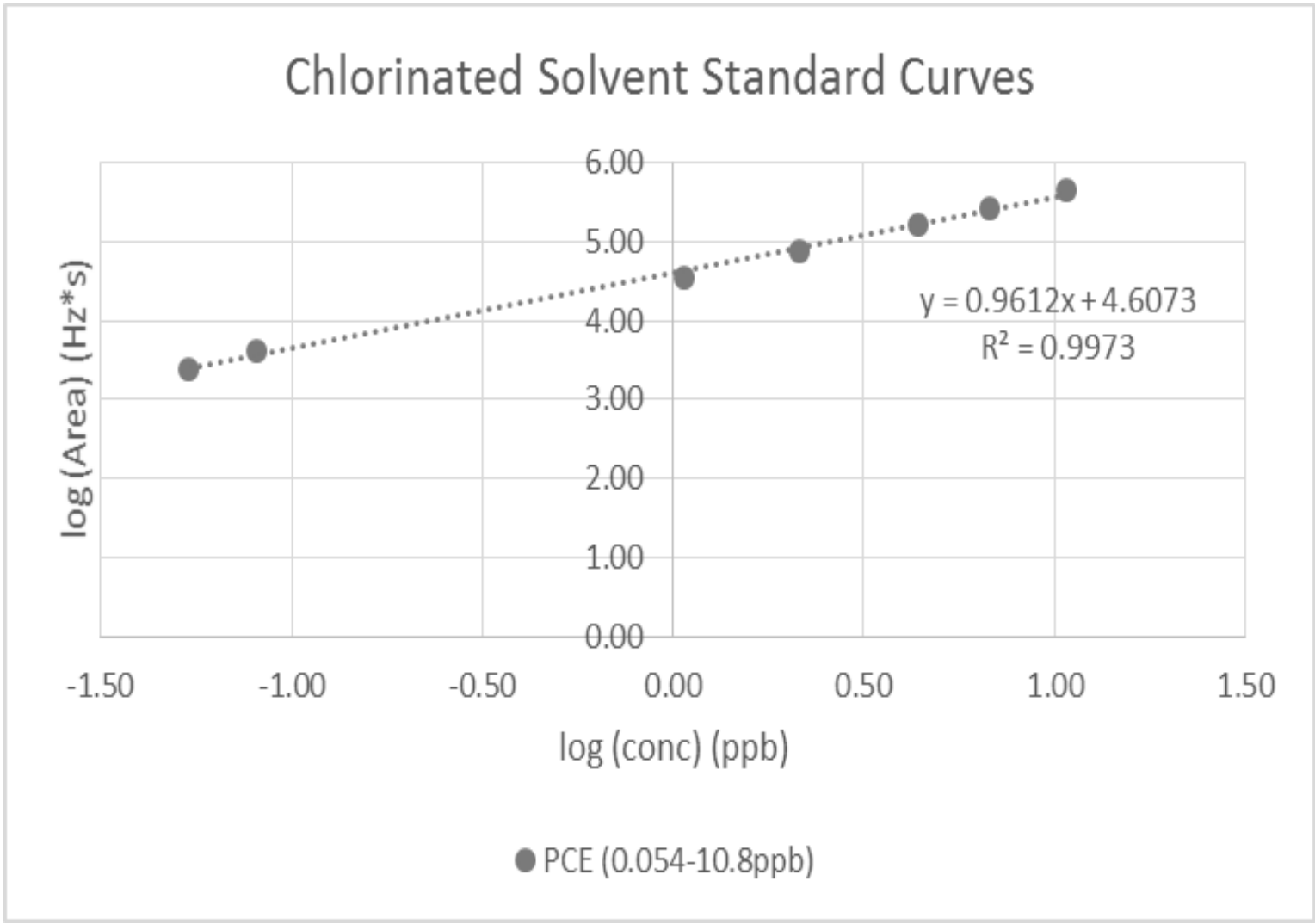
The SPSs in the reactors were mass transfer limited; circulation of water in the reactors laterally could allow SPSs in the reactors to reach equilibrium with a larger sample volume. Representative SPSs for different depths would be beneficial to produce a vertical contaminant profile in the reactor. A different reactor design may be needed to allow for more circulation in the subsurface.

A pilot scale experiment, which allowed for tree core sampling, would be useful for this concept. Destructive tree sampling only provides information at the end of the experiment. Producing a concentration over time profile of the relationship between the trees and the various remedial technologies would be beneficial to track the changes in reactivity of the *in-situ* reactive zones.

