CHARACTERIZATION OF CHLORINATED SOLVENT DEGRADATION PROFILE DUE TO MICROBIAL AND CHEMICAL PROCESSES IN A CONSTRUCTED WETLAND

THESIS

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Abstract

Perchloroethene (PCE) and its degradation products are among the most common organic groundwater contaminants in the United States. Constructed wetlands are a relatively new approach to dealing with this contamination problem. With their upward flow capability it is possible to introduce an aerobic and anaerobic environment with a consortium of microorganisms available to degrade the contaminants to within acceptable levels established by the Environmental Protection Agency (EPA).

This study is a follow-up to the previous two years of research on PCE degradation in cell 1 at Wright-Patterson Air Force Base. This thesis was conducted in order to study the wetland and determine the mechanisms that exist to degrade the chlorinated solvent contamination that is present. It also provided additional evidence that the constructed wetland is degrading PCE to its innocuous byproducts. A purge-and-trap gas chromatograph was used to determine the concentrations of PCE, TCE, DCE isomers, and VC throughout the three layers of the constructed wetland. Inflow and outflow were also sampled and analyzed.

In this year's data, PCE was detected at a level that was below the maximum contaminant level established by the EPA. However, it is clear that Cell 1 is still developing. This wetland cell has been in existence for three years and it is obvious that the development of a constructed wetland is a lengthy process. If a constructed wetland were to be used as a treatment process for contaminated water sources, time would have to be allowed for it to develop before it would reach maximum treatment efficiency.

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CHARACTERIZATION OF CHLORINATED SOLVENT DEGRADATION PROFILE DUE TO MICROBIAL AND CHEMICAL PROCESSES IN A CONSTRUCTED WETLAND

I. Introduction

The purpose of this study is to determine concentrations of chlorinated solvents and their biodegradation by-products contamination as they occur seasonally in two upward flow constructed wetland cells at Wright-Patterson Air Force Base (WPAFB), Ohio, constructed to investigate treatment of chlorinated solvents in the groundwater beneath. Earlier efforts have completed construction of the first wetland cell by installing a stratified sampling grid with drive point piezometers. Methodology has previously been developed for extracting samples of the contaminated groundwater from the wetland. This research follows up on the analysis done by Bryan Opperman (2002) and Nathan Clemmer (2003) that used gas chromatographs to distinguish the presence of chlorinated solvents and their daughter products in wetland samples. First, this effort will determine the seasonal concentrations of chlorinated solvents in three layers of the wetland by performing an analysis in 2003 in the fall. The second part of the analysis will be to provide further evidence that the constructed wetland is continuing to evolve and degrade the contamination.

This data will be used to enable further studies to develop more effective models and make improvements to the design and construction of wetlands to more efficiently remove chlorinated solvents from the groundwater.

Background

The exact number of groundwater contaminated sites is not known but is estimated by the National Research Council (NRC) to be between 300,000 and 400,000 with a cost for cleanup falling between $500 billion and $1 trillion in the next several decades (NRC, 1997). According to the Environmental Protection Agency (EPA), 1,236 sites were on the National Priority List (NPL) as of May 1, 2003. Sixty six additional sites have also been proposed to be placed on the NPL (EPA, 2003) which signifies that groundwater contaminated sites are a current and on-going problem.

In 1996, $9 billion dollars were spent on environmental remediation. Of this total cost the government was responsible for the largest portion at $3.8 billion, so it is clear that it is in the government's best interest to find innovative remediation technologies (NRC, 1997) that may end up saving money. According to the EPA, in 1996, conventional pump-and-treat methods were being used in 93 percent of the Superfund sites (EPA, 1996). While five percent of the Superfund sites used a combination of in-situ bioremediation and pump-and-treat methods, a mere one percent of the sites used solely in situ treatment.

The most common classes of hazardous substances that may be found in groundwater are volatile organic compounds (VOCs), toxic inorganic compounds, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), pesticides, and phthalates (NRC, 1997). The VOC perchloroethylene (PCE) and its degradation products will be the focus of this study. Industries such as textile cleaners, degreasers, and manufacturers of solvents for greases, oils, and waxes use chlorinated solvents. This

frequent use of chlorinated solvents has resulted in their being one of the most common contaminants found in groundwater systems throughout the country.

Chlorinated solvents have been produced and used in the United States for nearly a century. The production of chlorinated solvents began in the early nineteenth century in Germany. The United States started production of these solvents in 1906 and more extensively during World War II. Prior to the 1960s, it was assumed that groundwater was of good quality and essentially unharmed by man. As late as 1968 it was widely accepted that chlorinated solvents, such as TCE and PCE, may be poured directly onto the ground and absorbed into the atmosphere. It was believed that the subsurface could just absorb these contaminants without any negative effects to the environment. The first finding of chlorinated VOCs in groundwater and the recognition of the harmful effects that are associated with them occurred in the mid-1970s. In 1976 the Resource Conservation and Recovery Act (RCRA) and the 1980 Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA, or Superfund) eventually determined that there was a much more widespread contamination problem than originally expected (Pankow and Cherry, 1996).

Alkyl halide chemicals have high vapor pressures, fairly high aqueous solubilities, and a density that is greater than water (Table 1). While these properties make them invaluable in industrial applications they also result in a larger environmental problem because they are difficult to control as well as remediate. The fact that they are so useful has resulted both in their extensive use and ultimately their widespread groundwater contamination problem (Clemmer, 2003).

Compound	Density (g/ml) at 4° C	Solubility (mg/l) at 25° C	Henry's Constant (atm-m^3/mol)	Log K_{ow}	Vapor Pressure (mm Hg) at 25° C
Tetrachloroethylene (PCE)	1.620	150	.0153	2.60	17.8
Trichloroethylene (TCE)	1.460	1,100	.0091	2.38	57.9
cis-1,2-Dichloroethylene (DCE)	1.280	3,500	0.0037 atm-m^3/mol	0.70	208
trans-1,2-Dichloroethylene (DCE)	1.280	6,300	0.0072 atm-m^3/mol	0.48	324
1,1-Dichloroethylene (DCE)	1.210	2,250	.018	1.84	600
Vinyl Chloride (VC)	Gas	2,670	.315	1.38	2,660

Table 1. Physical Characteristics of PCE and its Daughter Products (EPA, 2000)

It was not until the late 1980s that groundwater scientists and engineers realized the difficulties associated with remediation of the dense non-aqueous phase liquid (DNAPL) solvents (Pankow and Cherry, 1996). The high densities of these DNAPLs result in the rapid percolation of the contaminant through the unsaturated soil zone and the vadose zone to form a lens on top of the water table. The water from the soil is displaced as the mass of the DNAPL increases and the DNAPL continues to sink until it penetrates the water table and eventually reaches an aquitard or an aquiclude (Domenico and Schwartz, 1998). The DNAPL lens rests on top of the aquitard or aquiclude and results in a reduced surface area to volume ratio to the groundwater flow which makes it more difficult for the contaminant to solubilize into the groundwater. That causes the removal rate of the contaminant to be lessened. (Johnson and Pankow, 1992). When the DNAPL moves into the subsurface and reaches the saturated zone it will have a more difficult time moving downward since the water is providing some resistance. However,

if the spill is large enough it will be able to continue to move downward and displace the groundwater (NRC, 1994).

Contaminants with high densities and lower viscosities result in a higher flow rate. This results in most liquid chlorinated solvents being able to move at rates that are comparable, or even quicker than, water (Pankow and Cherry, 1996).

Chlorinated solvents have low absolute solubilities which, when a large amount of the compound is spilled onto the ground, means the solvent will migrate as a DNAPL in the subsurface which may accumulate on top of areas with low permeability. The low solubilities result in the contaminants being able to reside in the subsurface for decades or even centuries (Johnson and Pankow, 1992). These contaminants may spread throughout a large area at high enough levels that are harmful to human health (Pankow and Cherry, 1996).

Chlorinated solvents also have low octanol-water partition coefficients (K_{ow}) indicating that the contaminants will not sorb very much to the soil. Because of this it moves with groundwater flow, another indication that the contaminant will affect a greater amount of the groundwater and, therefore, has a greater chance of affecting human health (Pankow and Cherry, 1996). However, if the soil has a relatively high organic content the contaminants may be able to adsorb to the soil more than in a soil with a higher clay content (Personal Communication, Amon).

Halogenated aliphatics, such as PCE and TCE, are very useful in many industries such as manufacturing and service industries. Because they are very effective as solvents and degreasers these compounds have become very prevalent in the environment. TCE

is the most frequently detected groundwater contaminant at hazardous waste sites and PCE is third on the list. Chlorinated aliphatic compounds comprise ten of the top twenty most frequently detected groundwater contaminants found at hazardous waste sites (Table 2).

Rank	Compound	Common Sources
1	**Trichloroethylene**	Dry cleaning; metal degreasing
2	Lead	Gasoline (prior to 1975); mining; construction material (pipes); manufacturing
3	**Tetrachloroethylene**	Dry cleaning; metal degreasing
4	Benzene	Gasoline; manufacturing
5	Toluene	Gasoline; manufacturing
6	Chromium	Metal plating
7	Methylene chloride	Degreasing; solvents; paint removal
8	Zinc	Manufacturing; mining
9	1,1,1-Trichloroethane	Metal and plastic cleaning
10	Arsenic	Mining; manufacturing
11	Chloroform	Solvents
12	1,1-Dichloroethane	Degreasing; solvents
13	**1,2-Dichloroethene, trans-**	Transformation products of 1,1,1-trichloroethane
14	Cadmium	Mining; plating
15	Manganese	Manufacturing; mining; occurs in nature as oxide
16	Copper	Manufacturing; mining
17	**1,1-Dichloroethene**	Manufacturing
18	**Vinyl Chloride**	Plastic and record manufacturing
19	Barium	Manufacturing; energy production
20	1,2-Dichloroethane	Metal degreasing; paint removal

Table 2. Twenty Most Frequently Detected Groundwater Contaminants at Hazardous Waste Sites (Adapted from NRC, 1994). The items in bold are the targets for analysis in present study.

The first legislation passed to protect drinking water quality in the United States was the Public Health Service Act of 1912. However, the Safe Drinking Water Act (SDWA) was the first act to require the establishment of drinking water quality standards by the EPA. The SDWA also required water treatment plant operators to monitor their

water before delivering to customers and treat it to ensure compliance with standards. In 1986, a series of amendments were passed which required the establishment of MCLs and MCLGs. MCLs are set by considering an acceptable public health risk as well as what is economically and technologically feasible. MCLGs are goals that are set by the EPA that establish a level of no risk to public health but may not be unattainable right now due to technology infeasibilities or cost issues (Masters, 2000). MCLs, as well as MCLGs, for TCE, PCE, 1,1-DCE, and Vinyl Chloride are shown in Table 3.

Compound	Maximum Contaminant Level Goals (MCLG) in water (mg/l)	Maximum Contaminant Level (MCL) in water (mg/l)	Potential Health Effects	Sources of Contaminants in Drinking Water
PCE	0	0.005	Liver problems, increased risk of cancer	Discharge from factories and dry cleaners
TCE	0	0.005	Liver problems, increased risk of cancer	Discharge from metal degreasing sites and other factories
1,1 DCE	0.007	0.007	Liver problems	Discharge from independent chemical factories
VC	0	0.002	Increased risk of cancer	Leaching from PVC pipes, discharge from plastic factories

Table 3. Contaminants and their respective MCLs (Adapted from EPA, 2002)

The Clean Water Act (CWA) of 1972 is the primary regulation that protects the United States' water sources including rivers, lakes, estuaries, and wetlands. It is the EPA's responsibility to ensure that all Federal facilities abide by the regulations (EPA, 2003). Under the CWA, permits are required under the National Pollutant Discharge

Elimination System (NPDES) to discharge wastewater. NPDES permits require the discharger to meet specific effluent limits and regularly monitor their effluent quality (Masters, 2000).

Under CERCLA, reportable quantities (RQ) of 100 pounds each have been established for any release of PCE or TCE. If a release of 100 pounds or greater occurs, the responsible party must notify the National Response Center, the local emergency planning commission, and the state emergency response commission (EPA, 2003). States have the authority to establish RQs that are lower than the national standards. The Emergency Planning and Community Right-to-Know Act (EPCRA), in accordance with the Superfund Amendments and Reauthorization Act (SARA) of 1986, states that anyone involved with the handling, storage or distribution of PCE and TCE is also required to maintain Material Safety Data Sheets (MSDS) and keep a strict inventory of these chemicals (EPA, 2003).

Wetlands

Natural wetlands have been found to have beneficial effects on waterborne contaminants. Wetlands have some exceptional properties that enable them to degrade chemical contaminants into more harmless products coming out of the wetlands. Another benefit of wetlands is that they can be constructed to provide an even more effective and efficient environment for the degradation of the chemical compounds (Lorah and Olsen, 1999). Use of constructed wetlands for remediation of chlorinated aliphatics is anticipated to have a cost that is only a fraction of conventional systems (NRC, 2000).

Research Objectives

The goal of this thesis is to follow up research done in the past two years to determine the level and mechanism of chlorinated solvent removal in each of the three layers in the constructed wetland. Methodology that has already been developed will be used for sampling cell one of the constructed wetland at WPAFB. The gas chromatograph will be used to determine concentrations of PCE and its daughter products.

Research Questions

1. Do the concentrations of PCE and its daughter products in the three layers of the constructed wetland give further evidence of biodegradation?
2. Do the concentrations of PCE and its daughter products give evidence of seasonal differences in biodegradation (i.e. summer, fall, and winter)?

II. Literature Review

Natural Attenuation

It was not until the past thirty years that it was determined that chlorinated solvents were hazardous and should be removed from the groundwater. It took almost ten years after that to realize the difficulties that were associated with removing these DNAPLs. Conventional methods for the remediation of sites with groundwater contamination are the frequently used pump-and-treat systems. There are several difficulties associated with these systems. Some of these difficulties are as follows: it is difficult to remove the contaminants with the groundwater because many of them have relatively low solubilities in water, the cost of these systems is very high, and it is difficult to determine the routes of travel of the contaminants because the subsurface is so heterogeneous (NRC, 1994).

Natural attenuation is defined as "naturally-occurring processes in soil and groundwater environments that act without human intervention to reduce the mass, toxicity, mobility, volume, or concentration of contaminants" (ITRC, 2002). The National Oil and Hazardous Substances Pollution Contingency Plan (NCP) describes natural attenuation as including any of the following processes: biodegradation, dilution, dispersion, and adsorption. It is important to realize that natural attenuation is not always appropriate but may be a feasible solution as long as the natural mechanisms in the groundwater can reduce contaminant concentrations to levels that are consistent with good health. The NCP also states that the timeframe required for natural attenuation

should not be significantly longer than what is required for conventional treatment methods (EPA, 1997).

When compared to more conventional methods, natural attenuation offers several advantages. One advantage is that the final byproducts of the process are harmless such as carbon dioxide and water. Also, since natural attenuation does not disturb the land and infrastructure above the contaminated groundwater it can continue to be used for other purposes. Natural attenuation is also less costly than current available technologies. Finally, this process can be used on the most mobile and toxic compounds without any added risk to human health. Incidentally, the compounds that are the most mobile and toxic are also the most likely to biodegrade (Wiedemeier, et al, 1997).

Several factors must also be considered as limitations when looking at natural attenuation. Groundwater is not homogeneous and changes in factors such as the gradient, velocity, pH, and electron acceptor and donor concentrations may affect the efficiency of the process. Also, it may take a relatively long time for natural attenuation to completely occur. Finally and most importantly, some of the intermediates of biodegradation may be more toxic than the original products (Wiedemeier, et al, 1997).

Phytoremediation and Biodegradation

Wetlands have a built-in capability to eliminate chemicals naturally from the groundwater by using phytoremediation and biodegradation. With phytoremediation plants are used to remove, transfer, stabilize, or destroy contaminants in soil, sediment, and groundwater. The four following mechanisms are included in phytoremediation:

enhanced rhizosphere biodegradation, phytoextraction, phytodegradation, and phytostabilization. Enhanced rhizosphere biodegradation takes place in the soil or in the groundwater that immediately surrounds the plant roots. Phytoextracation is when the plant roots uptake the contaminants and translocate or accumulate in the plant shoots and leaves. Phytodegradation is the metabolism of contaminants within plant tissues and phytostabilization is when plants produce chemical compounds to immobilize the contaminants at the interface of roots and soils. Plants can be used to mineralize toxic organic compounds as well as accumulate and concentrate heavy metals and additional inorganics from soil into aboveground shoots (EPA Treatment Technologies for Site Cleanup, 2001). Figure 1, on the following page, shows the basics of phytoremediation. Bioremediation uses microorganisms to degrade organic contaminants in soil, sludge, and solids. Bioremediation can either be done *ex situ* or *in situ*. The microorganisms use the contaminants as a food source or co metabolize them with a food source. *Ex situ* bioremediation requires a great amount of energy. Examples of *ex situ* bioremediation are slurry-phase bioremediation and solid-phase bioremediation. *In situ* bioremediation is performed in place. *In situ* procedures stimulate and create an environment where microorganisms can grow and use contaminants as food and energy. Oxygen, nutrients, moisture, temperature and pH must generally be controlled to make the in situ biodegradation process more efficient. In some instances microorganisms that have been adapted to degrade specific contaminants may be added to enhance the in situ process (EPA Treatment Technologies for Site Cleanup, 2001).

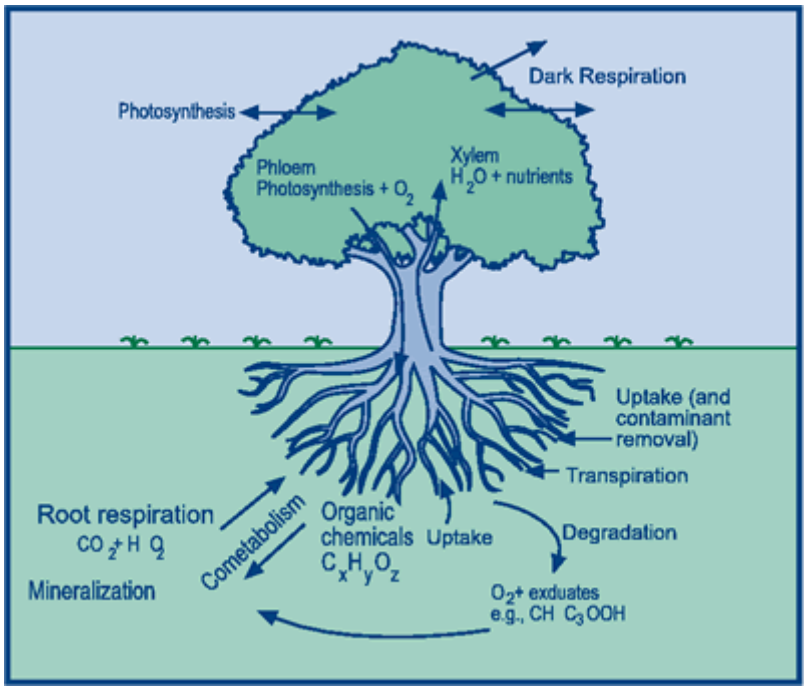


Figure 1. Processes in Phytoremediation (EPA Treatment Technologies for Site Cleanup, 2001)

Microbial Growth

Enzymes are used by microorganisms to increase the rates of chemical reactions. The most important reactions are redox reactions. Redox reactions transfer electrons from one molecule to another and result in the microorganisms' ability to generate energy and grow. Microorganisms are able to reproduce by organizing chemical reactions to create daughter cells. The enzymes enable the chemicals to come together and provide chemical reactions that react quickly. The reactions are completed by the expenditure of adenosine triphosphate (ATP). Microorganisms transfer electrons from the electron rich chemicals (electron donors) to the electron poor chemicals (electron acceptors) by expending ATP. They do not transfer them directly to the electron acceptors though but

rather to internal electron carriers. The main duty of the internal electron carriers is respiration which will generate ATP. In respiration, electrons are passed from carrier to carrier until they reach the terminal electron acceptor, capturing energy at each step (Clemmer, 2003).