Further incorporation of phytoforensics into classroom learning would be advantageous to students. Phytoforensics incorporates multiple classroom learning objectives such as mass transfer, partitioning, volatilization, groundwater quality, and organic chemistry. An entire course should be developed around a lab that explores each of the above learning objectives. One possible suggestion would be to incorporate a lab into the phytoremediation course that covers many aspects of different phytotechnologies.

APPENDIX A.

STANDARD CURVES


Chlorinated Solvent Standard Curves
6.00
5.00
4.00
3.00
2.00
1.00
0.00
log (Area) (Hz*s)
y = 0.9612x + 4.6073
R² = 0.9973
-1.50
-1.00
-0.50
0.00
0.50
1.00
1.50
log (conc) (ppb)
PCE (0.054-10.8ppb)

APPENDIX B.

STATISTICS FOR CONCENTRATION CONFIDENCE INTERVALS

Statistics for PCE in SPS.A for Reactor Series "Compost and DHC"

$n := 4$ Degree of Freedom

$ppb := 1 \cdot 10^{-6} \frac{gm}{L}$ Conversion Factor

$SPS_{21A} := 503.588 ppb$

$SPS_{22A} := 780.499 ppb$

$SPS_{23A} := 838.233 ppb$

$SPS_{24A} := 261.855 ppb$

Measured concentration of PCE in SPS.A of reactors 21-24

$Avg_A := \frac{SPS_{21A} + SPS_{22A} + SPS_{23A} + SPS_{24A}}{4} = 596.038 ppb$

Average PCE concentration in SPS.A for reactors 21-24

$Dev_{21A} := (SPS_{21A} - Avg_A)^2 = 8.548 \times 10^3 \cdot ppb^2$

$Dev_{22A} := (SPS_{22A} - Avg_A)^2 = 3.402 \times 10^4 \cdot ppb^2$

$Dev_{23A} := (SPS_{23A} - Avg_A)^2 = 5.866 \times 10^4 \cdot ppb^2$

$Dev_{24A} := (SPS_{24A} - Avg_A)^2 = 1.117 \times 10^5 \cdot ppb^2$

Deviation of the concentration of PCE from SPS.A in reactors 21-24 from the average

$Var_A := \frac{Dev_{21A} + Dev_{22A} + Dev_{23A} + Dev_{24A}}{n - 1} = 7.097 \times 10^4 \cdot ppb^2$

Variance of above deviations

$SDev_A := Var_A^{.5} = 266.402 ppb$

Standard Deviation of PCE in SPS.A in Reactors 21-24

$SErr_A := 2.132 \frac{SDev_A}{n^{0.5}} = 283.984 ppb$

T-Distribution for Standard Error of PCE in SPS.A in Reactors 21-24

$U95\%_A := Avg_A + SErr_A = 880 \cdot ppb$

Upper 90% CI

$L95\%_A := Avg_A - SErr_A = 312 \cdot ppb$

Lower 90% CI

The concentration of PCE in SPS.A is equal to 596 +/- 284 ppb

APPENDIX C.

DEHALOGENATING CULTURE INFORMATION

A serum bottle containing DHC was received from Terra Systems, Inc. Email correspondence was kept between the Vice President of Research and Development from Terra Systems, Inc. The serum bottle contained $4.1x10^{11}$ cells/L of DHC/L (Lee, 2015). The sample was kept at 2 °C, within the approved temperature range of -4 and 2 °C. The DHC stock solution was given an acetate and hydrogen addition after two weeks, and again after four weeks following the procedure in Loffler, et. al (2005). The stock solution was cultured and showed PCE degradation in a batch scale experiment. Unfortunately, and accidental exposure to air after three weeks resulted in the loss of the culture.

During week four, before the second addition of hydrogen and acetate, 2 mL of the stock solution was added to the reactor series that incorporated bioremediation as a method of degradation. This solution was added to the reactors from a dilution of 36 mL DHC stock in 900 mL of deoxygenated water. The water was deoxygenated by bubbling nitrogen gas through distilled water for thirty minutes, and dissolved oxygen was measured using an YSI Model 68 dissolved oxygen probe. 50 mL of the diluted DHC solution was added to reactors 21 through 32 by direct injection into the reactive media layer. On subsequent dosing occurrences a 5 mL/L molasses in distilled water solution was deoxygenated by nitrogen bubbling for thirty minutes before adding PCE. After adding PCE to the solution the solution was mixed and added to the anaerobic reactors. The dosing bottle lids were opened just enough to break the air seal and the solution was added through a sixteen-gauge needle used to maintain the dosing siphon to the reactor. The dosing bottle lid was sealed just before the end of the solution addition so that the last portion of the addition also acted to prime the siphon.