The amount of energy available to a microbial population depends largely on the electron donors and acceptors. Microbes are able to use a very wide range of electron donors which can be both organic and inorganic. The electron acceptors are much more limited and include the following: oxygen (O_2), nitrate (NO_3^-), nitrite (NO_2^-), sulfate (SO_4^{2-}), carbon dioxide (CO_2), iron (III), and manganese (IV). When oxygen is available many microbes will use it first and the process is called aerobic respiration. When any of the other electron acceptors are utilized it is called anaerobic respiration. The different ways that the microbes assist in biotransformation has been researched quite extensively and has come a long way in the past three decades (Clemmer, 2003). Table 4 summarizes what is currently known about the aforementioned chloroethenes.

	Primary Substrate			Co-metabolism	
Contaminants	Aerobic Donor	Anaerobic Donor	Anaerobic Acceptor	Aerobic	Anaerobic
Tetrachloroethene			X		X
Trichloroethene			X	X	X
cis-1,2,Dichloroethene	X	X	X	X	X
trans-1,2,Dichloroethene	X	X		X	X
1,1-Dichloroethene	X			X	X
Vinyl Chloride	X	X	X	X	X

Table 4. Known Biotransformation Reactions for Chloroethenes Found in Groundwater (Adapted from NRC, 2000, modified by EPA, 2000)
(Note: Known biotransformation reactions are indicated with an X. A blank space indicates that the reaction is not known to occur.)

Reductive Dechlorination

There are two types of reductive dechlorination: direct and cometabolic. The difference is difficult to determine so both processes are generally referred to as merely reductive dechlorination (EPA, 2000). Electron flow and energy gain for the microorganisms can result in reductive dechlorination of chlorinated aliphatics. Reductive dechlorination occurs in highly reduced environments such as the lower layers of the constructed wetlands. The chlorinated ethenes' chlorine atoms in their molecular structure make them somewhat oxidized compounds. The chlorinated ethenes can act as electron acceptors in microbial metabolism. The molecular hydrogen atom then replaces the chlorine in the chlorinated ethene molecule which results in a less chlorinated molecular structure (Chapelle, 2001). The reductive dechlorination process begins with PCE reducing to TCE. TCE then reduces to *cis*-DCE, *cis*-DCE reduces to VC, and VC may reductively dechlorinate to ethene (Flynn et al, 2000). The reductive dechlorination process is shown in Figure 2.

Unfortunately, as the molecule becomes less chlorinated, the tendency to undergo reductive dechlorination decreases. Under highly reducing conditions, PCE is the most rapidly reduced of the chlorinated ethenes and goes to TCE. The reduction of TCE to *cis*-DCE can occur in an Fe (III) reducing, sulfate reducing environment, or under methanogenic conditions. The reductive dechlorination of *cis*-DCE to VC may occur under sulfate reducing conditions, but the reduction occurs more readily under methanogenic conditions. Finally, the reduction of VC to ethene is extremely slow and requires highly reducing, methanogenic conditions. This can result in an accumulation of

VC in anaerobic regions of the groundwater (Chapelle, 2001). This is of particular concern because VC creates more of a human health risk than others (Distefano, 1999).

The microorganisms, known as halorespirers or dehalorespirers, in the anaerobic regions of the constructed wetlands are able to utilize the chlorinated ethenes as terminal electron acceptors. The microorganisms are able to receive energy and grow from the reductive dechlorination process. Halorespirers that are able to reduce PCE and TCE to cis-DCE are commonly found in anaerobic environments (Chapelle, 2001). For the most part though, in order for PCE to reductively dechlorinate all the way to ethene, more than one microbial community must be involved. At the present time the only known bacterial isolate that is able to complete the whole degradation process on its own is *Dehalococcoides ethenogenes*. Additionally, there are still problems with utilizing this isolate. The reduction of VC to ethene is the rate-limiting step and it does not appear as though the reduction of VC supports the growth of the microorganism (Flynn et al, 2000). It is important to realize that this is not a common occurrence in most reactions driven by microorganisms. Most redox conversions occur only with a consortium of bacteria with each of them acting on their own as a part of the total reaction sequence (Personal Communication, Amon).

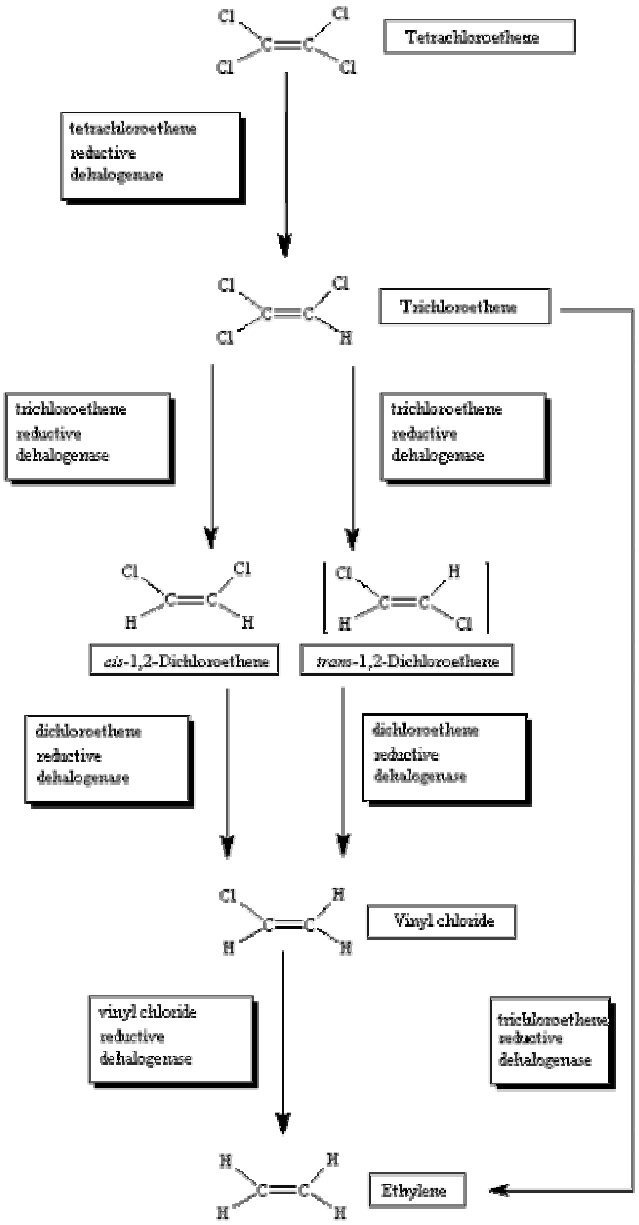


Figure 2. Known Pathway of Reductive Dechlorination of PCE (Ellis and Anderson, 2003)

Direct Oxidation

Direct oxidation occurs in the aerobic portions of the constructed wetland. In direct aerobic oxidation, the chlorinated hydrocarbon serves as an electron donor and the electron acceptor is oxygen. Generally, only chlorinated compounds with only one or two chlorines, such as VC and DCE, can be directly used by the microorganisms as electron donors. The VC and DCE can be oxidized into carbon dioxide, water, chlorine, and electrons. The oxygen is reduced to water. (EPA, 2000)

Cometabolic Oxidation

The difference between direct reactions and cometabolic reactions is that in direct reactions the microorganisms causing the reaction gain energy as the chlorinated solvent is reduced or oxidized. In cometabolic reactions the reduction or oxidation of the chlorinated solvent is caused by an enzyme produced during the microbial metabolism of another compound. The microbe does not benefit from the metabolic reaction. Direct reactions are generally more rapid than cometabolic mechanisms (EPA, 2000).

Cometabolic aerobic oxidation occurs when a contaminant is fortuitously oxidized by any enzyme that is produced during the microbial metabolism of another compound. Oxygen is the electron acceptor in cometabolic aerobic oxidation with electron donors of compounds such as TCE, DCE, VC, methane, ethane, ethene, propane, butane, aromatic hydrocarbons, and ammonia. The electron donor reaction is mediated, for example, by a methane monooxygenase (MMO) enzyme in the case of primary

oxidation of methane. The presence of methane is an effective means of stimulating the cometabolic biodegradation of chlorinated aliphatic hydrocarbons (EPA, 2000).

Redox Reactions

Microorganisms need to use electron transfer processes in order to maintain their life functions such as reproducing, growing, etc. When discussing groundwater, redox reactions should be described as kinetic processes instead of by using the traditional equilibrium approach (Chapelle, 2001).

In a highly reducing environment the chlorinated ethenes will most often serve as electron acceptors in the microorganisms' metabolism. The microorganisms will transfer electrons to the chlorinated ethenes and thereby reduce the compound. In environments that are not highly reducing the microorganisms will oxidize the chlorinated ethene and use it as an electron donor (Chapelle, 2001).

There are three steps that are required to describe kinetic redox processes. The first step is to document the source of the electron donor that is supporting the microbial metabolism. Identifying the electron donors helps to determine which processes are dominant in the groundwater. Most often carbon is the electron donor but it is also important to determine which of the carbon species is dominating. The second step is the documentation of the final sink for electron acceptors supporting the microorganisms' metabolism. Identifying the electron acceptors is much more challenging because in groundwater systems there is usually a variety of electron acceptors. As was mentioned in a previous section electron acceptors can be O_2, NO_3, Mn(IV), Fe(III), SO_4, and CO_2.

The microorganisms are constantly competing for available resources. The microbes that are physiologically suited to the environment have a greater advantage and a much better chance of survival whereas the microbes that are not physiologically suited to the environment will have a difficult time competing and may eventually be eliminated. The final step in the description of the kinetic process is to document the rates of electron transfer that are occurring within the system (Chapelle, 2001).

Nitrate and sulfate reducing bacteria may compete with each other and limit both methanogenic activity and the extent of the anaerobic reductive dechlorination. The maximum redox potential that has been found for the reductive dechlorination of PCE to TCE is +580 mV and the maximum for the degradation of TCE to DCE is +490 mV which indicates that these reactions are possible under manganese or iron reducing conditions. (EPA, 2000)

III. Methodology

Introduction

Two theses have already been accomplished sampling and monitoring cell 1 of a constructed wetland specifically designed to treat groundwater that has been contaminated with PCE. This thesis effort is following up on a research effort completed by Clemmer in 2003 to study the characterization of chlorinated solvent degradation in a constructed upward-flow treatment wetland at WPAFB, Ohio. The methodology that will be used to determine the contaminant and daughter product concentrations will be very similar to the methodology used last year. This similarity will allow for an accurate comparison of both sets of results which will enable the wetland's development to be tracked and its ability to degrade the chlorinated solvents. The wetland contains 66 piezometers that are used for sampling purposes. In addition to the piezometers, there are six monitoring wells that were used last year. The monitoring wells will be used again to determine dissolved oxygen, pH, temperature, conductivity, and redox potential. This additional data can also be used for comparison to last year's results.

For the past two years an additional student has performed research to determine the levels of several organic acids and inorganic ions (Bugg, 2002 and Kovacic, 2003). In a parallel study this year, BonDurant examined organic acids and inorganic ions in the wetland.

Experimental Constructed Wetland Cells

In August of 2000 in Area A of WPAFB two experimental wetland cells were constructed in order to study the degradation of chlorinated solvents into innocuous byproducts. The wetlands were built over an aquifer contaminated with PCE. The water is pumped into the wetland and the wetland acts as an upward flow treatment system. The two cells of the wetland are approximately 120 feet long, 60 feet wide, and the liner is approximately six feet deep (Clemmer, 2003). The wetland was designed to consist of three layers that are each approximately 18 inches deep with 66 piezometers installed in each layer for sampling purposes. However, recent findings from core studies at Wright State University discovered that the actual layers are estimated to be only about 12 inches deep (Personal Communication, Amon). Figure 3 shows a cross section of the constructed wetland.

There are three six-inch perforated PVC pipes that run parallel along the bottom of the cell that are enclosed in a bed of gravel that is nine inches deep. The crushed gravel layer allows the water to be distributed evenly within the lowest layer of the wetland. A geo-membrane is in place in order to separate the wetland from the surrounding soil. Above the gravel layer is 54 inches of lightly compacted wetland soil. After the original construction of the wetland, 10% wood chips (by volume) were added to the bottom 18 inches of the soil to act as a compost layer. The wood chips were designed to provide an initial source of organic carbon for the microbes to use as energy yielding reactions (Chapelle, 2001). The constructed wetland was divided into three

horizontal layers for sampling reasons. Native wetland vegetation was planted in separate plot areas on the surface.

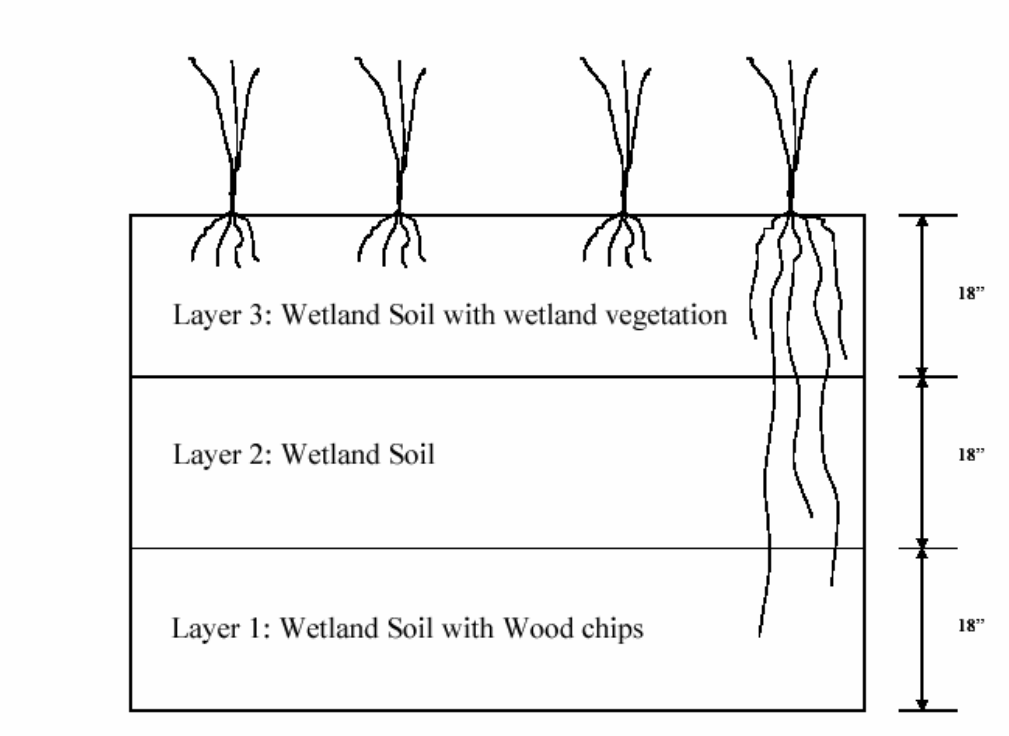


Figure 3. Cross Section of the Constructed Wetland, Cell 1 (Clemmer, 2003)

There is an exit weir across from the water inlet pipe at the opposite end of the cell. The level of the weir could be altered to control the surface water depth. After the water exited the wetland via the weir it was sent to the local sanitary sewer. Figure 4 on the following page shows a three dimensional drawing of the wetland cell depicting the idealistic flow of water. The walls of the wetland cell were actually angled out at a 1:1 slope to avoid collapse.

66 piezometer nests were installed in the summer of 2001. Each nest included a piezometer that reached into the lower layer, the middle layer, and the top layer. The piezometer nests are depicted as small circles in Figure 5 on the following page. Six larger well nests were also installed in order to attain other water parameters with a water monitoring sonde. The larger wells, which are depicted by large circles in Figure 5, were designed so that a sonde could be lowered into the water to take water quality measurements that are close to the soil.

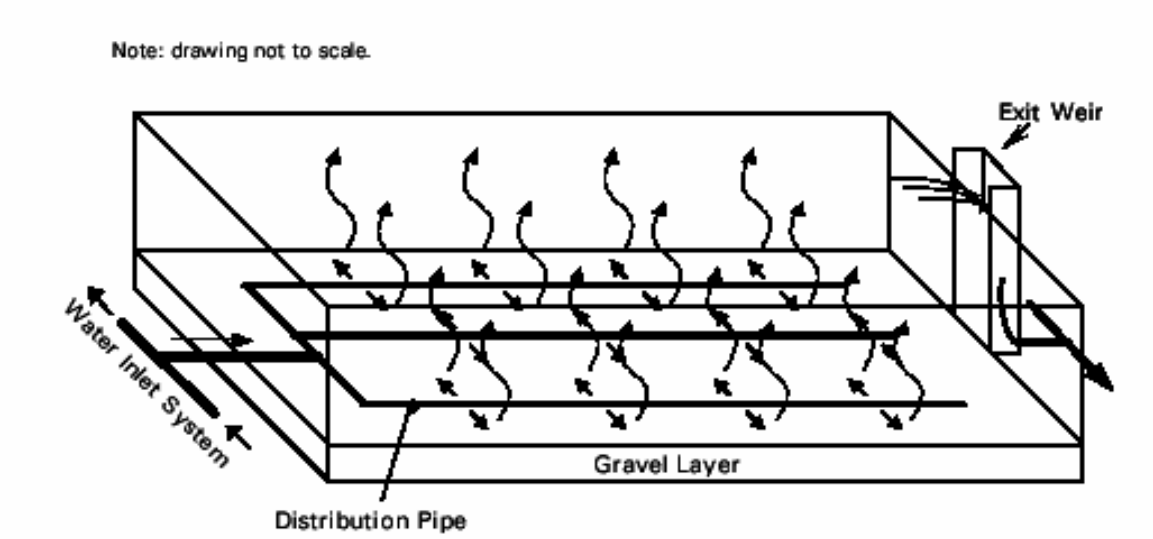


Figure 4. Water Flow Through Constructed Wetland (Entingh, 2002)

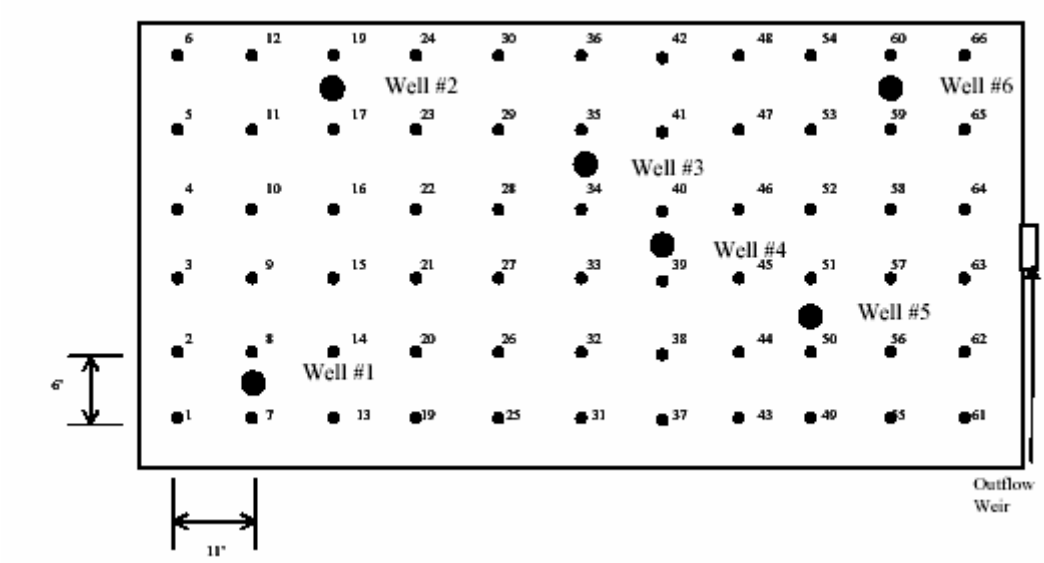


Figure 5. Plan View of Piezometer and Well Locations (Clemmer, 2003)

Sampling Procedure

The sampling method that was used in this thesis was identical to that of Clemmer (2003). This method was first developed by Opperman and Bugg (2002) and slightly refined by Clemmer (2003). This year's thesis effort used a virtually identical sampling procedure to last year's.