Michael D. Lee, Ph.D. <m_d_lee@msn.com> 11/17/15

to Joel, me

Tommy and Joel:

I am shipping the TSI-DC culture out today for delivery on Thursday, Nov. 19 by Federal Express tracking number 774991897266.

The *Dehalococcoides* (DHC) culture is grown under anaerobic conditions on PCE and lactate. The culture contains 4E11 DHC/L. For a laboratory study, we would typically add 1 mL of culture per L of groundwater which would provide approximately 4E8 DHC/L. This volume of culture would be sufficient to inoculate at least 100 L of water. The culture is sensitive to oxygen and I not sure how well it will do in an open pot with plants growing in it. You would want to dose the water in the pot with a substrate like sodium lactate (recommended dosage of 1,000 mg/L TOC or about 3.9 mL 60% sodium lactate/L of water). Other substrates can be used such as an emulsified vegetable oil product like Terra Systems SRS, but may complicate your experimental design and analyses. Let me know if you have any questions.

Michael D. Lee, Ph.D.
Vice President Research and Development
Terra Systems, Inc.
130 Hickman Road Suite 1
Claymont DE 19703
P 302-798-9553
F 302-798-9554
Email mlee@terrasystems.net
Web www.terrasystems.net

Email certification of the DHC culture used in this study (USEPA, 1997).

>1x10[11] *Dehalococcoides* cells/L

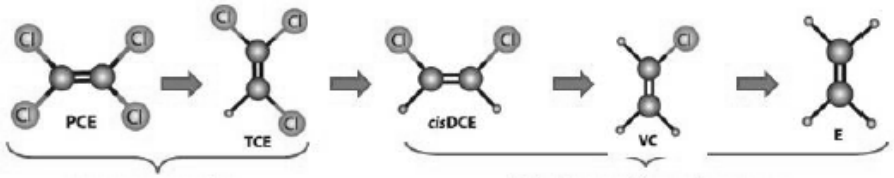
Terra Systems INCORPORATED

TSI DC *Dehalococcoides mccartyii* Bioaugmentation Culture®

TSI DC *Dehalococcoides mccartyii* Bioaugmentation Culture® is an enriched natural bacteria culture that contains *Dehalococcoides mccartyi* (formerly *ethenogenes*) for bioaugmentation. This culture dechlorinates tetrachloroethene (PCE) and trichloroethene (TCE) to the non-toxic products ethene or ethane. The culture also biodegrades 1,1,1-trichloroethane to 1,1-dichloroethene, 1,1-dichloroethane, and chloroethane. It also can biodegrade carbon tetrachloride and chloroform to methylene chloride and innocuous products. It can be used at sites where bacteria capable of complete reductive dechlorination are not present, are present at low numbers, or there is a need to decrease the remediation time frame. It is estimated that *Dehalococcoides* are not present in 10 to 40 percent of chlorinated solvent contaminated sites.

Key Benefits of TSI DC *Dehalococcoides mccartyi* Bioaugmentation Culture®

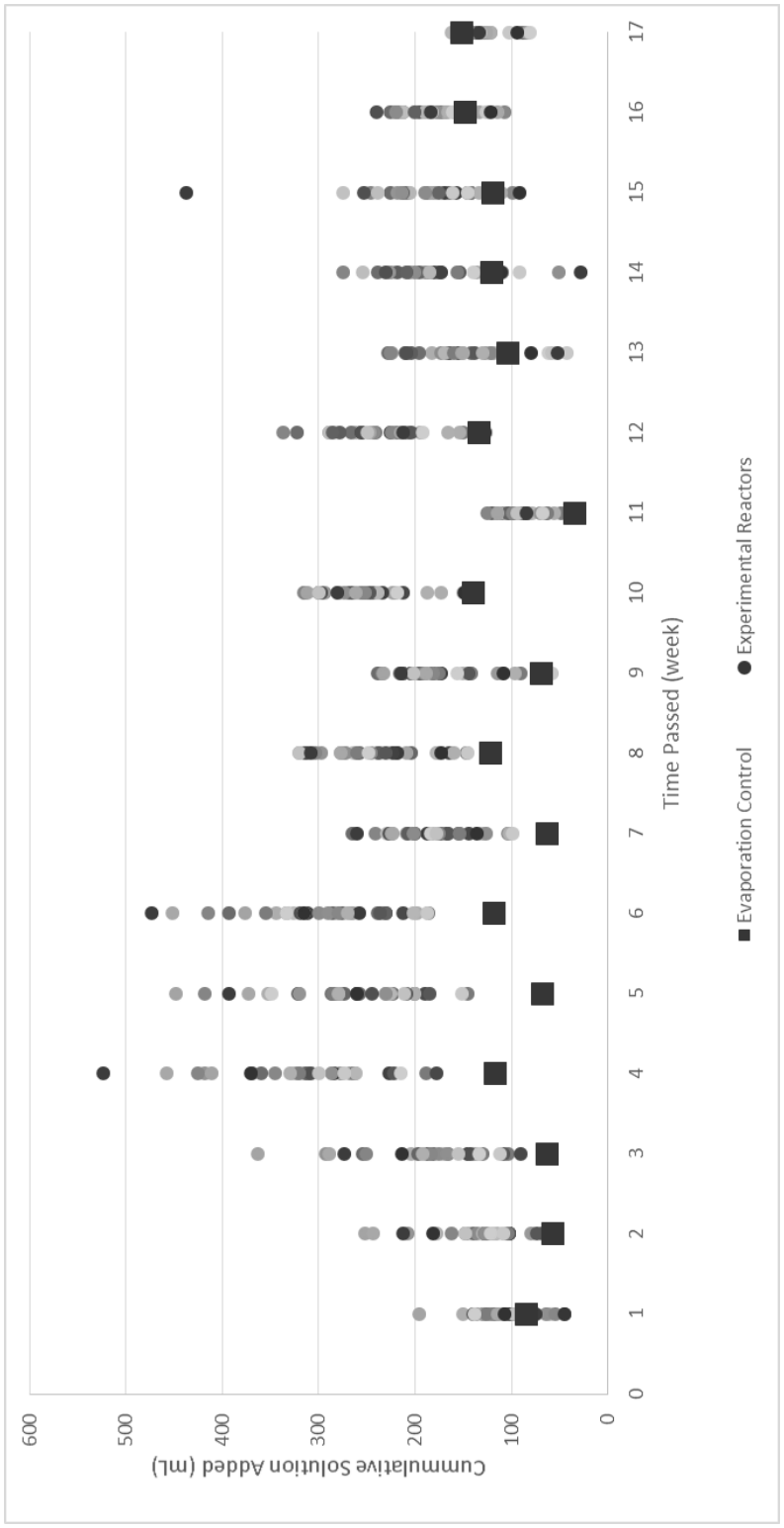
There is a growing body of laboratory and field data demonstrating that the *Dehalococcoides* group of microorganisms are solely responsible for the complete dechlorination of PCE and TCE to ethene. At sites where *Dehalococcoides* microorganisms are not present or are found at low numbers, the process will often "stall" at cis-1,2-dichloroethene. The TSI-DC® Bioaugmentation Culture will promote the complete dechlorination of PCE or TCE. The TSI-DC® Bioaugmentation Culture contains greater than **1 x 10[11]** *Dehalococcoides*/L.