The piezometers were purged each time before taking samples in the field. This was done in order to remove the stagnant water from the piezometer and allow the fresh water from the soil matrix to infiltrate. A peristaltic pump fitted with Teflon® tubing was used to purge the constructed wetland. The Teflon® tubing used for purging the piezometers was identical to the tubing that was used when sampling. It was possible to purge the wells dry in the top two layers of the wetland. However, since the bottom layer had a considerably higher flow into it, it was not possible to purge it dry. The bottom

layer was purged for three well volumes. Twenty four hours was allowed after purging for the water levels to replenish in all three of the wetland layers.

After purging, the actual sampling was performed with a 100 mL glass syringe connected to the Teflon® tubing with a three-way cock stop connector. Before sampling, the 40 mL sampling vials were labeled by piezometer number and a letter corresponding to the layer of the wetland. For example, piezometer 44 in the bottom layer would be labeled "44C". The vials were transported to the wetland cell in a cardboard box that kept the vials separated.

The sampling tube was inserted in the piezometer in the center of the screened area. Approximately 10 to 20 mL of wetland water was pulled into the syringe in order to prime the sampling tube. The water and any air that may have been in the syringe were ejected. This was done to eliminate the air in the syringe and also to rinse the syringe of any remaining de-ionized water from the last rinse. Next, a minimum of 60 mL of water from the wetland was pulled into the syringe with care taken to avoid air bubbles. The sample was then ejected into a 40 mL sampling vial. It was important to be cautious to pour the water down the side of the bottle to eliminate turbulence and air bubbles in the sampling vial. The sample was intentionally poured until the vial overflowed and a lens of water above the vial remained. The vial was capped rapidly with a screw top PTFE septum cap to minimize exposure to the atmosphere and to avoid the presence of air bubbles. After each sample, the syringe was rinsed with de-ionized water to avoid any residual effects from previous samples.

As soon as possible, the samples were taken to the lab and analyzed. Due to this urgency, there was no need for sample preservation. One note, however, is that only 51 samples could be run at one time in the autosampler and the remaining samples were kept in the lab refrigerator until the autosampler was free. Each sample took approximately 30 minutes to run in the GC so the last sample may have been kept in the autosampler at room temperature for as much as 25 hours.

All three layers of the 66 piezometers were sampled once throughout this research effort. The top layer of each piezometer was taken first, followed by the middle and bottom layers. This was done due to the upward flow in the constructed wetland so that the sampling of the two underlying layers did not cause any undesirable effects. All of the piezometers in each layer were taken before moving onto the lower layers. Furthermore, pH and temperature were taken from each sample and recorded onsite in a field notebook.

In addition to sampling the 66 piezometers, the inflow and outflow of the constructed wetland were also sampled. The inflow sampling procedure was consistent with that of Clemmer and Kovacic (2003). Inflow was sampled in the pump house from a valve in the pipe that led to cell 1. The valve was opened and sample was collected with care taken to prevent the introduction of air bubbles. The outflow was altered slightly from the procedure that Clemmer and Kovacic (2003) used. The outflow was sampled just as the water flowed over the weir as opposed to last year's method where the outflow was sampled from a pool of water just before the water spilled over the weir.

Additional care was taken to ensure that the sampler did not disturb the surrounding vegetation and cause any unnecessary soil or debris to be introduced into the vial.

Data collected from the 66 piezometers was entered into Excel spreadsheets for data analysis. Using the GC the following analytes were analyzed: PCE, TCE, *cis*-DCE, *trans*-DCE, *1,1*-DCE, and VC. This raw data can be found in Appendix A.

Preparation of Standards

Standard solutions for each analyte were prepared using the pure phase of each compound including PCE, TCE, 1, 1-DCE, trans-DCE, cis-DCE, and vinyl chloride. These pure phase compounds were used in place of the custom stocks in a methanol solution that were used last year.

EPA 72 mL bottles were used to prepare all stocks and EPA 40 mL bottles were used to prepare all standards. De-ionized water was used in both cases as were Teflon-lined septa to cap the vials. The 72 mL bottles used to prepare the stocks were crimped with an aluminum top and the 40 mL bottles used to prepare the standards were capped with a plastic screw top. A fresh needle was inserted through the septum when excess pressure needed to be released. Gas-tight syringes of various volumes were used to transfer the pure compounds to the vials as well as to transfer the stocks to the standards. The stock and standard vials were placed in a rotator for 24 hours to equilibrate.

A concentration to volume ratio (equation 1) was used to determine the volume of the pure compounds required as well as the stock volumes required to develop the desired concentrations. The following equation was used to determine the concentrations:

$$C_1 \times V_1 = C_2 \times V_2 \qquad \text{(Equation 1)}$$

where: C_1 = concentration of pure compound or stock solution
V_1 = volume of pure compound or stock solution
C_2 = concentration of desired stock or standard
V_2 = volume of 72 mL or 40 mL vial

To begin with, 5 µL of pure compounds (PCE, TCE, *cis*-DCE, *trans*-DCE, *1,1*-DCE, and VC) were injected into 72 mL of de-ionized water resulting in stock A with a concentration of 112.71 mg/L. Stock A was equilibrated for 24 hours in a rotator. The exception to this was PCE. PCE was equilibrated for approximately 48 hours because on inspection of PCE stock A after 24 hours there was still some pure phase compound that had not dissolved. Stock A was then diluted by injecting 1 mL of stock A into 72 mL vial of de-ionized water to form stock B. Stock B was also equilibrated for 24 hours in a rotator. Stock B was ultimately used to make standard solutions using 40 mL EPA VOC vials. The following amounts of stock B were injected into 40 mL of de-ionized water and equilibrated to form the desired standards: 4 µL, 40 µL, and 400 µL. Four separate gastight syringes (GLENCO—Houston, TX) were used for the transfers with volumes of 5 µL, 100 µL, and 1 mL, respectively. All syringes used were rinsed three times with methanol and dried between each use. Calibration curves were created from the three various concentrations using Microsoft Excel software (see Appendix B). The curves were forced through zero to result in an improved R-squared value for each of the analytes.

Purge-and-Trap Methodology

All standards as well as wetland samples were analyzed using purge and trap gas chromatography. The Archon AutoSampler (Varian Analytical Instruments) held 51 sample vials at one time and mechanically sent 5 mL of the samples or standards to the Encon Purge-and-Trap concentrator. The AutoSampler required the use of 40 mL vials. EPA 40 mL glass VOC sample vials were used for all samples and standards. The vials were all topped with a polytetrafluoroethylene (PTFE) septum and then capped with a plastic open top screw-on cap. The AutoSampler sent the sample to the Purge-and-Trap which then passed the concentrated gaseous sample to the gas chromatograph. The AutoSampler also flushed the syringe and tubing once with 1 mL of deionized water and Helium between each sample's analysis.

The theory of purge and trap can be found in Opperman's (2002) thesis. The operating parameters for the Purge-and-Trap system can be found in Table 5. They are identical to what Clemmer (2003) used in his analysis.

Sample Volume (mL)	5
Purge Gas	Helium
Purge Gas Flow Rate (mL/min)	40
Purge Time (min)	11
Purge Temp (deg C)	Ambient
Dry Purge Time (min)	2
Desorb Preheat Temp (deg C)	245
Desorb Temp (deg C)	250
Desorb Time (min)	2
Bake Time (min)	10
Bake Temp (deg C)	250
Moisture Reduction Bake (deg C)	260

Table 5. Operating Parameters for the Encon Purge-and-Trap

Blank samples were run at the beginning of each sample run to make sure there were no impurities in the system. At the end of the chromatograph there was a slight tailing effect which is assumed to be due to the natural breakdown of the column. After time, a column naturally breaks down and does not compromise integrity of the samples until the tailing effect coincides with the analytes' peaks. If this were to happen the column would need to be replaced (Agrawal, Personal Communication).

The entire process for each sample took approximately 33 minutes. 50 samples and a blank water sample could be put into the AutoSampler at a time and it took just over 28 hours to complete. When those were completed the last 18 samples (which includes inflow and outflow samples) could be processed which took roughly an additional ten hours. The purge-and-trap concentrator limited how many samples could be processed at a time. Each sample took approximately 25 minutes in the purge and trap to complete and the AutoSampler could not take the next sample until the purge and trap had gotten through with its portion and reset to the beginning of the program.

Gas Chromatograph Methodology

An Agilent 6890 Series GC was used to analyze the components of each sample, with a micro-Electron Capture Detector (μECD) and a Flame Ionization Detector (FID). A detailed description of the GC operation and theory can be found in Opperman's thesis (2002).

The GC used a splitter to send the sample to both the μECD and the FID after a single injection. A 30m Restek RTX-VRX (Model 49314) column was used for the connection to the μECD and a 20m J&W 113-4332 GS-GASPRO column was used for

the FID. The μECD was intended to detect the heavier chlorinated compounds which include PCE, TCE, and the three isomers of DCE. The FID was intended to detect the lighter non-chlorinated compounds which include methane, ethane, and ethene as well vinyl chloride. The GC analytical operating parameters were similar to what both Opperman (2002) and Clemmer (2003) used. The only difference was that the oven maximum temperature was increased from 220 to 225 degrees Celsius. This change was made in order to ensure seeing all of the analytes of interest (Dawes, Personal Communication). The parameters are listed on the following page in Table 6.

ChemStation software version 4.1 was used to run all the aforementioned equipment. The software was installed on a desktop computer and operated the AutoSampler, the purge-and-trap, and the GC. The software had the ability to plot the chromatogram and integrate the chromatogram peaks. Although the program could auto-integrate the peaks there was also an option where the user could integrate the peaks. This was used in some cases where the peaks were too small for the auto-integrator to distinguish. Microsoft Excel was used to determine the concentration of each analyte. The calibration curves were run, best-fit lines were plotted, and the equation of the line was used with the area found by the ChemStation software to determine the concentrations of each analyte.

Oven	
Initial Temp (deg C)	50
Initial Time (min)	1.50
Ramp (deg C/min)	10.00
Final Temp (deg C)	225 * (Previous effort 220)
Hold Time at Final Temp (min)	1.0

Post Temp (deg C)	50
Total Run Time (min)	19.5

Front Inlet (Split/Splitless)

Mode:	Split
Initial Temp (deg C)	200
Pressure (psi)	15.00
Split Ratio:	5:1
Split Flow (mL/min)	20.6
Total Flow (mL/min)	27.6
Gas Saver:	On
Saver Flow (mL/min)	20.0
Saver Time (min)	2.00
Gas Type:	Helium

Column 1 (Restek 49314 RTX-VRX)		**Column 2 (J&W 113-4332 GS-GASPRO)**	
Max Temp (deg C)	260	Max Temp (deg C)	260
Nominal Length (m)	20	Nominal Length (m)	30
Nominal Diameter (µm)	180	Nominal Diameter (µm)	320
Nominal Film Thickness (µm)	1.00	Nominal Film Thickness (µm)	n/a
Mode	Const Press	Mode	Const Press
Pressure (psi)	15.00	Pressure (psi)	15.00
Nominal Init Flow (mL/min)	0.5	Nominal Init Flow (mL/min)	3.6
Average Velocity (cm/sec)	24	Average Velocity (cm/sec)	52
Inlet	Front	Inlet	Front
Outlet	Front	Outlet	Back
Outlet Pressure	Ambient	Outlet Pressure	Ambient

Front Detector (µECD)		**Back Detector (FID)**	
Temp (deg C)	250	Temp	250
Mode	Constant makeup flow	Hydrogen Flow (mL/min)	40.0
Combined Flow (mL/min)	45	Air Flow (mL/min)	400.0
Makeup Flow (mL/min)	25.0	Mode	Constant Makeup Flow
Makeup Gas Type	Nitrogen	Makeup Flow (mL/min)	45.0
Electrometer	On	Makeup Gas Type	Nitrogen
		Flame & Electrometer	On
		Lit Offset	2.0

Table 6. Gas Chromatograph Operating Parameters

IV. Results and Discussion

The results of the laboratory and field analyses laid out in Chapter 3 are discussed here in detail. Whenever possible, the results from this effort and the past two years' efforts were compared. This comparison shows how the wetland has matured in its degradation characteristics. The results allow an even more detailed evaluation of the degradation processes that are occurring and provide more evidence of the effectiveness of the constructed wetland. Additionally, the assessment of the data should enable a better understanding in the design of further constructed wetlands.

The first year of this constructed wetland study utilized the FID to analyze VC. It was determined this year and last year that the μECD gave a stronger signal so it was used to detect VC. The GC FID was set up to detect ethane, ethene, and methane. However, due to reasons that have yet to be determined, no measurable concentrations of ethane or ethene were shown. Very small concentrations of what is thought to be methane were determined but calibration curves at such low concentrations were very inaccurate and difficult to develop. Much thought went into what could be done to detect ethane and ethene and more accurate measurements of methane.

The first step required to analyze the data using the GC was to determine the retention times for each chlorinated solvent. This year's retention times were very similar to what Clemmer (2003) determined due to the fact that the exact same columns were used. The variation between the 2002 and 2003 data was caused by a maintenance

requirement to cut off a length of each column on the GC (Clemmer, 2003). Table 7 lists the retention times for all three years.

	Current Effort	Previous Effort (2003)	Previous Effort (2002)
Analyte	**Retention Time (min, detector)**	**Retention Time (min, detector)**	**Retention Time (min, detector)**
PCE	7.911 (μECD)	7.920 (μECD)	9.010 (μECD)
TCE	5.501 (μECD)	5.509 (μECD)	6.402 (μECD)
cis-DCE	3.806 (μECD)	3.818 (μECD)	4.496 (μECD)
trans-DCE	3.240 (μECD)	3.283 (μECD)	3.856 (μECD)
1,1-DCE	2.830 (μECD)	2.830 (μECD)	3.228 (μECD)
VC	2.746 (μECD)	2.750 (μECD)	6.709 (FID)
Ethene	N/A	N/A	2.715 (FID)
Ethane	N/A	N/A	1.893 (FID)
Methane	N/A	N/A	1.359 (FID)

Table 7. Characteristic Retention Times for All Analyses (Modified from Clemmer, 2003). N/A indicates analytes that were not detected in this analysis.

The original goal of this study was to take cell 1 data in the summer and fall of 2003. However, due to maintenance problems with the GC, it was not possible to collect data in the summer months. A complete pass of the constructed wetland was performed in the fall during the months of October and November. All wells were sampled with only a very small percentage (5 wells out of 298 or 2.5%) that did not have adequate water flow to take a sample. These wells were not factored into the average concentrations and were marked with an N/A in the data.

PCE, TCE, *cis*-DCE, *1,1*-DCE, *trans*-DCE, and VC concentrations were found throughout the constructed wetland. This raw data in Excel spreadsheet form can be found in Appendix A.

In each layer, the average concentration of each analyte and its particular confidence interval was calculated using functions in Excel. This was done mainly to

compare the data from the previous two years' efforts and this year's effort. This comparison can be seen in Table 8 on the following page. All of the statistics were calculated without eliminating any of the outliers. From this year's spreadsheet it can be seen that there is a fairly large variability amongst several of the analytes. VC has the largest variability in Layer C. It can be seen by the contour plots that there are two wells that have an extremely high concentration. This large variability may have been due to the fact that only one data set was taken. It could also be due to the fact that several areas of the wetland seem to be colonizing resulting in higher concentrations in some areas and negligible concentrations in other areas. The constructed wetland is a very heterogeneous environment so it stands to reason that the concentrations of chlorinated solvents will not be uniform throughout the 66 piezometers and will result in a confidence interval with a large range.

In the sampling run that was taken in December of 2001, only PCE and TCE were detected. Last year's sampling run detected a presence of cis-DCE and VC as well. This year's data resulted in the detection of the two additional analytes of trans-DCE and 1,1-DCE. This year's data determined a presence of trans-DCE in both the inflow and outflow of the wetland and VC in the inflow. The previous years' data did not identify either of these in the outflow or the inflow.

8a. Data from Oct/Nov 2003

Average Concentration (ppb ± 95% Confidence Interval)						
	PCE	TCE	cis-DCE	trans-DCE	1,1-DCE	VC
Outflow	0.796±0.486	0.821±1.132	ND	2.441±2.699	ND	ND
A	0.289±0.419	2.383±2.155	0.150±0.211	0.483±0.323	ND	10.025±9.541
B	0.294±0.379	2.241±1.905	0.330±0.322	4.473±0.530	0.241±0.457	5.849±3.094
C	7.790±1.779	1.583±0.300	6.780±1.716	1.978±0.660	0.430±0.383	ND
Inflow	23.933±1.142	0.278±0.110	ND	2.441±2.699	ND	0.310±0.608

ND – None Detected

Averages and confidence intervals were computed with 3 samples for the inflow, 3 samples for the outflow, and 66 samples for each layer sampling each piezometer once.

8b. Data from Jan 2003

Average Concentration (ppb ± 95% Confidence Interval)						
	PCE	TCE	cis-DCE	trans-DCE	1,1-DCE	VC
Outflow	8.637±0.807	0.509±0.041	ND	ND	ND	ND
A	1.178±0.938	0.381±0.192	1.105±0.585	ND	ND	0.256±0.130
B	1.492±0.743	0.721±0.270	1.770±0.724	ND	ND	8.701±6.691
C	25.533±1.726	0.754±0.194	0.311±0.275	ND	ND	0.021±.0023
Inflow	32.59±0.699	0.170±.0.011	ND	ND	ND	ND

ND – None Detected

Averages and confidence intervals were computed with 9 samples for the inflow, 11 samples for the outflow, and 66 samples for each layer sampling each piezometer once.

8c. Data from Dec 2001

Average Concentration (ppb ± 95% Confidence Interval)						
	PCE	TCE	cis-DCE	trans-DCE	1,1-DCE	VC
Outflow	5.593±0.615	2.138±2.177	ND	ND	ND	ND
A	2.422±0.557	0.342±0.343	ND	ND	ND	ND
B	1.797±0.165	0.349±0.031	ND	ND	ND	ND
C	26.821±0.383	0.806±0.034	ND	ND	ND	ND
Inflow	33.97±0.920	0.627±0.194	ND	ND	ND	ND

ND – None Detected

Averages and confidence intervals were computed with 12 samples for the inflow and 4 samples for the outflow. Each piezometer was sampled three times and averaged. The piezometer averages in the three layers were then averaged to arrive at the average concentration for the entire layer.

Table 8. Analyte Average Concentrations (Outliers not removed and zero response by GC is included in the calculations as zero)

The largest reduction in PCE concentration this year was found going from the bottom layer to the middle layer. Using the average concentrations, the PCE was reduced by 96% from layer C to layer B. This is comparable to the 94% reduction that was detected last year. While there was a slight reduction from layer B to layer A in the PCE concentrations, it is essentially negligible due to the large confidence intervals that are associated with these concentrations. Looking at the difference from the inflow to the top layer PCE concentrations, a reduction of nearly 99% can be calculated.

Overall, there was a much lower concentration of PCE detected this year as compared to the previous two years. From January 2003 until the fall of 2003 when these samples were taken, PCE was reduced by approximately 27% in the inflow. While that is a significant reduction, the most notable reduction of PCE concentration was in layer C. The average concentration from last year's data to this year's was reduced from 32.59 ppb to 7.79 ppb which is 76%.

From the inflow to the outflow weir, PCE was reduced by 96.7%. The outflow concentration was actually higher than the concentration of PCE in layer A. This may be due to areas in the constructed wetland where water is bypassing the three treatment layers and flowing directly to the weir without being degraded (Clemmer, 2003).

Another notable observation is that the PCE in the outflow weir reduced from an average concentration of 8.637 ppb in January of 2003 to 0.796 ppb in the fall of 2003. This is a 91 percent reduction which signifies that the constructed wetland has shown enormous improvements in the amount of PCE that is degraded between the time that it

enters and exits the constructed wetland. This can be seen graphically on the following page in Figure 6.

In layer C of the constructed wetland there is a relatively high concentration of PCE throughout the stratum. This is to be expected because the contaminated water comes in through this layer and as it makes its way up and towards the outflow weir it is treated. There is an extraordinarily high concentration of PCE along rows 2 and 5 which is where the PVC pipes were laid during the construction of the wetland. However, from looking at the contour plots in Figure 7, between rows 3 and 4 where the remaining PVC pipe was laid, there is an area where very small concentrations of PCE were detected (wells 33, 34, 39, 40, 45, and 46). This may be due to the reductive dechlorination at these points occurring so rapidly that the PCE is disappearing before it can even be detected. The flow at this area may be so low that there is sufficient time for the PCE to dechlorinate.

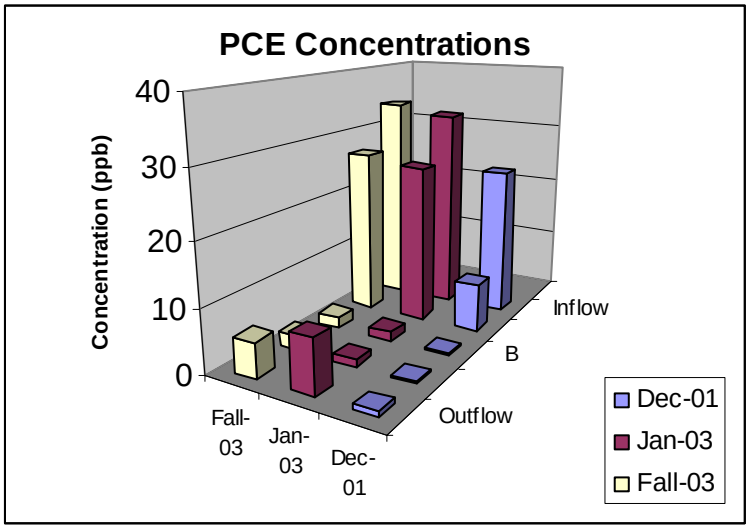


Figure 6. PCE Concentrations (Past three years' data)

	Fall 03	Jan-03	Dec-01	Jan-03	Dec-01
Outflow	0.796	8.637	5.593	8.637	5.593
A	0.289	1.178	2.422	1.178	2.422
B	0.294	1.492	1.797	1.492	1.797
C	7.79	25.533	26.821	25.533	26.821
Inflow	23.933	32.59	33.97	32.59	33.97

Table 9. PCE Concentrations (Numerical data for past three years' data)

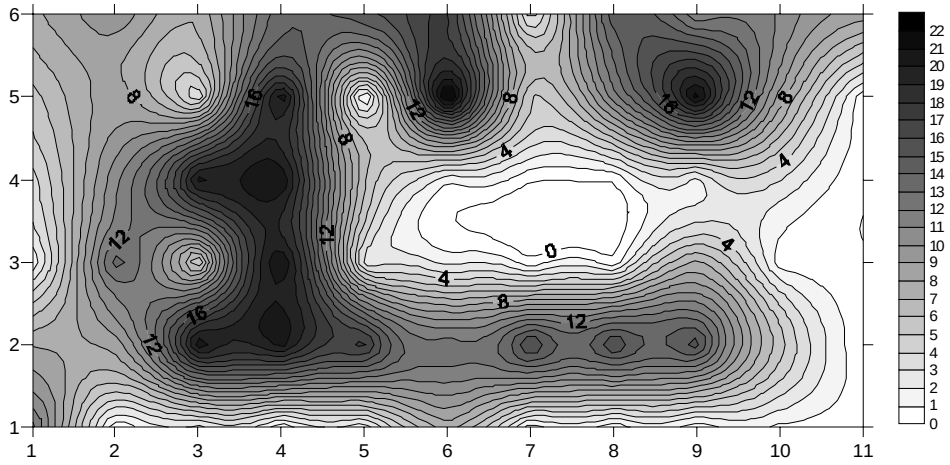


Figure 7. Contour Plot of PCE Concentrations in Layer C (Oct-Nov 03)

Additionally, there is a notably high concentration of PCE along the row that includes wells 20 through 24 (see Figure 7). This area of elevated PCE concentration occurs where the pipe is beginning to be perforated. This observation indicates that the constructed wetland may not be following a vertical flow pattern until it reaches the perforations in the pipes.

There was also a significant concentration of PCE in well 23 in both layers A and B. A concentration of 14.1 ppb was detected at layer A and 12.4 ppb was detected at layer B. This is considerably higher than the average concentrations of 0.289 ppb for layer A and 0.294 ppb for layer B. The area in the constructed wetland where well 23 is located has historically been an area where water has flowed through the wetland without

degrading. Due to the presumed quick flow rate in this area, it is possible that no electron donors are able to exist here that will aid in the reductive dechlorination of the contaminant.

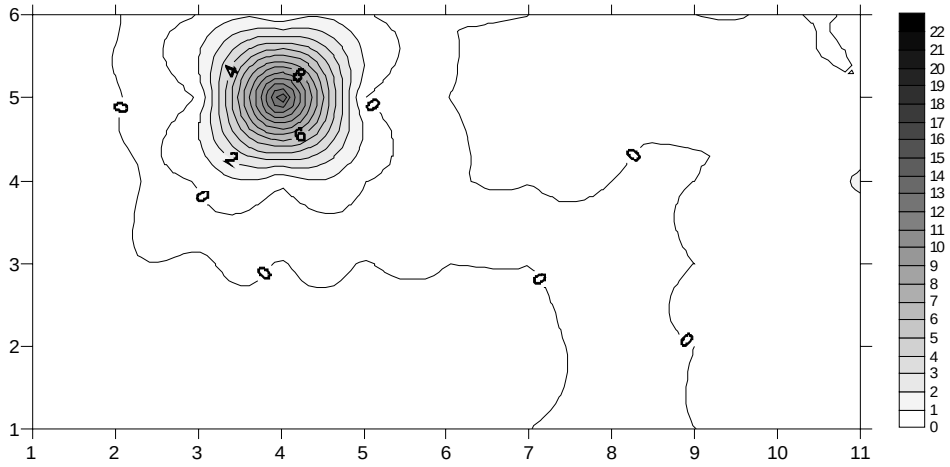


Figure 8. Contour Plot of PCE Concentrations in Layer A (Oct-Nov 03)

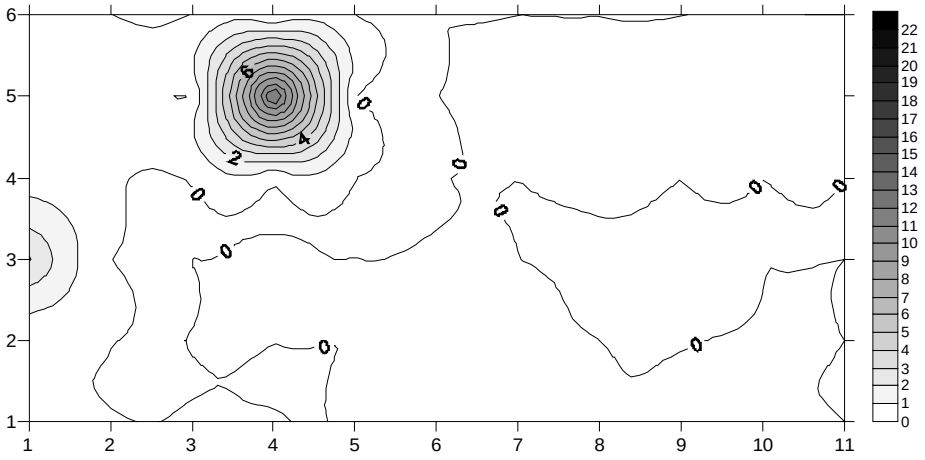


Figure 9. Contour Plot of PCE Concentrations in Layer B (Oct-Nov 03)

The average concentration of TCE throughout the wetland was significantly higher than what was found last year and the year before (see Figure 10). For example, in layer A the average concentration jumped from 0.381 ppb to 2.383 ppb which is over a

600% increase. This indicates that more TCE is being formed this year from the reductive dechlorination of PCE. Average TCE concentrations also increase 50% from Layer C to Layer A in this year's data. However, it is important to realize that the concentrations of TCE in the constructed wetland are still very minute. From the relatively small concentrations of TCE, it can be determined that the degradation from PCE to *cis*-DCE is fairly rapid because the TCE does not persist long enough to be detected in our sampling procedures.

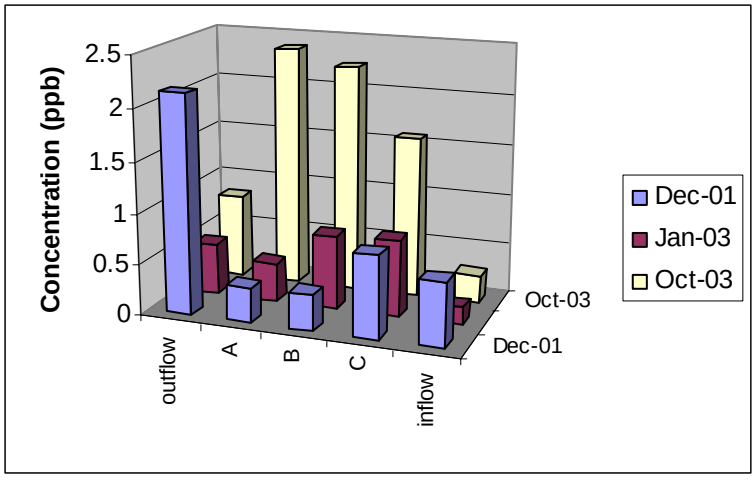


Figure 10. TCE Concentrations (Past three years' data)

	Fall 03	Jan-03	Dec-01
Outflow	0.821	0.509	2.138
A	2.383	0.381	0.342
B	2.241	0.721	0.349
C	1.583	0.754	0.806
Inflow	0.278	0.17	0.627

Table 10. TCE Concentrations (Numerical data for past three years' data)

In layer C there were relatively low concentrations of TCE present. The highest concentration of TCE was 4.5 ppb in this layer. It seems likely that the microorganisms are rapidly reducing PCE to a DCE isomer so quickly in this layer that no significant concentrations of TCE are able to endure.

In Layer B, there is one area where there is a concentration of TCE that is extremely high (over 40 ppb). This can be seen in Figure 11 at wells 53 and 59. This is an area where there are apparently no microorganisms available to reductively dechlorinate the TCE to its daughter products. Since TCE is more likely to reduce in an anaerobic environment by means of reductive dechlorination it is also possible that this region is an aerobic environment.

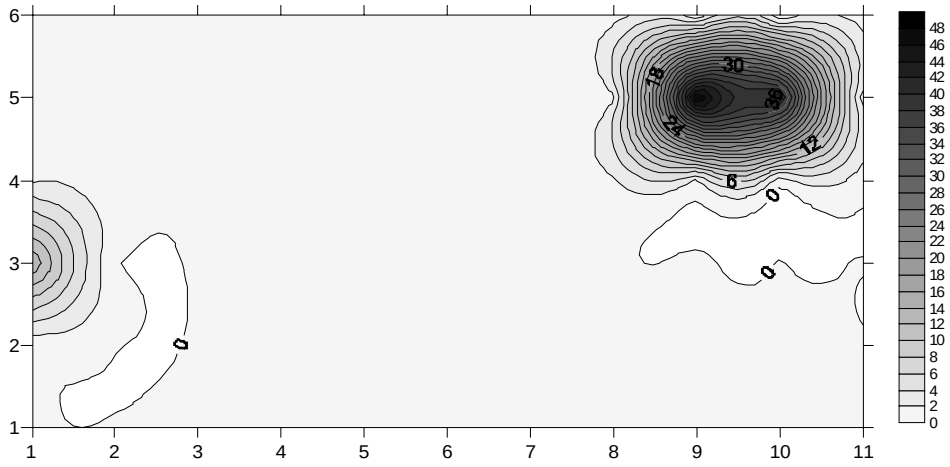


Figure 11. Contour Plot of TCE Concentrations in Layer B (Oct-Nov 03)

In Layer A, there were relatively high concentrations of TCE near the inflow of the constructed wetland (wells 3 and 10) and near the outflow weir (wells 57 and 64). This indicates that the water is making its way up through the wetland without the TCE reducing. The PCE is rapidly dechlorinating to TCE in this area of the wetland but

getting trapped at TCE because the environment is more than likely aerobic in these areas. Something to note is that in Appendix H the cis-DCE concentration is low in these two previously noted areas. This observation backs up the previous conclusion that the dechlorination of PCE is not proceeding past the intermediate product of TCE instead of being reduced all the way to an innocuous byproduct.

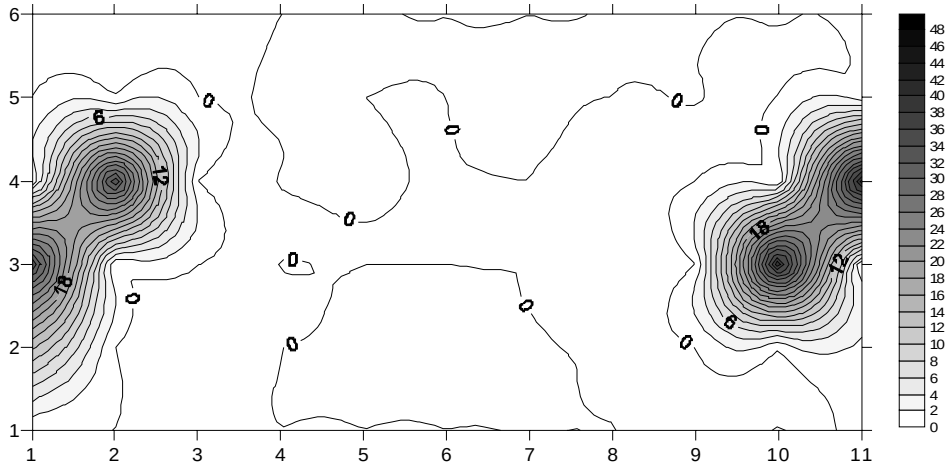


Figure 12. Contour Plot of TCE Concentrations in Layer A (Oct-Nov 03)

This was the first year that any DCE isomers other than *cis*-DCE were detected. All three isomers (*cis*-, *trans*-, and *1,1*-) of DCE were detected in this year's thesis effort. A much greater amount of *cis*-DCE was detected in layer C this year than the previous year. The average *cis*-DCE concentration in layer C increased from 0.311 ppb last year to 6.780 ppb this year (see Figure 13 on the following page). This could be due to the continuous development of the wetland. As more of the PCE is reductively dechlorinated it stands to reason that higher concentrations of the intermediate and end products will be present in the constructed wetland.

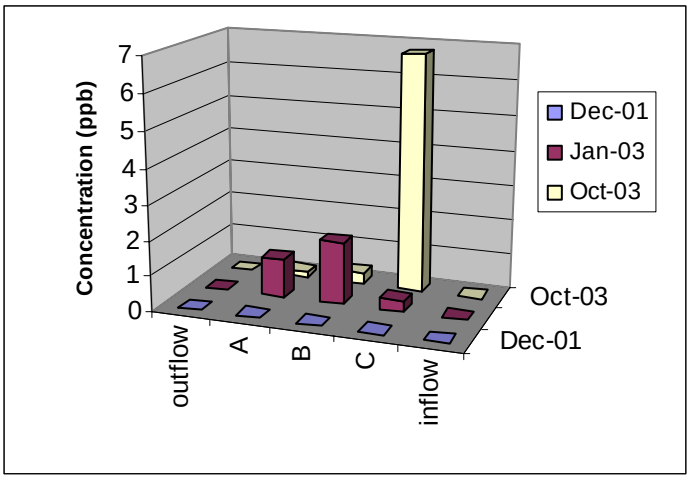


Figure 13. *cis*-DCE Concentrations (Past three years' data)

	Dec-01	Jan-03	Oct-03
Outflow	0	0	0
A	0	1.105	0.15
B	0	1.77	0.33
C	0	0.311	6.78
Inflow	0	0	0

Table 11. *cis*-DCE Concentrations (Numerical data for past three years' data)

There were several notable observations regarding the *cis*-DCE data. The first of which is in layer C. There is a very high concentration of *cis*-DCE in the same area that there are very small concentrations of PCE present at wells 34, 40, and 45 (see Figures 14 and 15). These two contour plots give a very strong indication that the PCE reduction is occurring so rapidly that we cannot detect any PCE. The reaction is moving so quickly from PCE to *cis*-DCE that there is no evidence of any TCE either.

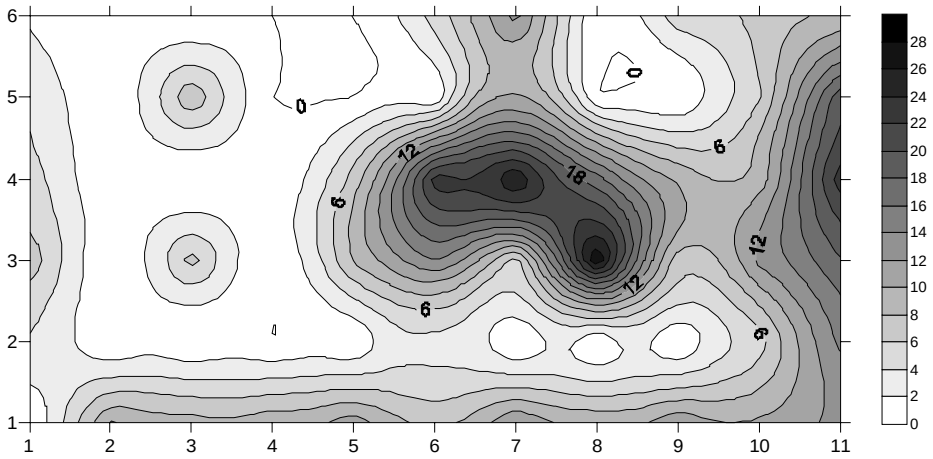


Figure 14. Contour Plot of *cis*-DCE Concentrations in Layer C (Oct-Nov 03)

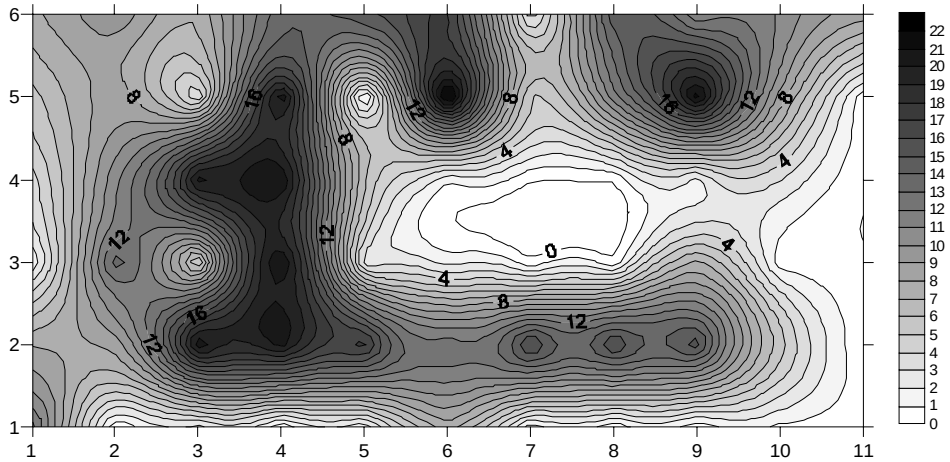


Figure 15. Contour Plot of PCE Concentrations in Layer C (Oct-Nov 03)

The reciprocal observation to the aforementioned is that there is no *cis*-DCE where there were high concentrations of PCE in layer C (see Figures 14 and 15). This indicates that since there is still a high concentration of PCE in those areas that the original contaminant is not degrading at a very brisk rate and there is virtually no evidence of intermediate by-products in these areas.