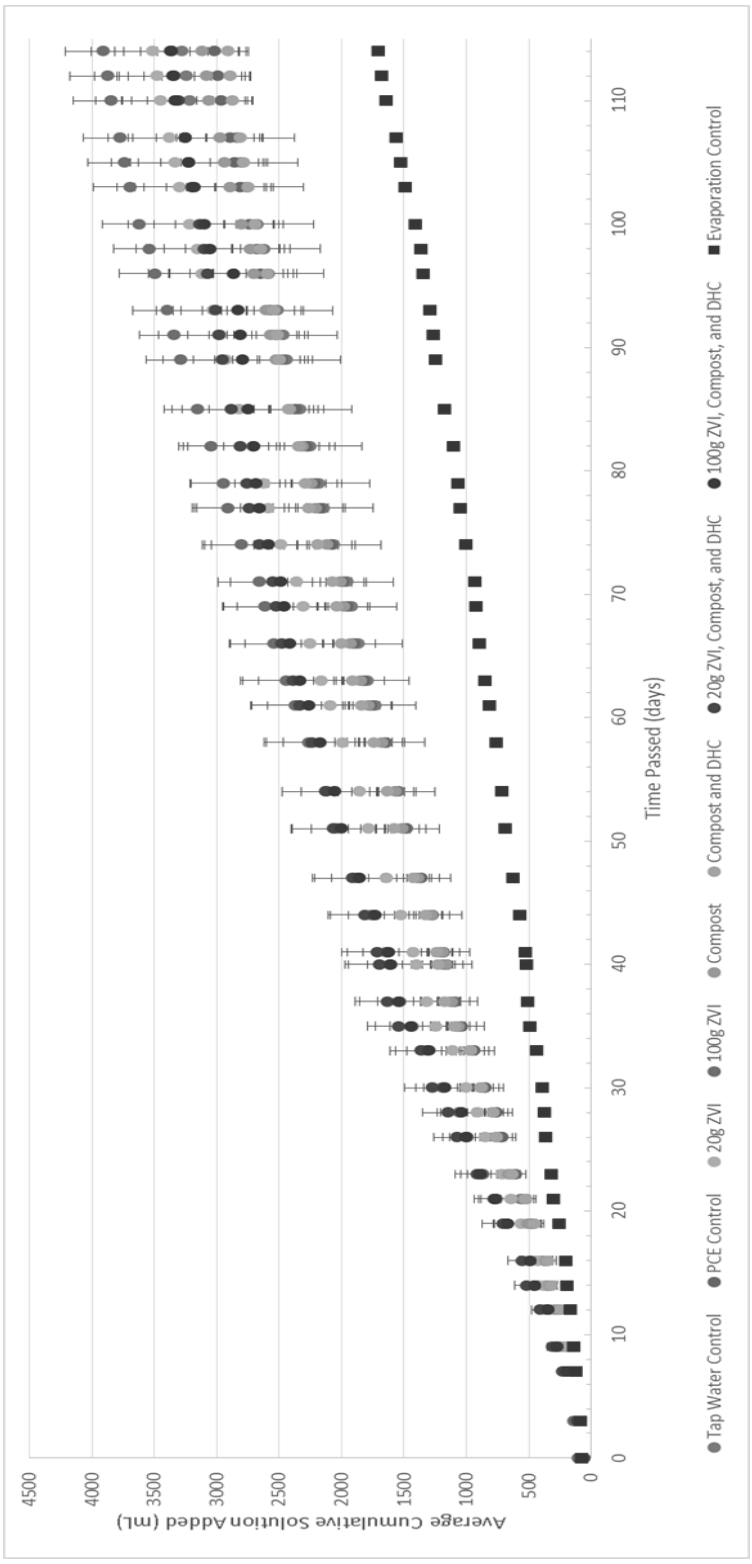
130 Hickman Road, Suite 1
Claymont Delaware 19703
Phone: 302-798-9553
Email: mfree@terrasystems.net
On the Web: www.terrasystems.net

APPENDIX D.

REACTOR SOLUTION UPTAKE RATES



Weekly Evapotranspiration Volumes for All Reactors



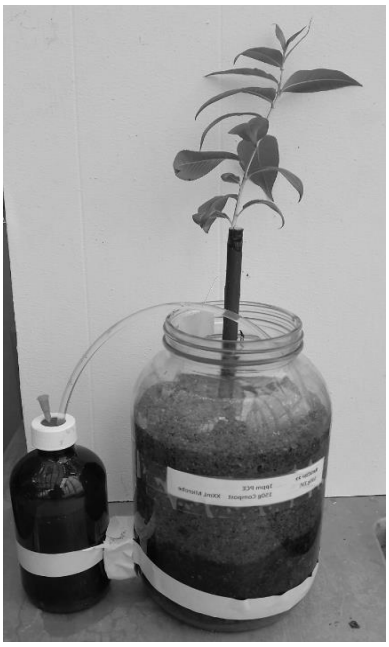
Cumulative Evapotranspiration Volumes for All Reactors with 90% CI

APPENDIX E.

EXPERIMENTAL PHOTOS

Inspecting Reactors during Spider Mite Infestation

PCE Control Reactor on Day 1



100 g ZVI, Compost, and DHC Reactor on Day 1

Increased Venation Stress in 100 g ZVI Reactors
for Introductory Course Experiment

Challenge Reactor for Introductory Course Experiment

APPENDIX F.