In layer A (see Appendix H), there are no significant concentrations of *cis*-DCE. The *cis*-DCE is most likely being oxidized in this layer since it is mainly an aerobic

environment. The oxidation of *cis*-DCE results in the production of VC or more innocuous end products.

This year was the first time that the remaining two isomers of DCE were detected (see Figures 16 and 20). There was a rather prevalent finding of *trans*-DCE in all three layers of the constructed wetland and also in the inflow and outflow. In both layers A and C the *trans*-DCE did not show up until the far end of the constructed wetland towards the outflow weir (see Figures 17 and 18). It can be hypothesized that as the water is moving down the wetland it is reductively dechlorinating the PCE which results in the intermediate product of *trans*-DCE.

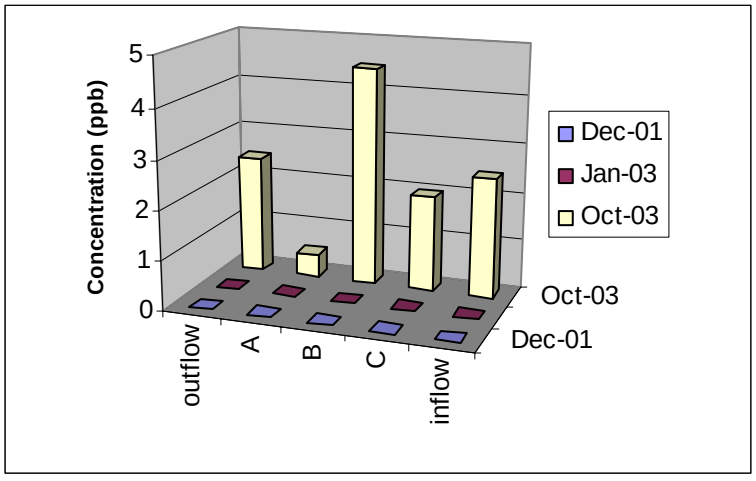


Figure 156. *trans*-DCE Concentrations (Past three years' data)

	Dec-01	Jan-03	Oct-03
Outflow	0	0	2.441
A	0	0	0.483
B	0	0	4.473
C	0	0	1.978
Inflow	0	0	2.441

Table 12. *trans*-DCE Concentrations (Numerical data for past three years' data)

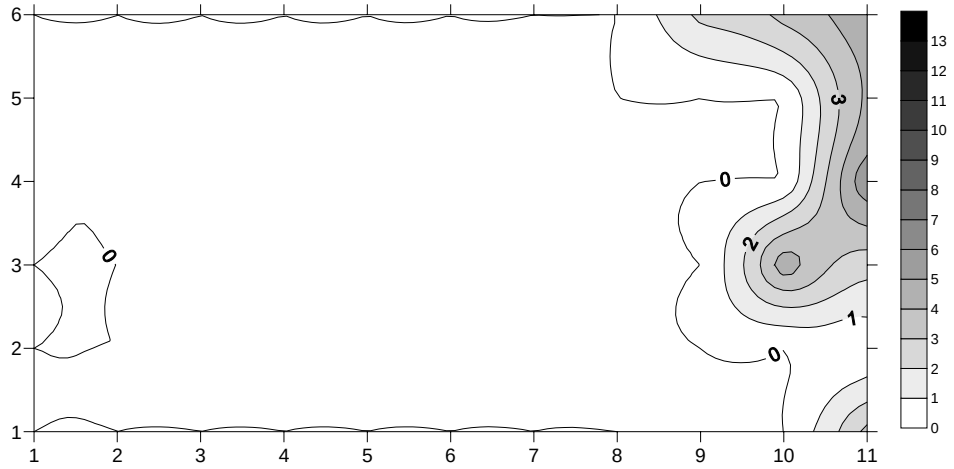


Figure 17. Contour Plot of *trans*-DCE Concentrations in Layer A (Oct-Nov 03)

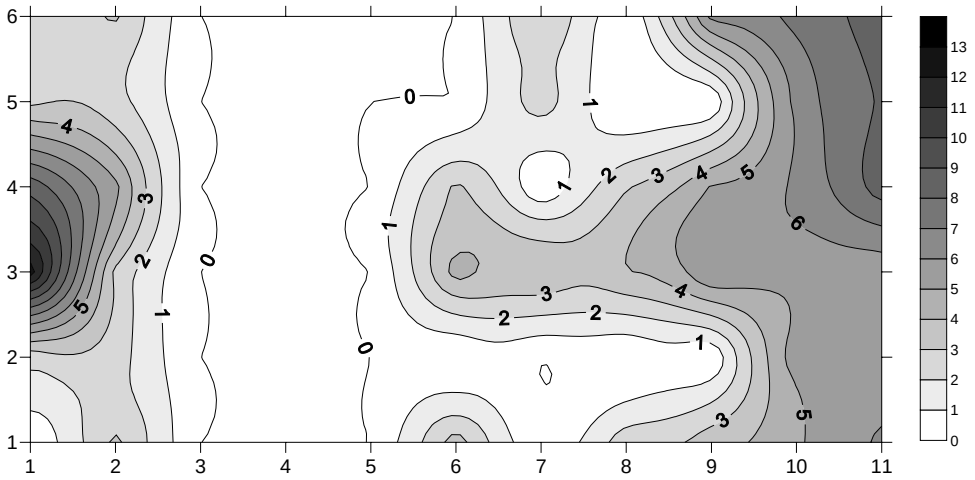


Figure 18. Contour Plot of trans-DCE Concentrations in Layer C (Oct-Nov 03)

In layer C, the existence of *trans*-DCE is consistent with the PCE contour plot (see Figures 18 and 19). Where there are low concentrations of PCE in layer C there are high concentrations of *trans*-DCE and vice versa. It is possible that there are microbial communities in the constructed wetland that transform TCE into *trans*-DCE.

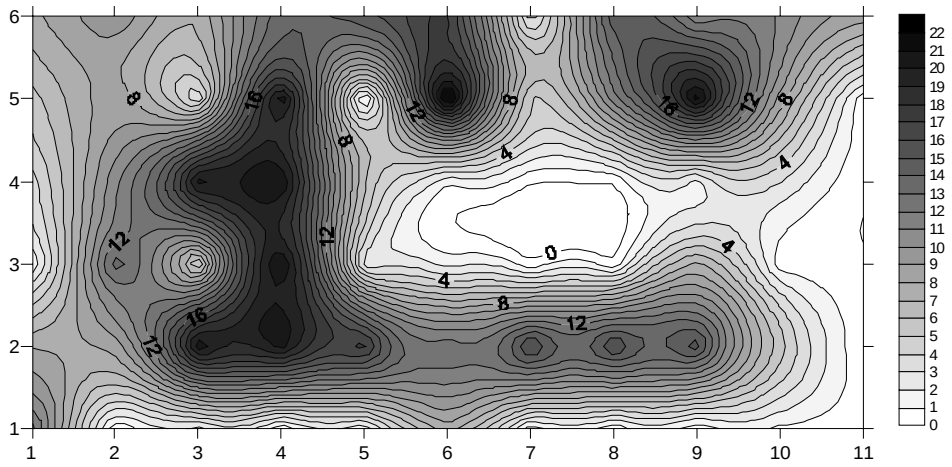


Figure 19. Contour Plot of PCE Concentrations in Layer C (Oct-Nov 03)

There was no *1,1*-DCE found in layer A (see Appendix J and Figure 20 on the following page). Finally, the *1,1*-DCE concentrations were the highest in layer C in the areas that there is no *cis*-DCE present. It would be beneficial in future data analysis to determine when conditions are favorable for *1,1*-DCE to form instead of the other DCE isomers.

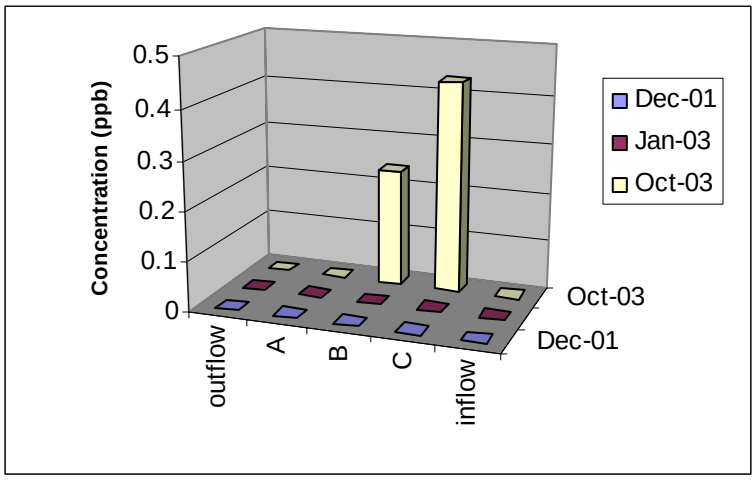


Figure 20. *1,1*-DCE Concentrations (Past three years' data)

	Dec-01	Jan-03	Oct-03
Outflow	0	0	0
A	0	0	0
B	0	0	0.241
C	0	0	0.43
Inflow	0	0	0

Table 13. *1,1*-DCE Concentrations (Numerical data for past three years' data)

In this year's data there was a significantly higher concentration of VC found in layer A (see Figure 21 on the following page). The average concentration in layer A increased from 0.256 ppb to 10.025 ppb in the fall of 2003 which is a considerable increase. This is most likely due to the fact that as the wetland is maturing, more of the PCE is being reductively dechlorinated and getting trapped at the intermediate by-product of VC. Most likely there are still some areas in the wetland that do not have the correct conditions available to oxidize or reductively dechlorinate the VC into a more harmless end product.

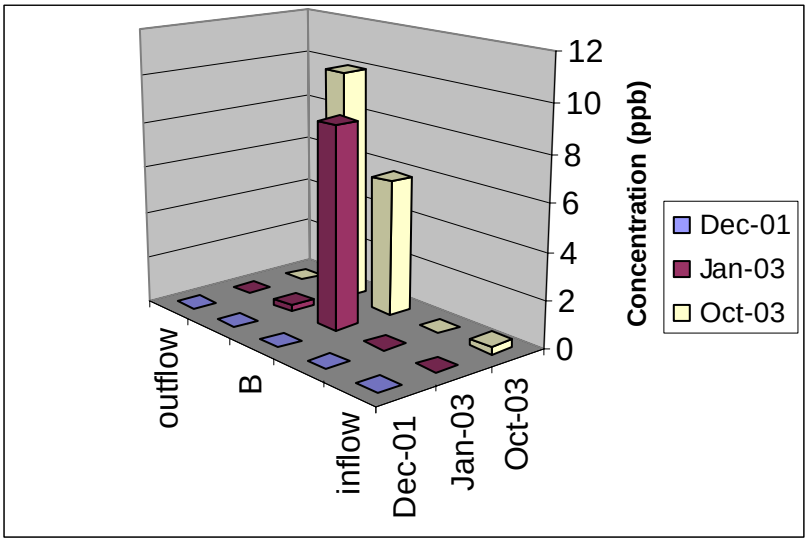


Figure 21. VC Concentrations (Past three years' data)

	Dec-01	Jan-03	Oct-03
Outflow	0	0	0
A	0	0.256	10.025
B	0	8.701	5.849
C	0	0.021	0
Inflow	0	0	0.31

Table 14. VC Concentrations (Numerical data for past three years' data)

The most notable observations regarding VC concentrations occurred in layer A. There is an extremely high concentration of VC at well 39 (298 ppb) and a lower but still significant concentration at well 47 (114 ppb). Both of these anomalies are relatively close to each other (see Figure 22). At first glance, it seems likely that these two points are outliers, possibly caused by a false reading on the GC. This is a possibility; however, there is a curious correlation between this area of the constructed wetland and the vegetation that is present in this region. Since VC is an extremely volatile compound, the presence of a lot of vegetation above it would act as a vent to volatilize the VC by transpiration. There is a sweetflag patch in the area of well 39 and it is the only area in cell 1 that has this type of vegetation. The sweet flag's rhizomes are approximately 2 centimeters in diameter. As the colony of sweet flag grows the rhizomes overlap and form a tight meshwork. The roots that come off of the rhizomes are not very fine, relatively, and have a much smaller surface area for the VC to penetrate. Since the roots also do not penetrate deeply they have even less contact with the VC. This is possibly what is keeping the VC trapped and resulting in an extremely high concentration in this area (Personal Communication, Amon).

The large concentration at well 47 could also be caused by vegetation. At well 47 there is a plot of *Juncus effusus* which is a commonly found wetland plant in the Midwest

region of the United States. *Juncus effusus* is a plant that stays green almost all year round with an extremely low water loss potential. Most of its dense roots are quite near the surface which means that there is little opportunity to uptake or transport the VC. This is possibly an explanation for the abnormally high concentration of VC that is at this location in the constructed wetland (Personal Communication, Amon).

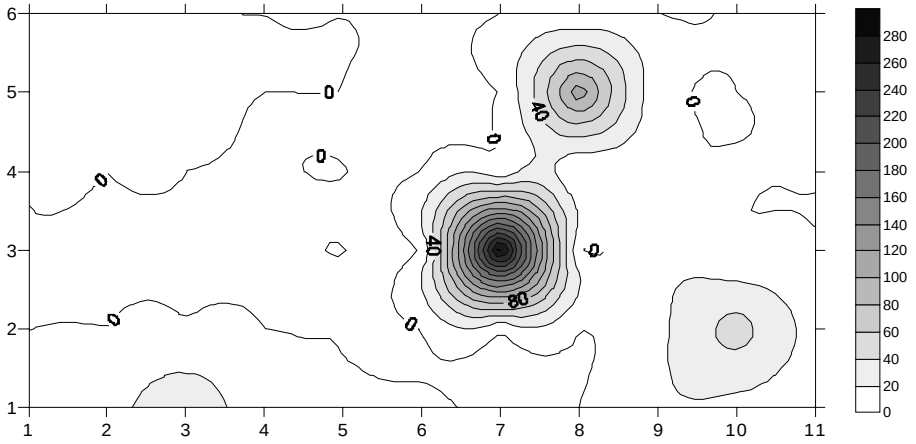


Figure 22. Contour Plot of VC Concentrations in Layer A (Oct-Nov 03)

In layer B, there were also several areas that had an unusually large concentration of VC. These areas were at wells 19, 25, and 54. Due to the highly reducing environment in layer B, DCE is reduced to VC. In the areas that VC concentrations are high at layer B, there is a negligible concentration of VC in layer A (see Appendix K). This may be due to the vegetation, *Eleocharis erythropoda*, which is present in this area. *Eleocharis erythropoda* is commonly known as bald spikerush and is a species of wetland plant. The root mass exists at the depth of layer B which means that it is possible that the VC is being vented through the plants. The VC could have entered the tissue of the plant while it was in layer B and transported through the tissue into the air.

There also is a very dense root system at all three of the aforementioned locations. That would provide an explanation as to why there is no VC present directly above this concentration in layer B (Personal Communication, Amon).

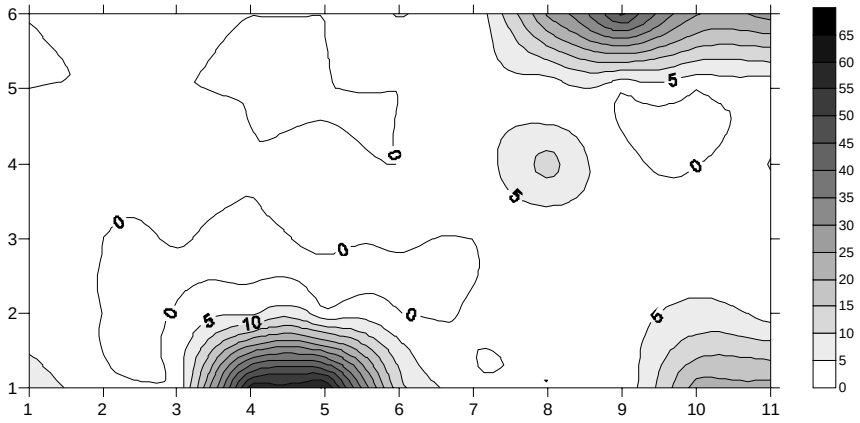


Figure 23. Contour Plot of VC Concentrations in Layer B (Oct-Nov 03)

An interesting observation between this year's data and last year's data is that although concentrations of intermediate products such as DCE and VC were higher on average, they were actually present in fewer of the piezometers (see Table 15 on the following page). A possible explanation for this is that the microorganisms that are reductively dechlorinating the PCE are colonizing as the constructed wetland develops. There are relatively large sections of the wetland where the PCE is reducing almost completely to *cis*-DCE in layer C. This may be the cause for the increase from 5 wells to 41 wells this year in layer C that detected *cis*-DCE.

There were significantly fewer wells in layers A and B that detected PCE and *cis*-DCE in this year's data. This may signify that more activity is occurring deeper within the wetland and reducing the contaminants in a quicker manner than in the previous

years. Essentially, the constructed wetland may be becoming more efficient at degrading the chlorinated solvents.

15a. Data from Jan 2003

	Number of Piezometers Analyte was Detected (Ave Conc. ppb ± 95% CI)					
Layer	PCE	TCE	*cis*-DCE	*trans*-DCE	*1,1*-DCE	VC
A	65 (1.20 ± 0.94)	41 (0.61 ± 0.30)	13 (5.61 ± 1.15)	ND	ND	26 (0.65 ± 0.27)
B	66 (1.49 ± 0.74)	46 (1.04 ± 0.35)	20 (5.84 ± 1.05)	ND	ND	43 (13.36 ± 10.03)
C	66 (25.53 ± 1.73)	65 (0.75 ± 0.20)	5 (4.05 ± 1.08)	ND	ND	3 (0.45 ± 0.08)

15b. Data from Fall 2003

	Number of Piezometers Analyte was Detected (Ave Conc. ppb ± 95% CI)					
Layer	PCE	TCE	*cis*-DCE	*trans*-DCE	*1,1*-DCE	VC
A	43 (0.424 ± 0.22)	34 (4.485 ± 1.403)	2 (4.785 ± 1.095)	8 (3.860 ± 0.307)	ND	37 (17.341 ± 5.749)
B	38 (0.495 ± 0.225)	64 (2.241 ± 0.676)	4 (5.285 ± 0.65)	62 (4.618 ± 0.167)	1 (15.40 ± 0)	42 (8.913 ± 1.583)
C	65 (7.790 ± 0.617)	65 (1.583 ± 0.104)	41 (10.748 ± 0.644)	25 (5.142 ± 0.239)	5.590 ± 0.663)	ND

Table 15. Frequency of Analyte Detection and Average Concentrations Calculated with Non-zero Measurements Only (Outliers not removed)

This year it was decided to eliminate outliers in order to provide a comparison between this year's data and the previous two years' data (See Table 16). Overall from this comparison it is evident that there are considerably more outliers this year. This is partially due to the fact that more chlorinated compounds were detected this year such as the various isomers of DCE, but it also confirms the fact that the constructed wetland is colonizing somewhat. Considering how many outliers were determined using a standard convention of ±2 standard deviations, it is obvious that these "outliers" may not be what is conventionally considered an outlier. In last year's analysis the outliers were eliminated to simulate that of an ideally behaving constructed wetland. It was suggested that the elimination of outliers was not a good way to gain insight into an idealistic wetland (Clemmer, 2003) and this year's data reinforces that suggestion. The constructed

wetland is an extremely heterogeneous system which is evident from examination of the contour plots in the Appendix.