DOSING PROCEDURES

Dosing Instructions for All Reactor Series

Supplies:

2L Jar for Molasses Solution with cap

Molasses

50mL beaker for measuring molasses

1L Erlenmeyer flask with Saturated PCE (under fume hood)

Paper to record solution added per reactor

50mL "clean" syringe for dosing tap water to control reactors

50mL syringe for dosing chlorinated solution to test reactors

10mL manual propipetter for sat. PCE

1L brown bottle with Teflon coated cap

400mL beaker for tap water

Procedure:

Deoxygenate the Molasses Solution

- Add 10mL of molasses to the 2L jar using the 50mL beaker to measure volume.
- Fill the remainder of the 2L jar with distilled water up nearly to the bottle neck.
- Deoxygenate the molasses solution using nitrogen gas bubble stone for at least 30 minutes; while solution is deoxygenating proceed to dose the aerobic reactors in the greenhouse (Tap water control, PCE control, 20 g ZVI, 100 g ZVI, and Compost reactor series).

Tap Water Control Reactors (including evaporation control)

- Fill the 400mL clean beaker with tap water to dose the control reactors.
- Add X mL into each control reactor of clean tap water using the clean 50 mL syringe until the water level is at the shoulder in the dosing bottle of the test reactor.
- Record the volume of mixture that was added to each of the test reactors on the form.
- Prime the control reactors with the 50 mL "clean" syringe.
- Excess water after filling the 5 control reactors can be dumped down the drain.

PCE Control, 20 g ZVI, 100 g ZVI, and Compost Reactors (aerobic)

- Fill the 1L brown bottle with 993.3 mL of tap water; Approximate at near bottom of the bottle neck.
- Using the 10mL propipetter, add 6.7mL sat. PCE into the 1L brown bottle. Be sure not to obtain the free product PCE at the bottom of the 1L Erlenmeyer flask.
- Cap the sat. PCE Erlenmeyer flask immediately.
- Cap the brown bottle and shake to mix the chemicals into the water; ~60 seconds.
- Add X mL into each test reactor of dosing solution using the chlorinated 50 mL syringe until the water level is at the shoulder in the 250 mL dosing bottle of the test reactor.
- Record the volume of mixture that was added to each of the test reactors on the form.
- Prime the control reactors with the 50 mL chlorinated syringe.

Reactor Series that contain DHC (anaerobic)

- Return to the molasses solution and remove the bubble stone from the jar before turning off the nitrogen stream.
- Turn off the nitrogen stream and cap the 2L anaerobic molasses solution.
- Using the 10mL propipetter, add 13.4mL sat. PCE into the 2L molasses solution. Be sure not to obtain the free product PCE at the bottom of the 1L Erlenmeyer flask.
- Cap the sat. PCE Erlenmeyer flask immediately.
- Cap the 2L jar and shake to mix the chemicals into the water; ~60 seconds.
- Add X mL into each test reactor of dosing solution using the chlorinated 50 mL syringe until the water level is at the shoulder in the 250 mL dosing bottle of the test reactor.
- Record the volume of mixture that was added to each of the test reactors on the form.
- Prime the control reactors with the 50 mL chlorinated syringe.

Once finished, clean out the equipment and return them to where they belong.

APPENDIX G.

GAS CHROMOTAGRAPHY METHOD INFORMATION

Method Information

Method:

C:\CHEM32\1\METHODS\BURKEN\LIMMER_SPME_ECD_PA_SPLIT.M

Modified: 4/13/2014 at 2:48:07 PM

This is a SPME-ECD method for PCE, TCE, DCE detection

Run Time Checklist

Pre-Run Cmd/Macro:	off
Data Acquisition:	on
Standard Data Analysis:	on
Customized Data Analysis:	off
Save GLP Data:	of
Post-Run Cmd/Macro:	off
Save Method with Data:	on

Injection Source and Location

Injection Source: Manual

Injection Location: Back

==========

Agilent Agilent 7890A

==========

Oven

Equilibration Time	1 min
Oven Program	On

40 °C for 0.75 min then 20 °C/min to 160 °C for 0 min

Run Time	6.75 min

Front SS Inlet N2

Excluded from Affecting GC's Readiness State

Mode	Splitless
Heater	Off
Pressure	On 8.5123 psi
Total Flow	On 52 mL/min
Septum Purge Flow	Off
Gas Saver	Off
Purge Flow to Split Vent	50 mL/min at 2 min

Back SS Inlet N2

Mode	Split
Heater	On 280 °C
Pressure	On 9.4603 psi
Total Flow	On 54 mL/min

Septum Purge Flow	On 3 mL/min
Gas Saver	Off
Split Ratio	50 :1
Split Flow	50 mL/min