16a. Data from Fall 2003
(Outliers for layers A, B, and C removed [± 2 standard deviations])

	Average Concentration (ppb ± 95% Confidence Interval)					
Location	**PCE**	**TCE**	**cis-DCE**	**trans-DCE**	**1,1-DCE**	**VC**
Outflow	0.796 ± 0.486	0.821 ± 1.132	ND	2.441 ± 2.699	ND	ND
A	0.066 ± 0.015	0.070 ± 0.013	0	0.078 ± 0.037	ND	3.692 ± 0.776
B	0.101 ± 0.035	0.853 ± 0.152	0.043 ± 0.029	4.345 ± 0.156	0	3.419 ± 0.592
C	7.551 ± 0.604	1.451 ± 0.095	5.582 ± 0.474	1.671 ± 0.207	0.036 ± 0.024	ND
Inflow	23.933 ± 0.608	0.278 ± 0.110	ND	2.441 ± 2.699	ND	0.310 ± 0.608

ND – None Detected

Note: A "0" indicates that all positive readings were eliminated as outliers.
Averages and confidence intervals were determined with 3 samples from the inflow and 3 samples from the outflow. 64 samples were taken for layer A, 64 samples were taken for layer B, and 65 samples were taken for layer C sampling each piezometer once. Number of outliers removed: PCE in A-23, PCE in B-23, PCE in C-35, TCE in A-3, TCE in A-10, TCE in A-57, TCE in A-64, TCE in B-53, TCE in B-59, TCE in C-34, TCE in C-59, TCE in C-66, *cis*-DCE in A-22, *cis*-DCE in A-37, *cis*-DCE in B-11, *cis*-DCE in B-26, *cis*-DCE in B-61, *cis*-DCE in C-34, *cis*-DCE in C-40, *cis*-DCE in C-45, *cis*-DCE in C-64, *trans*-DCE in A-57, *trans*-DCE in A-60, *trans*-DCE in A-61, *trans*-DCE in A-64, *trans*-DCE in A-65, *trans*-DCE in A-66, *trans*-DCE in B-3, *trans*-DCE in C-64, *trans*-DCE in C-65, *trans*-DCE in C-66, *1,1*-DCE in B-31, *1,1*-DCE in C-47, *1,1*-DCE in C-48, *1,1*-DCE in C-50, *1,1*-DCE in C-53, VC in A-39, VC in A-47, VC in B-19, VC in B-25, and VC in B-54.

16b. Data from Jan 2003
(Outliers for layers A, B, and C removed [± 2 standard deviations])

	Average Concentration (ppb ± 95% Confidence Interval)					
Location	**PCE**	**TCE**	**cis-DCE**	**trans-DCE**	**1,1-DCE**	**VC**
Outflow	8.637 ± 0.807	2.138 ± 2.117	ND	ND	ND	ND
A	0.171 ± 0.079	0.173 ± 0.030	0	ND	ND	0.113 ± 0.046
B	0.319 ± 0.139	0.172 ± 0.029	1.346 ± 0.573	ND	ND	0.495 ± 0.171
C	26.266 ± 0.872	0.706 ± 0.044	0	ND	ND	0.021 ± 0.023
Inflow	32.59 ± 0.699	0.627 ± 0.194	ND	ND	ND	ND

ND – None Detected

Note: A "0" indicates that all positive readings were eliminated as outliers.
Averages and confidence intervals were computed with 9 samples for the inflow and 11 samples for the outflow. 66 samples were taken for each layer sampling each piezometer once. Number of outliers removed: PCE in A-7, PCE in B-11, PCE in C-4, TCE in A-10, TCE in B-6, *cis*-DCE in A-13, *cis*-DCE in B-3, *cis*-DCE in C-5, VC in A-6, VC in B-8, and VC in C-3.

16c. Data from Dec 2001
(Outliers for layers A, B, and C removed [± 2 standard deviations])

	Average Concentration (ppb ± 95% Confidence Interval)					
Location	PCE	TCE	cis-DCE	trans-DCE	1,1-DCE	VC
Outflow	5.593 ± 0.615	2.138 ± 2.117	ND	ND	ND	ND
A	0.813 ± 0.083	0.173 ± 0.030	ND	ND	ND	ND
B	1.145 ± 0.105	0.172 ± 0.029	ND	ND	ND	ND
C	27.431 ± 1.358	0.706 ± 0.044	ND	ND	ND	ND
Inflow	33.97 ± 0.920	0.627 ± 0.194	ND	ND	ND	ND

ND – None Detected

Averages and confidence intervals were computed with 12 samples for the inflow and 4 samples for the outflow. Each piezometer was sampled three times and averaged. The piezometer averages in the three layers were then averaged to arrive at the average concentration for the entire layer. Number of outliers removed: PCE in A-10, PCE in B-7, PCE in C-2, TCE in A-9, TCE in B-9, and TCE in C-6.

Table 16. Analyte Average Concentrations (Outliers removed and zero response by GC is included in the calculations as zero)

Trends in Contaminant Concentration

To continue the comparison of data with the previous two efforts, both to determine any seasonal conclusions and to show how the wetland has been maturing, a plot of PCE concentrations was developed with error bars representing the 95% confidence intervals (CI). This also serves as an additional way to visualize the data that was found and draw conclusions.

The PCE concentration in the inflow has significantly decreased in the three years that data has been collected in cell 1 (see Figure 24). At all three layers of the constructed wetland, the concentration of PCE was detected to be significantly less than the previous two efforts. Most importantly, the outflow concentration of PCE was much lower than the December 2001 data and the January 2003 data.

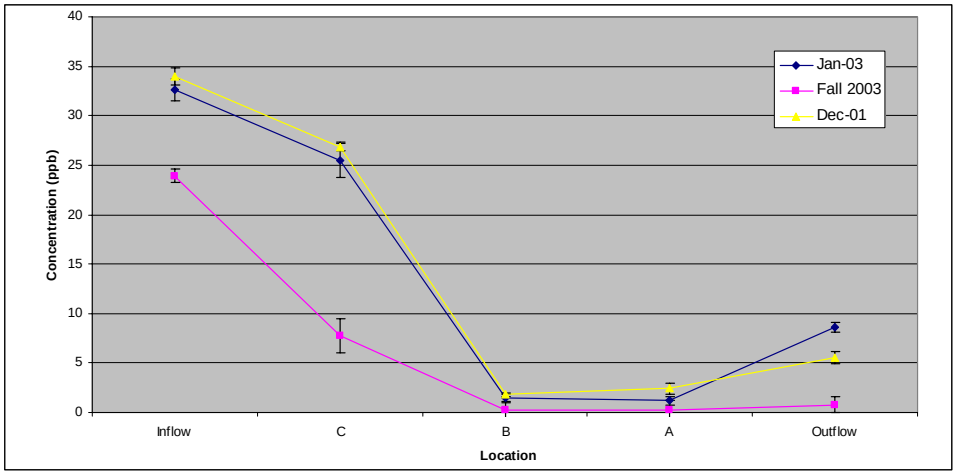


Figure 24. PCE Average Contaminant Trends throughout Three Years (with 95% CIs; including outliers)

V. Conclusions and Recommendations

This study is a follow-up to the previous two years of research on PCE degradation in cell 1 (Opperman, 2002 and Clemmer, 2003) of the constructed wetland at WPAFB. This thesis was conducted in order to study the wetland and determine the mechanisms that exist to degrade the chlorinated solvent contamination that is present. It is also intended to provide additional evidence that the wetland is degrading PCE to its innocuous byproducts. The previous studies included the installation of sampling piezometers, development of a sampling methodology, and the utilization of the purge-and-trap GC to determine contaminant concentrations (Clemmer, 2003). This research very closely followed the previously developed methodologies to determine the concentrations of PCE, TCE, DCE isomers, and VC throughout the three layers of the constructed wetland. Inflow and outflow were also sampled and analyzed. All three data collection efforts thus far have been successful. Each of the three efforts have provided additional insights to the many processes that are happening within the wetland and proving that the PCE is indeed degraded to a level that is within MCLs according to the EPA.

This year's effort provided several sources of information to be added to the previous two years' thesis efforts. The most significant was the discovery of *trans*-DCE and *1,1*-DCE. Additionally, the levels of PCE coming into the wetland through the pump have lessened possibly due to natural processes within the aquifer. The additional data

proved invaluable to provide more insights into the trends that are occurring and to what is happening within the wetland.

PCE has been consistently reduced at the inflow of the wetland indicating that a process is occurring before the water is pumped to the cell. This could be due to more PCE being adsorbed to the soil, natural attenuation in the movement from the aquifer to the wetland cell, or the normal phenomenon associated with pump and treat systems. In pump and treat systems when there are high groundwater concentrations in equilibrium the sorbed chemical is being purged from the system and the lower concentrations represent a desorption rate limitation (Personal Communication, Shelley).

Based on the data collected during this thesis effort, and the data from previous years' efforts, it is clear that Cell 1 is still developing. This wetland cell has been in existence for three years and it is obvious that the development of a constructed wetland is a lengthy process. If a constructed wetland were to be used as a treatment process for contaminated water sources, time would have to be allowed for it to develop before it would reach maximum treatment efficiency.

Answers to Specific Research Questions

1. Do the concentrations of PCE and its daughter products in the three layers of the constructed wetland give further evidence of biodegradation?

Yes, the concentrations of PCE and its daughter products all give evidence that PCE is being degraded. The PCE and TCE concentrations continue to decrease from the

previous years which provide conclusive proof that cell 1 is continuing to develop and continuing to improve in efficiency.

The evidence of additional daughter products of PCE, namely the DCE isomers of *trans*-DCE and *1,1*-DCE, offers more evidence that the PCE is degrading to these intermediate products. Additionally, there was a more prevalent finding of VC throughout layer A in the wetland.

The finding of *cis*-DCE in layer C increased from an average concentration in January of 2003 of 0.311 ppb to 6.780 ppb in the fall of 2003. That is a significant increase which can possibly be correlated with the significant decrease of PCE that was found in layer C this year. Last year an average concentration of 25.533 ppb in layer C was detected compared with an average concentration of 7.790 ppb this year. The comparison of the *cis*-DCE concentration and PCE concentration may provide evidence that the PCE is being reductively dechlorinated to *cis*-DCE in the lower layer of the constructed wetland now that it has matured.

The final and most important development in this year's research is that the water that is coming out of the outflow weir into the wastewater treatment system is now at a level of contamination that is below MCLs. Last year the average PCE concentration being emitted out of the wetland was 8.637 ppb and this year the average PCE concentration was 0.796 ppb with the highest concentration (of three samples) being 1.292 ppb. 1.298 ppb is well below the MCL of 5 ppb set by the EPA. The only other intermediate products that were detected flowing out of the wetland were TCE and *trans*-

DCE. TCE was detected during all three sampling periods and *trans*-DCE was only detected once.

There is no doubt that the PCE is demonstrating further evidence of biodegradation. The constructed wetland is performing in an effective manner and reducing the PCE to a level that is accepted by the EPA.

2. *Do the concentrations of PCE and its daughter products give evidence of seasonal differences in biodegradation (i.e. summer, fall, and winter)?*

As mentioned in Chapter 3, no data was collected in the summer due to equipment inoperability. However, the data collected this year was the first attempt at collecting samples within the fall season. There was a significant decrease in contaminant concentrations when comparing this data with the data of the past two years. Without additional seasonal data though, this contaminant reduction cannot be proven to be due to seasonal differences. It could be due to the wetland's maturation throughout the years or any number of things. It may be important to note that the data in this thesis effort was taken in October and November which was just as the vegetation began to die off.

Effort Strengths

This thesis effort was able to further demonstrate the effectiveness of the previously developed sampling methods and analytical procedures. Only very slight

modifications were deemed necessary to improve the effectiveness of the GC's measurements.

This study also provided connections between the separate analyte's profiles, specifically the PCE profile and the *cis*-DCE profile. It was indicated that the PCE is degrading more significantly within the lower layer of the wetland than had been seen in previous results. A better understanding of the processes occurring within the wetland was determined.

Additionally, this thesis effort demonstrated that the PCE is degrading within the wetland to a level that is acceptable by EPA standards. The daughter products of PCE were also emitted from the wetland at levels below their respective MCLs.

Effort Limitations

The most significant limitation of this thesis effort was the failure to detect ethane, methane, or ethylene. A detailed characterization of the processes that are occurring in the wetland is not possible without these lighter compounds. At the present time it does not seem as though the GC method is set up correctly to detect these compounds. Methane, ethane, and ethylene are generally more easily detected using head space analysis rather than direct sample injection. However, research is being done to provide a method that would enable the FID to detect the aforementioned compounds using direct sample injection (Personal Communication, Amos).

Another limitation to this effort was the inability to determine the flow pattern of the wetland. Insight into how the water is flowing through the wetland would prove

invaluable. It may provide definitive knowledge as to why there are apparent pockets of VC in layer A of the wetland and why PCE is not being degraded at layer C.

There were not any core studies performed to determine how much of the contamination may be adsorbing to the soil. Also, no studies were performed to determine how much of the VC and DCE was volatilized into the atmosphere.

There was no literature found to suggest why the separate isomers (*cis-*, *trans-*, *and 1,1-*) of DCE occurred in specific areas. This year there was the first presence of *trans*-DCE, and *1,1*-DCE and it is unknown as to why they were detected in certain areas of the wetland.

After samples were taken, the last samples remained in the autosampler for up to thirty hours before analysis by the GC. No attempt was made to see if there was an effect on these samples from sitting at room temperature for this significant period of time.

Recommendations for Further Study

1. Sample the wetland during the spring and summer to provide a full picture of the seasonal differences that are occurring in cell 1. Seasonal data from the spring and summer could enable researchers to determine relationships between the microorganisms' activity throughout the year. Conclusions could be drawn to determine when the microorganisms are more actively degrading the contamination and when they are more dormant. Plant activity could also possibly be determined by the seasonal data.

2. Collect and analyze inflow and outflow samples more frequently throughout the year to determine seasonal trends. From the data collected in the past two years there is a possibility that there could be seasonal differences (Clemmer, 2003).
3. Determining the flow pattern of Cell 1 would add much information to this thesis effort. A correlation could be made between the flow pattern and more definite conclusions could possibly be drawn from the data that has already been collected in the previous efforts.
4. Hydrogen data could also be collected to indicate where the hydrogen is being used as an electron donor. Iron concentrations could also be collected to indicate if/where it is being used as an electron acceptor.
5. Methane, ethane, and ethylene data need to be collected. The method used on the GC likely needs to be modified to allow the presence of these three analytes to be detected using the FID.
6. Carbon dioxide concentrations would be valuable to this research study to determine the mechanisms that are occurring to degrade the PCE.
7. Redox data could be collected on a regular basis (i.e. once a week) to determine overall trends that are occurring in specific regions of the constructed wetland. Research shows that redox data needs to be collected frequently over a longer period of time in order to make a legitimate correlation.
8. Determine whether or not the VC concentrations found in layer A are legitimate findings or if there is a reason that the GC is providing a false reading. If the VC is accumulating at such high concentrations in certain areas in the wetland due to

vegetation that may need to be changed in the future or in the design of additional constructed wetlands.

Appendix A: Chemical Concentrations Raw Data for Layer A

Layer A													
Date Collected	Well	PCE (Area Under the Curve)	PCE Concentration (ppb)	TCE (Area Under the Curve)	TCE Concentration (ppb)	*cis*-DCE (Area Under the Curve)	*cis*-DCE Concentration (ppb)	*trans*-DCE (Area Under the Curve)	*trans*-DCE Concentration (ppb)	1,1-DCE (Area Under the Curve)	1,1-DCE Concentration (ppb)	VC (Area Under the Curve)	VC Concentration (ppb)
16-Nov-03	1A	1454.517	0.102	703.419	0.211	0	0	0	0	0	0	482.139	2.445
16-Nov-03	2A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
16-Nov-03	3A	618.840	0.043	112531	33.759	0	0	0	0	0	0	0	0
16-Nov-03	4A	3857.627	0.270	192.566	0.058	0	0	0	0	0	0	114.907	0.583
16-Nov-03	5A	877.105	0.061	93.221	0.028	0	0	0	0	0	0	0	0
16-Nov-03	6A	1328.051	0.093	0	0	0	0	0	0	0	0	188.023	0.954
16-Nov-03	7A	694.104	0.049	0	0	0	0	0	0	0	0	2501.620	12.687
16-Nov-03	8A	758.442	0.053	69.958	0.021	0	0	0	0	0	0	0	0
16-Nov-03	9A	316.077	0.022	0	0	0	0	0	0	0	0	0	0
16-Nov-03	10A	2133.997	0.149	113709.000	34.113	0	0	0	0	0	0	0	0
16-Nov-03	11A	973.233	0.068	229.781	0.069	0	0	0	0	0	0	3696.391	18.747
16-Nov-03	12A	565.497	0.040	0	0	0	0	0	0	0	0	261.971	1.329
16-Nov-03	13A	480.780	0.034	0	0	0	0	0	0	0	0	7740.248	39.256
16-Nov-03	14A	502.292	0.035	0	0	0	0	0	0	0	0	145.201	0.736
16-Nov-03	15A	825.299	0.058	131.013	0.039	0	0	0	0	0	0	0	0
16-Nov-03	16A	413.544	0.029	0	0	0	0	0	0	0	0	0	0
16-Nov-03	17A	485.307	0.034	79.447	0.024	0	0	0	0	0	0	231.566	1.174
16-Nov-03	18A	85.731	0.006	0	0	0	0	0	0	0	0	186.006	0.943
16-Nov-03	19A	0	0	0	0	0	0	0	0	0	0	975.430	4.947
16-Nov-03	20A	462.268	0.032	0	0	0	0	0	0	0	0	0	0
16-Nov-03	21A	465.964	0.033	133.641	0.040	0	0	0	0	0	0	0	0
16-Nov-03	22A	1155.971	0.081	328.720	0.099	34.522	3.162	0	0	0	0	0	0
16-Nov-03	23A	201391.000	14.097	1492.615	0.448	0	0	0	0	0	0	0	0
16-Nov-03	24A	1280.907	0.090	0	0	0	0	0	0	0	0	0	0
16-Nov-03	25A	629.447	0.044	0	0	0	0	0	0	0	0	758.546	3.847
16-Nov-03	26A	1159.757	0.081	153.413	0.046	0	0	0	0	0	0	0	0
16-Nov-03	27A	311.563	0.022	0	0	0	0	0	0	0	0	118.568	0.601
16-Nov-03	28A	313.166	0.022	108.500	0.033	0	0	0	0	0	0	100.821	0.511
16-Nov-03	29A	193.502	0.014	0	0	0	0	0	0	0	0	0	0
16-Nov-03	30A	1452.428	0.102	152.880	0.046	0	0	0	0	0	0	0	0
16-Nov-03	31A	222.457	0.016	0	0	0	0	0	0	0	0	723.773	3.671
16-Nov-03	32A	609.092	0.043	162.697	0.049	0	0	0	0	0	0	0	0
16-Nov-03	33A	0	0	0	0	0	0	0	0	0	0	388.673	1.971
16-Nov-03	34A	0	0	0	0	0	0	0	0	0	0	0	0
16-Nov-03	35A	0	0	0	0	0	0	0	0	0	0	0	0
16-Nov-03	36A	0	0	0	0	0	0	0	0	0	0	0	0
16-Nov-03	37A	0	0	87.631	0.026	69.953	6.408	0	0	0	0	0	0
16-Nov-03	38A	57.681	0.004	96.955	0.029	0	0	0	0	0	0	201.598	1.022
16-Nov-03	39A	0	0	0	0	0	0	0	0	0	0	58812.800	298.279
16-Nov-03	40A	0	0	0	0	0	0	0	0	0	0	298.290	1.513
16-Nov-03	41A	3633.549	0.254	237.862	0.071	0	0	0	0	0	0	171.798	0.871
16-Nov-03	42A	0	0	0	0	0	0	0	0	0	0	0	0
16-Nov-03	43A	0	0	91.065	0.027	0	0	0	0	0	0	0	0
16-Nov-03	44A	0	0	0	0	0	0	0	0	0	0	0	0
16-Nov-03	45A	0	0	0	0	0	0	0	0	0	0	138.738	0.704
16-Nov-03	46A	0	0	0	0	0	0	0	0	0	0	0	0
16-Nov-03	47A	0	0	0	0	0	0	0	0	0	0	22557.700	114.405
16-Nov-03	48A	933.237	0.065	74.048	0.022	0	0	0	0	0	0	197.645	1.002
16-Nov-03	49A	0	0	0	0	0	0	0	0	0	0	3456.086	17.528
16-Nov-03	50A	0	0	0	0	0	0	0	0	0	0	3039.997	15.418
16-Nov-03	51A	0	0	0	0	0	0	0	0	0	0	3816.139	19.354
16-Nov-03	52A	0	0	0	0	0	0	0	0	0	0	211.642	1.073
16-Nov-03	53A	639.164	0.045	721.286	0.216	0	0	0	0	0	0	784.210	3.977
16-Nov-03	54A	0	0	606.266	0.182	0	0	57.953	2.283	0	0	0	0
16-Nov-03	55A	241.001	0.017	314.479	0.094	0	0	0	0	0	0	2806.009	14.231
16-Nov-03	56A	224.285	n/a	198.312	0.059	0	0	0	0	0	0	9998.658	50.710
16-Nov-03	57A	11210.100	0.785	131804.000	39.541	0	0	119.929	4.725	0	0	182.224	0.924
16-Nov-03	58A	274.620	0.019	279.025	0.084	0	0	0	0	0	0	187.682	0.952
16-Nov-03	59A	162.693	0.011	310.930	0.093	0	0	0	0	0	0	0	0
16-Nov-03	60A	212.346	0.015	731.737	0.220	0	0	84.373	3.324	0	0	300.191	1.522
16-Nov-03	61A	20.238	0.001	881.452	0.264	0	0	84.656	3.335	0	0	238.238	1.208
16-Nov-03	62A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
16-Nov-03	63A	0	0	316.629	0.095	0	0	57.402	2.262	0	0	192.473	0.976
16-Nov-03	64A	16698.400	1.169	136283.000	40.885	0	0	149.320	5.883	0	0	128.276	0.651
16-Nov-03	65A	146.947	0.010	2287.640	0.686	0	0	106.480	4.195	0	0	176.049	0.893
16-Nov-03	66A	255.441	0.018	2683.414	0.805	0	0	123.669	4.873	0	0	0	0

Appendix B: Chemical Concentrations Raw Data for Layer B

Layer B													
Date Collected	Well	PCE (Area Under the Curve)	PCE Concentration (ppb)	TCE (Area Under the Curve)	TCE Concentration (ppb)	*cis*-DCE (Area Under the Curve)	*cis*-DCE Concentration (ppb)	*trans*-DCE (Area Under the Curve)	*trans*-DCE Concentration (ppb)	*1,1*-DCE (Area Under the Curve)	*1,1*-DCE Concentration (ppb)	VC (Area Under the Curve)	VC Concentration (ppb)
11-Nov-03	1B	3300.361	0.231	919.205	0.276	0	0	0	0	0	0	1620.752	8.220
11-Nov-03	2B	1060.861	0.074	1934.734	0.580	0	0	58.409	2.301	0	0	474.421	2.406
11-Nov-03	3B	44052.700	3.084	46737.600	14.021	0	0	318.046	12.531	0	0	606.686	3.077
11-Nov-03	4B	6393.968	0.448	6002.052	1.801	0	0	219.405	8.645	0	0	555.373	2.817
11-Nov-03	5B	408.999	0.029	921.461	0.276	0	0	69.378	2.733	0	0	0	0
11-Nov-03	6B	134.108	0.009	233.638	0.070	0	0	54.662	2.154	0	0	0	0
11-Nov-03	7B	209.735	0.015	265.381	0.080	0	0	82.467	3.249	0	0	287.679	1.459
11-Nov-03	8B	226.280	0.016	210.821	0.063	0	0	62.020	2.444	0	0	0	0
11-Nov-03	9B	129.710	0.009	262.940	0.079	0	0	68.188	2.687	0	0	0	0
11-Nov-03	10B	945.582	0.066	945.582	0.284	0	0	133.942	5.277	0	0	0	0
11-Nov-03	11B	13900.300	0.973	1025.448	0.308	56.910	5.213	58.158	2.291	0	0	0	0
11-Nov-03	12B	85.750	0.006	377.751	0.113	0	0	79.982	3.151	0	0	202.581	1.027
11-Nov-03	13B	190.857	0.013	1602.380	0.481	0	0	141.899	5.591	0	0	120.672	0.612
11-Nov-03	14B	147.047	0.010	301.373	0.090	0	0	76.805	3.026	0	0	82.406	0.418
11-Nov-03	15B	94.729	0.007	604.180	0.181	0	0	83.829	3.303	0	0	60.347	0.306
11-Nov-03	16B	601.135	0.042	1448.548	0.435	0	0	126.341	4.978	0	0	183.735	0.932
11-Nov-03	17B	126.720	0.009	439.020	0.132	0	0	63.360	2.496	0	0	0	0
11-Nov-03	18B	0	0	1569.821	0.471	0	0	111.637	4.399	0	0	150.992	0.766
11-Nov-03	19B	54.389	0.004	358.920	0.108	0	0	65.704	2.589	0	0	11516.900	58.410
11-Nov-03	20B	13.811	0.001	1927.579	0.578	0	0	131.759	5.191	0	0	623.948	3.164
11-Nov-03	21B	3394.675	0.238	2739.258	0.822	0	0	66.358	2.614	0	0	0	0
11-Nov-03	22B	1270.322	0.089	3033.339	0.910	28.410	2.602	134.851	5.313	0	0	0	0
11-Nov-03	23B	177833.000	12.448	1608.431	0.483	0	0	77.849	3.067	0	0	0	0
11-Nov-03	24B	565.678	0.040	3614.852	1.084	0	0	149.869	5.905	0	0	0	0
11-Nov-03	25B	115.352	0.008	761.912	0.229	0	0	81.770	3.222	0	0	12011.700	60.919
11-Nov-03	26B	28.015	0.002	1236.708	0.371	75.784	6.942	85.021	3.350	0	0	0	0
11-Nov-03	27B	92.415	0.006	992.415	0.298	0	0	98.730	3.890	0	0	264.698	1.342
11-Nov-03	28B	0	0	2435.605	0.731	0	0	122.082	4.810	0	0	102.128	0.518
11-Nov-03	29B	0	0	3141.466	0.942	0	0	141.771	5.586	0	0	0	0
11-Nov-03	30B	0	0	2694.340	0.808	0	0	137.441	5.415	0	0	0	0
11-Nov-03	31B	23.598	0.002	4096.112	1.229	0	0	149.551	5.892	153.89	15.40	2187.416	11.094
11-Nov-03	32B	4877.587	0.341	5136.547	1.541	0	0	134.971	5.318	0	0	0	0
11-Nov-03	33B	1039.897	0.073	3277.496	0.983	0	0	148.463	5.849	0	0	142.397	0.722
11-Nov-03	34B	0	0	667.336	0.200	0	0	94.791	3.735	0	0	0	0
11-Nov-03	35B	0	0	137.183	0.041	0	0	0	0	0	0	0	0
11-Nov-03	36B	0	0	3395.739	1.019	0	0	150.011	5.910	0	0	1084.054	5.498
11-Nov-03	37B	1128.239	0.079	5279.434	1.584	0	0	149.673	5.897	0	0	113.941	0.578
11-Nov-03	38B	299.553	0.021	5492.901	1.648	0	0	145.224	5.722	0	0	98.951	0.502
11-Nov-03	39B	0	0	5191.333	1.557	0	0	169.042	6.660	0	0	0	0
11-Nov-03	40B	0	0	4248.953	1.275	0	0	139.446	5.494	0	0	543.321	2.756
11-Nov-03	41B	2951.511	0.207	1541.967	0.463	0	0	93.023	3.665	0	0	768.939	3.900
11-Nov-03	42B	0	0	3525.576	1.058	0	0	131.563	5.184	0	0	172.111	0.873
11-Nov-03	43B	0	0	863.232	0.259	0	0	73.617	2.900	0	0	0	0
11-Nov-03	44B	0	0	1397.272	0.419	0	0	81.806	3.223	0	0	738.013	3.743
11-Nov-03	45B	0	0	546.763	0.164	0	0	83.958	3.308	0	0	105.584	0.535
11-Nov-03	46B	146.229	0.010	5860.734	1.758	0	0	172.622	6.801	0	0	2521.439	12.788
11-Nov-03	47B	72.012	0.005	5134.726	1.540	0	0	155.430	6.124	0	0	192.391	0.976
11-Nov-03	48B	0	0	4006.861	1.202	0	0	132.444	5.218	0	0	5119.547	25.965
11-Nov-03	49B	128.656	0.009	4881.823	1.465	0	0	147.508	5.812	0	0	136.340	0.691
11-Nov-03	50B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
11-Nov-03	51B	0	0	1468.862	0.441	0	0	79.307	3.125	0	0	225.922	1.146
11-Nov-03	52B	0	0	4493.515	1.348	0	0	136.696	5.386	0	0	273.002	1.385
11-Nov-03	53B	1083.065	0.076	163362.000	49.009	0	0	137.501	5.418	0	0	0	0
11-Nov-03	54B	0	0	1212.128	0.364	0	0	80.057	3.154	0	0	9163.575	46.475
11-Nov-03	55B	0	0	290.328	0.087	0	0	60.899	2.399	0	0	4424.442	22.439
11-Nov-03	56B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
11-Nov-03	57B	0	0	336.887	0.101	0	0	83.013	3.271	0	0	416.192	2.111
11-Nov-03	58B	0	0	1254.941	0.376	0	0	144.664	5.700	0	0	0	0
11-Nov-03	59B	1014.032	0.071	138496.000	41.549	0	0	85.933	3.386	0	0	0	0
11-Nov-03	60B	0	0	1060.204	0.318	0	0	122.556	4.829	0	0	4986.427	25.290
11-Nov-03	61B	0	0	403.598	0.121	69.695	6.384	88.330	3.480	0	0	4257.658	21.593
11-Nov-03	62B	0	0	322.017	0.097	0	0	83.470	3.289	0	0	498.706	2.529
11-Nov-03	63B	0	0	343.543	0.103	0	0	77.083	3.037	0	0	124.098	0.629
11-Nov-03	64B	0	0	3271.640	0.981	0	0	212.061	8.355	0	0	1081.792	5.486
11-Nov-03	65B	415.495	0.029	3439.048	1.032	0	0	197.191	7.769	0	0	208.659	1.058
11-Nov-03	66B	0	0	3363.663	1.009	0	0	222.621	8.771	0	0	5362.012	27.194

Appendix C: Chemical Concentrations Raw Data for Layer C

Layer C

Well	PCE (Area Under the Curve)	PCE Concentration (ppb)	TCE (Area Under the Curve)	TCE Concentration (ppb)	*cis*-DCE (Area Under the Curve)	*cis*-DCE Concentration (ppb)	*trans*-DCE (Area Under the Curve)	*trans*-DCE Concentration (ppb)	*1,1*-DCE (Area Under the Curve)	*1,1*-DCE Concentration (ppb)	VC (Area Under the Curve)	VC Concentration (ppb)
1C	177725.000	12.441	11006.800	3.302	0	0	0	0	0	0	0	0
2C	113217.000	7.925	9676.089	2.903	42.266	3.872	0	0	0	0	0	0
3C	15836.500	1.109	5287.254	1.586	78.852	7.223	0	0	0	0	0	0
4C	51019.900	3.571	8043.515	2.413	54.079	4.954	0	0	0	0	0	0
5C	80097.200	5.607	9592.052	2.878	42.941	3.933	0	0	0	0	0	0
6C	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
7C	1062.540	0.074	4370.977	1.311	111.807	10.242	0	0	0	0	0	0
8C	119514.000	8.366	6038.340	1.812	0	0	0	0	0	0	0	0
9C	193690.000	13.558	2170.054	0.651	0	0	0	0	0	0	0	0
10C	152916.000	10.704	3357.386	1.007	0	0	0	0	0	0	0	0
11C	123411.000	8.639	6124.899	1.837	0	0	0	0	0	0	0	0
12C	142253.000	9.958	3458.915	1.038	0	0	0	0	0	0	0	0
13C	24399.400	1.708	4839.583	1.452	96.760	8.863	0	0	0	0	0	0
14C	299207.000	20.944	823.478	0.247	0	0	0	0	0	0	0	0
15C	66170.800	4.632	6680.895	2.004	75.070	6.876	0	0	0	0	0	0
16C	291499.000	20.405	1115.442	0.335	0	0	0	0	0	0	0	0
17C	42654.500	2.986	10545.300	3.164	84.149	7.708	0	0	0	0	0	0
18C	102013.000	7.141	7622.846	2.287	0	0	0	0	0	0	0	0
19C	1544.752	0.108	514.591	0.154	102.222	9.363	0	0	0	0	0	0
20C	293218.000	20.525	1394.422	0.418	0	0	0	0	0	0	0	0
21C	302223.000	21.156	809.491	0.243	0	0	0	0	0	0	0	0
22C	301043.000	21.073	510.140	0.153	0	0	0	0	0	0	0	0
23C	298004.000	20.860	1845.597	0.554	0	0	0	0	0	0	0	0
24C	195842.000	13.709	2308.927	0.693	0	0	0	0	0	0	0	0
25C	17464.200	1.222	1244.185	0.373	122.159	11.190	0	0	0	0	0	0
26C	250193.000	17.514	1888.457	0.567	0	0	0	0	0	0	0	0
27C	32152.300	2.251	11431.300	3.429	90.625	8.301	0	0	0	0	0	0
28C	87312.000	6.112	8116.506	2.435	74.571	6.831	0	0	0	0	0	0
29C	4852.168	0.340	1490.856	0.447	0	0	0	0	0	0	0	0
30C	211856.000	14.830	1514.922	0.454	0	0	0	0	0	0	0	0
31C	108629.000	7.604	8583.082	2.575	78.008	7.146	89.855	3.540	0	0	0	0
32C	179895.000	12.593	6396.873	1.919	37.956	3.477	0	0	0	0	0	0
33C	21869.400	1.531	2428.562	0.729	154.853	14.185	112.700	4.440	0	0	0	0
34C	16032.000	1.122	15010.000	4.503	251.249	23.014	80.836	3.185	0	0	0	0
35C	329487.000	23.064	1038.273	0.311	0	0	0	0	0	0	0	0
36C	247790.000	17.345	2663.968	0.799	53.798	4.928	0	0	0	0	0	0
37C	5550.573	0.389	1059.766	0.318	131.657	12.060	0	0	0	0	0	0
38C	234795.000	16.436	3118.808	0.936	0	0	0	0	0	0	0	0
39C	2775.797	0.194	627.158	0.188	53.263	4.879	100.498	3.960	0	0	0	0
40C	1717.447	0.120	1867.028	0.560	284.116	26.025	0	0	0	0	0	0
41C	68951.700	4.827	10745.200	3.224	111.998	10.259	64.508	2.542	0	0	0	0
42C	31115.400	2.178	12264.000	3.679	139.179	12.749	78.942	3.110	0	0	0	0
43C	1649.648	0.115	1969.914	0.591	105.499	9.664	67.887	2.675	0	0	0	0
44C	230469.000	16.133	5352.187	1.606	0	0	0	0	47.27	2.18142	0	0
45C	2715.279	0.190	2637.599	0.791	305.908	28.021	101.382	3.994	0	0	0	0
46C	1079.732	0.076	771.770	0.232	181.992	16.670	73.979	2.915	0	0	0	0
47C	166923.000	11.685	7338.568	2.202	0	0	0	0	178.31	8.22785	0	0
48C	212995.000	14.910	6721.571	2.016	0	0	0	0	121.46	5.60476	0	0
49C	2230.312	0.156	576.323	0.173	127.756	11.702	91.749	3.615	0	0	0	0
50C	222919.000	15.604	6114.510	1.834	0	0	0	0	139.46	6.43545	0	0
51C	103638.000	7.255	12194.000	3.658	73.011	6.688	153.484	6.047	0	0	0	0
52C	21727.100	1.521	9127.234	2.738	106.023	9.712	129.377	5.097	0	0	0	0
53C	310158.000	21.711	877.004	0.263	0	0	0	0	119.15	5.49814	0	0
54C	158814.000	11.117	5352.875	1.606	57.123	5.233	160.683	6.331	0	0	0	0
55C	11258.400	0.788	4068.214	1.220	112.696	10.323	122.954	4.844	0	0	0	0
56C	78987.500	5.529	13190.200	3.957	55.365	5.071	142.211	5.603	0	0	0	0
57C	7095.455	0.497	7669.268	2.301	137.084	12.557	131.397	5.177	0	0	0	0
58C	43647.000	3.055	10741.900	3.223	94.060	8.616	157.171	6.193	0	0	0	0
59C	122828.000	8.598	13579.200	4.074	61.978	5.677	160.428	6.321	0	0	0	0
60C	155332.000	10.873	6586.129	1.976	74.222	6.799	186.226	7.337	0	0	0	0
61C	3598.830	0.252	3945.382	1.184	138.873	12.721	156.139	6.152	0	0	0	0
62C	2684.269	0.188	3179.705	0.954	157.967	14.470	132.910	5.237	0	0	0	0
63C	2208.183	0.155	926.649	0.278	194.708	17.835	133.411	5.256	0	0	0	0
64C	3366.277	0.236	3039.969	0.912	252.312	23.112	216.170	8.517	0	0	0	0
65C	5237.361	0.367	2900.198	0.870	212.680	19.481	204.923	8.074	0	0	0	0
66C	121021.000	8.471	14578.300	4.373	89.997	8.244	212.643	8.378	0	0	0	0

Appendix D: Inflow and Outflow Concentrations

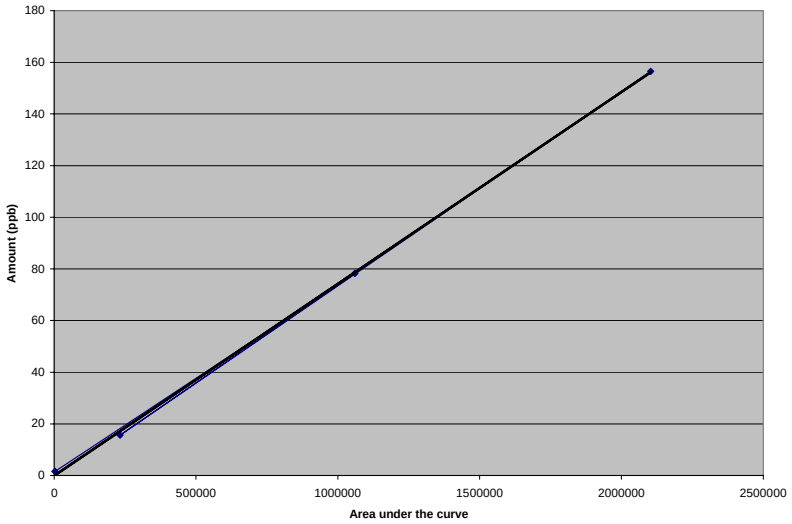
Inflow Concentrations (Analytes not shown were not detected)								
	PCE Area	PCE Concentration	TCE Area	TCE Concentration	t-DCE Area	t-DCE Concentration	VC Area	VC Concentration
24-Oct-03	355850	24.910	656.86	0.197	0	0	0	0
11-Nov-03	342790	23.995	1286.58	0.386	120.98	4.767	0	0
16-Nov-03	327049	22.893	832.97	0.250	64.878	2.556	183.5	0.930734
Average	341896	23.933	925.47	0.278	61.95	2.441	61.167	0.310
Outflow Concentrations (Analytes not shown were not detected)								
	PCE Area	PCE Concentration	TCE Area	TCE Concentration	t-DCE Area	t-DCE Concentration		
24-Oct-03	18452	1.292	739.76	0.222	0	0		
11-Nov-03	7693.5	0.539	6587.16	1.976	236.28	4.767		
16-Nov-03	7948.2	0.556	885.36	0.266	59.89	2.556		
Average	11364.6	0.796	2737.43	0.821	98.72	2.441		

Appendix E: Calibration curves for PCE, TCE, *cis*-DCE, *trans*-DCE, *1,1*-DCE, and VC

These calibration curves were prepared using Microsoft Excel. Each curve was generated using four concentrations of a standard solution (1.565, 15.65, 78.25, 156.5 ppb). Each curve was forced through zero which resulted in R-squared values of over 0.99 for each of the analytes.