Column #1

HP-5 5% Phenyl Methyl Siloxan: 530.62969

HP-5 5% Phenyl Methyl Siloxan

325 °C: 30 m x 320 µm x 0.25 µm

In: Front SS Inlet N2

Out: Front Detector FID

(Initial)	40 °C
Pressure	8.5123 psi
Flow	2 mL/min
Average Velocity	33.302 cm/sec
Holdup Time	1.5014 min
Flow Program	On
2 mL/min for 0 min	
Run Time	6.75 min

Column #2

10mx0.20ID, 1.2um10mx0.20ID, 1.2um

325 °C: 10 m x 200 µm x 1.2 µm

In: Back SS Inlet N2

Out: Back Detector µECD

(Initial)	40 °C
Pressure	9.4603 psi
Flow	1 mL/min
Average Velocity	42.346 cm/sec
Holdup Time	0.39358 min
Flow Program	On
1 mL/min for 0 min	
Run Time	6.75 min

Front Detector FID

Excluded from Affecting GC's Readiness State

Heater	Off
H2 Flow	Off
Air Flow	Off
Makeup Flow	Off
Const Col + Makeup	Off
Flame	Off
Electrometer	Off

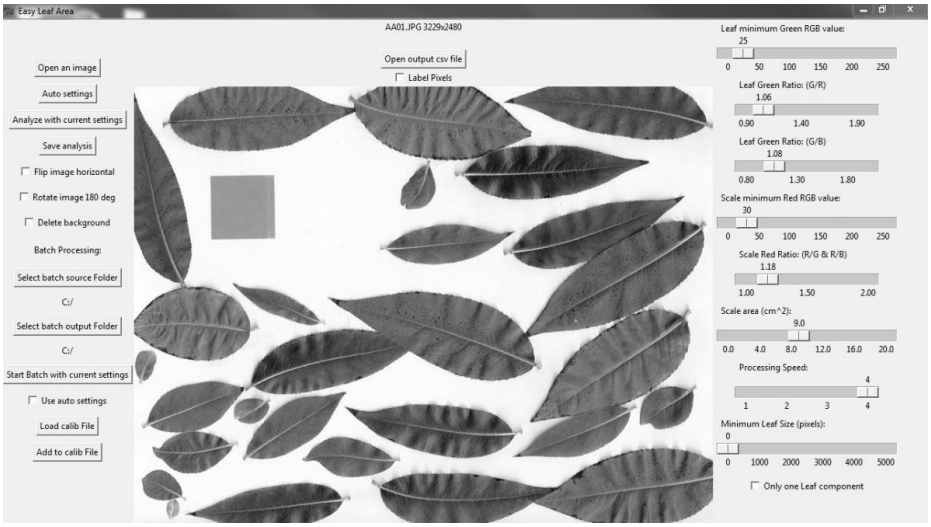
Back Detector μECD		
Heater	On	250 °C
Anode Flow	Off	
Makeup Flow	On	36.5 mL/min
Const Col + Makeup	On	0 mL/min
Electrometer	Off	
Signals		
Back Signal	Save On	
	20 Hz	

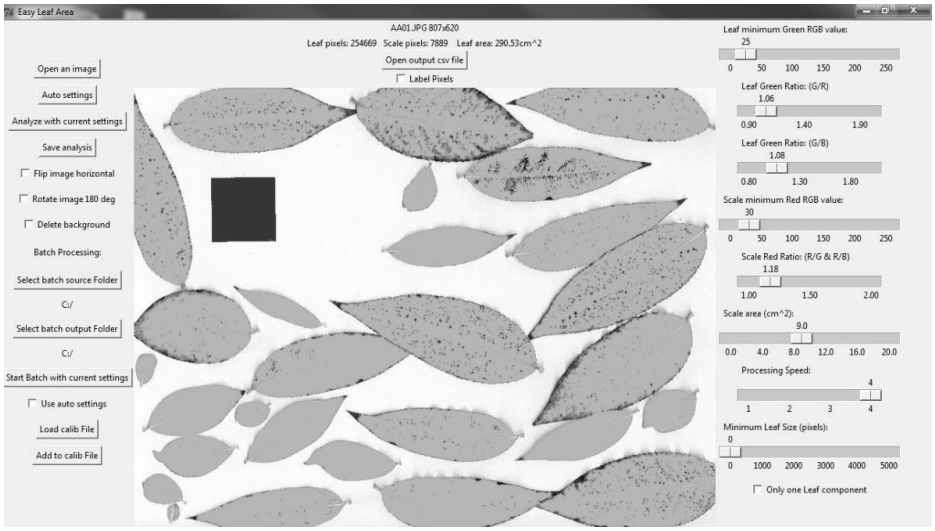
APPENDIX H.

EASY LEAF AREA SOFTWARE METHOD INFORMATION

Instructions:

Remove leaves from plant and arrange on scanner so that when you place the paper with the red square on the scanner the red square is not covered up. Scan in the image in as at least a 300 dpi color jpg file. Open the image in Easy Leaf area at the top left of the options, and adjust the settings to where they match the settings in the following image. If using a new red square paper be sure to adjust the scale area options. When ready click “analyze with current settings” and wait for it to output the leaf area. Images of the leaf area settings are shown in the following images.


Easy Leaf Area
AA01.JPG 3229x2480
Open an image
Auto settings
Analyze with current settings
Save analysis
Flip image horizontal
Rotate image 180 deg
Delete background
Batch Processing:
Select batch source Folder
C:/
Select batch output Folder
C:/
Start Batch with current settings
Use auto settings
Load calib File
Add to calib File
Open output csv file
Label Pixels
Leaf minimum Green RGB value:
25
0 50 100 150 200 250
Leaf Green Ratio: (G/R)
1.06
0.90 1.40 1.90
Leaf Green Ratio: (G/B)
1.08
0.80 1.30 1.80
Scale minimum Red RGB value:
30
0 50 100 150 200 250
Scale Red Ratio: (R/G & R/B)
1.18
1.00 1.50 2.00
Scale area (cm^2):
9.0
0.0 4.0 8.0 12.0 16.0 20.0
Processing Speed:
4
1 2 3 4
Minimum Leaf Size (pixels):
0
0 1000 2000 3000 4000 5000
Only one Leaf component


Easy Leaf Area
AA01.JPG 807x620
Leaf pixels: 254669 Scale pixels: 7889 Leaf area: 290.53cm^2
Open an image
Auto settings
Analyze with current settings
Save analysis
Flip image horizontal
Rotate image 180 deg
Delete background
Batch Processing:
Select batch source Folder
C:/
Select batch output Folder
C:/
Start Batch with current settings
Use auto settings
Load calib File
Add to calib File
Open output csv file
Label Pixels
Leaf minimum Green RGB value:
25
0 50 100 150 200 250
Leaf Green Ratio: (G/R)
1.06
0.90 1.40 1.90
Leaf Green Ratio: (G/B)
1.08
0.80 1.30 1.80
Scale minimum Red RGB value:
30
0 50 100 150 200 250
Scale Red Ratio: (R/G & R/B)
1.18
1.00 1.50 2.00
Scale area (cm^2):
9.0
0.0 4.0 8.0 12.0 16.0 20.0
Processing Speed:
4
1 2 3 4
Minimum Leaf Size (pixels):
0
0 1000 2000 3000 4000 5000
Only one Leaf component

APPENDIX I.

REPRESENTATIVE REACTOR IMAGES

Tap Water Control
(Reactor 1)

PCE Control
(Reactor 6)

20 g ZVI
(Reactor 10)

100 g ZVI
(Reactor 15)

Compost
(Reactor 20)

Compost and DHC
(Reactor 22)

20 g ZVI, Compost, and DHC
(Reactor 24)

100 g ZVI, Compost, and DHC
(Reactor 28)

Evaporation Control
(Reactor 33)

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8. VITA

Tommy Joe Goodwin Jr was born on March 25, 1991 to Tommy Joe Goodwin and Ruth Ann Coble in Kankakee, IL. Tommy graduated from Niangua R-V high school in Niangua, MO in May 2009. He attended Missouri University of Science and Technology (Missouri S&T) where he received dual B.S. degrees in Environmental Engineering and Biological Sciences in December 2014. In May 2016, Tommy Goodwin graduated with his M.S. in Environmental Engineering from Missouri S&T.

Tommy has been a member of American Society of Microbiology (ASM) since 2010. He has been a member of Water Environment Federation (WEF) since 2012. He has been a member of Engineers without Borders (EWB) since 2013. He has been a member of American Society of Civil Engineers (ASCE) since 2014. He has been a member of Association of Environmental and Engineering Geologists (AEG) since 2014. He was inducted into the honor societies Chi Epsilon and Tau Beta Pi in 2014 and 2015, respectively. Tommy was elected President of the Council of Graduate Students, the governing organization of the Missouri S&T graduate student body, for the 2015-2016 school year.