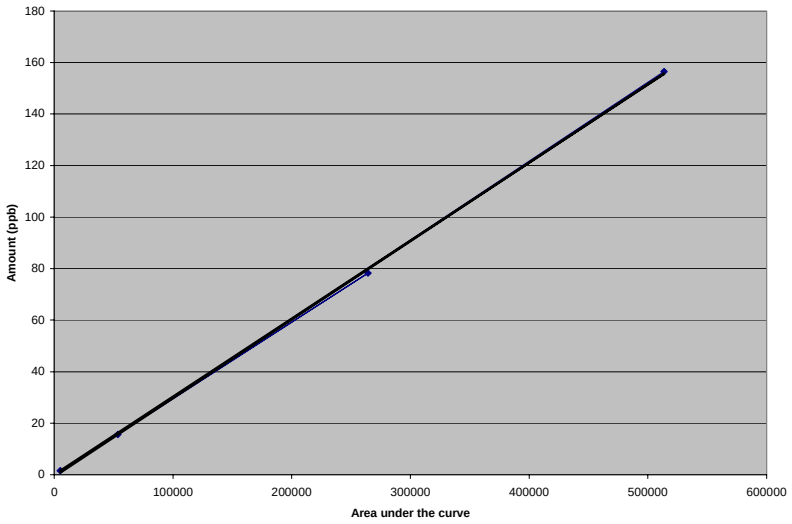
I. Calibration curve for PCE

Amount (ppb) = 7E-5*(Area under the curve), R^2=0.9997



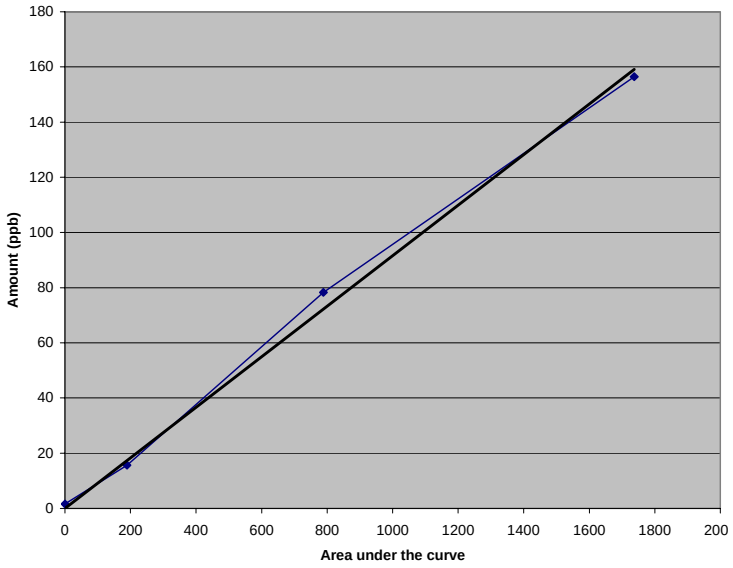
II. Calibration curve for TCE

Amount (ppb) = 0.0003*(Area under the curve), R^2=0.9997



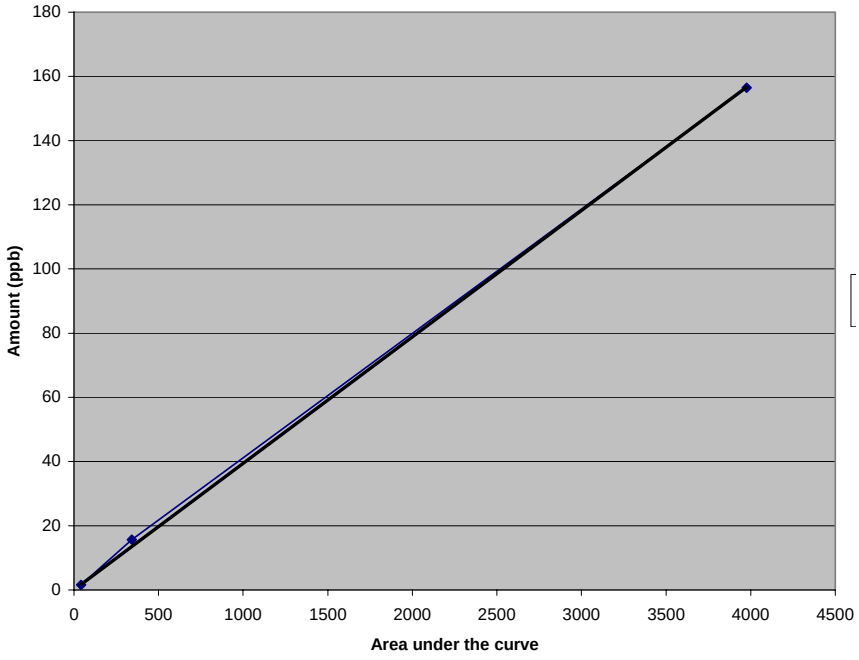
III. Calibration curve for *cis*-DCE

Amount (ppb) = 0.0916*(Area under the curve), R^2=0.9968

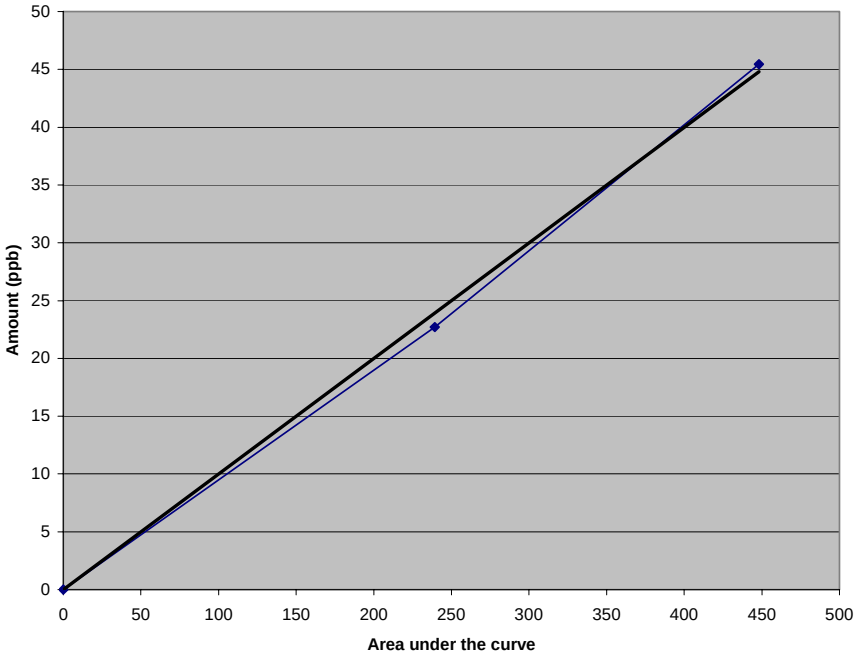


IV. Calibration curve for *trans*-DCE

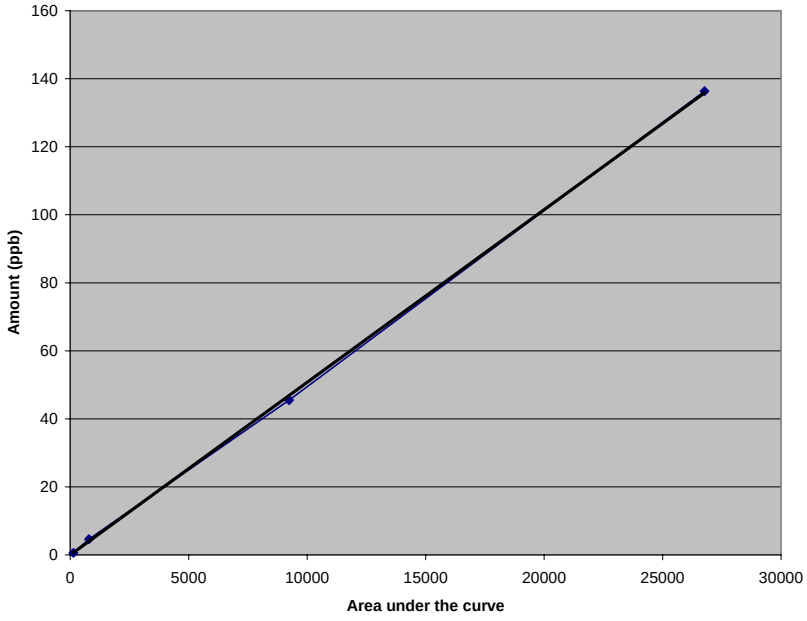
Amount (ppb) = 0.0394*(Area under the curve), R^2=0.9997



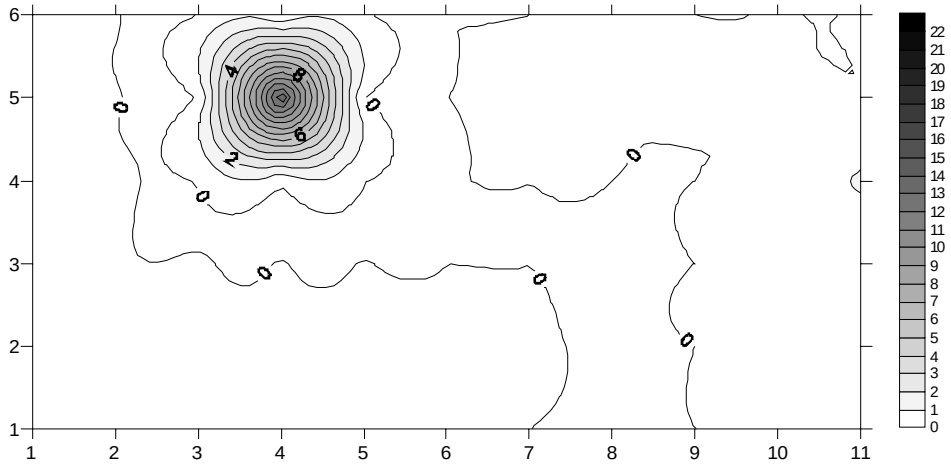
V. Calibration curve for *1,1*-DCE
Amount (ppb) = 0.1*(Area under the curve), R^2=0.9982



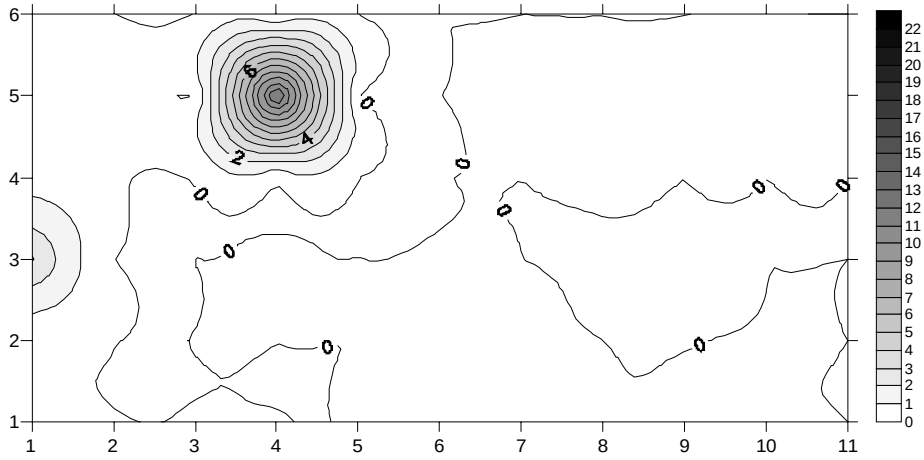
VI. Calibration curve for VC
Amount (ppb) = 0.0051*(Area under the curve), R^2=0.9998



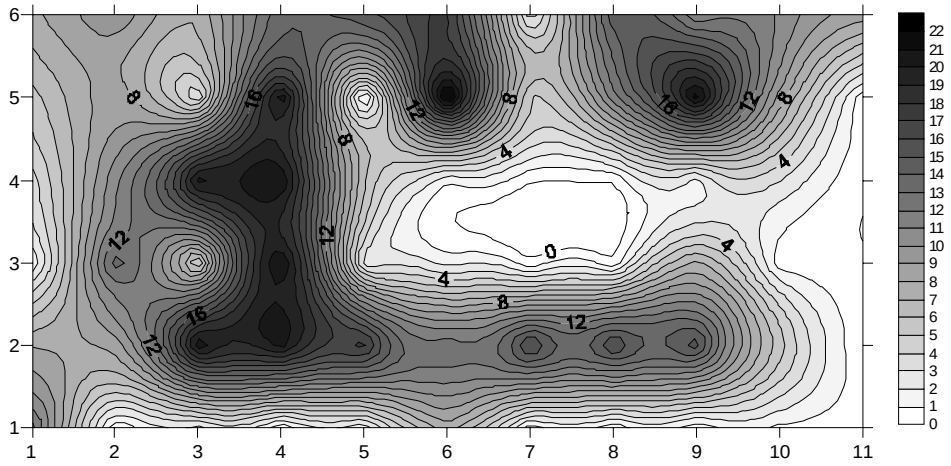
Appendix F: PCE Contour Plots (Oct-Nov 03)



PCE Layer A

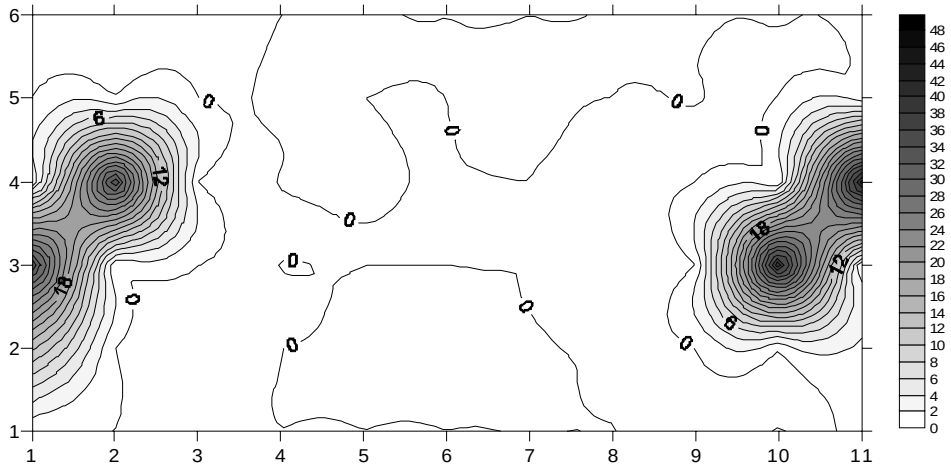


PCE Layer B

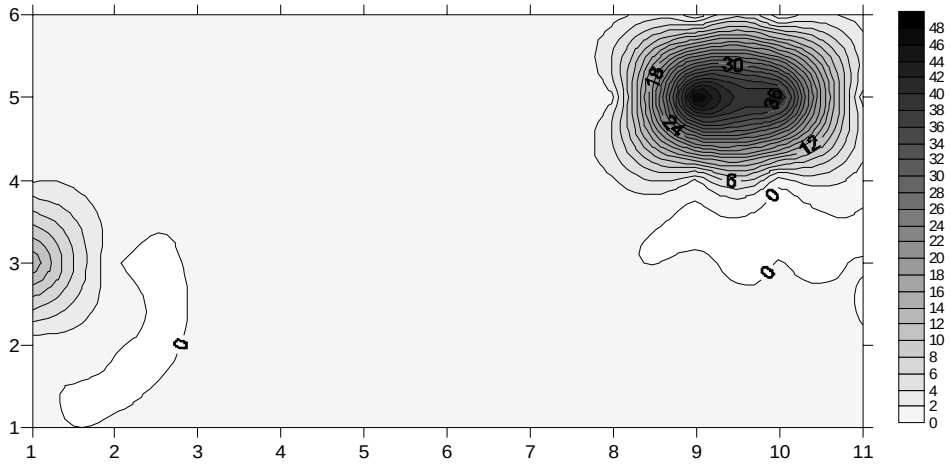


PCE Layer C

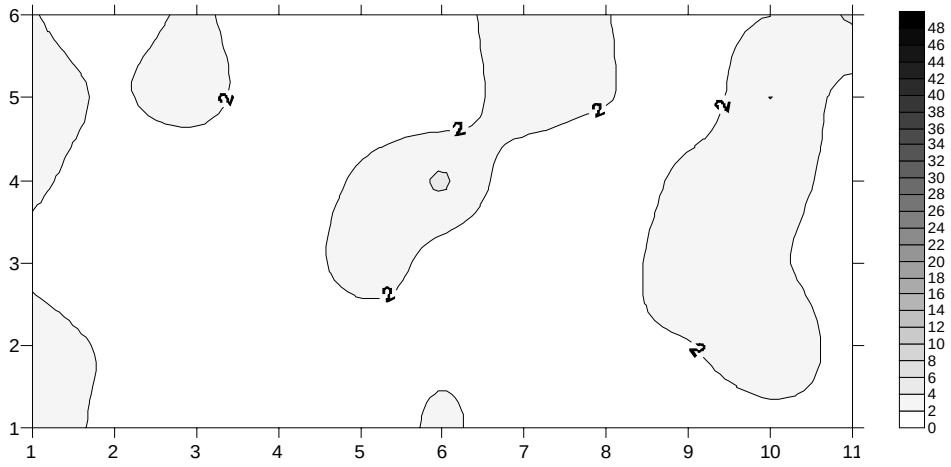
Appendix G: TCE Contour Plots (Oct-Nov 03)



TCE Layer A

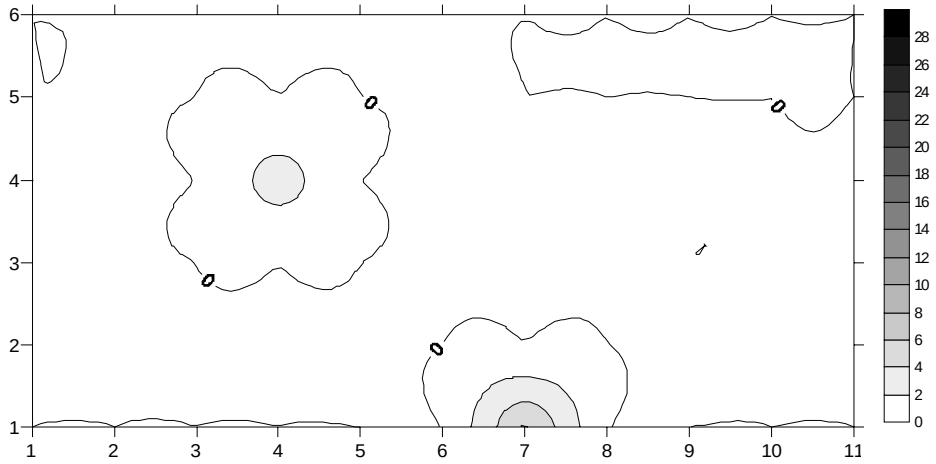


TCE Layer B

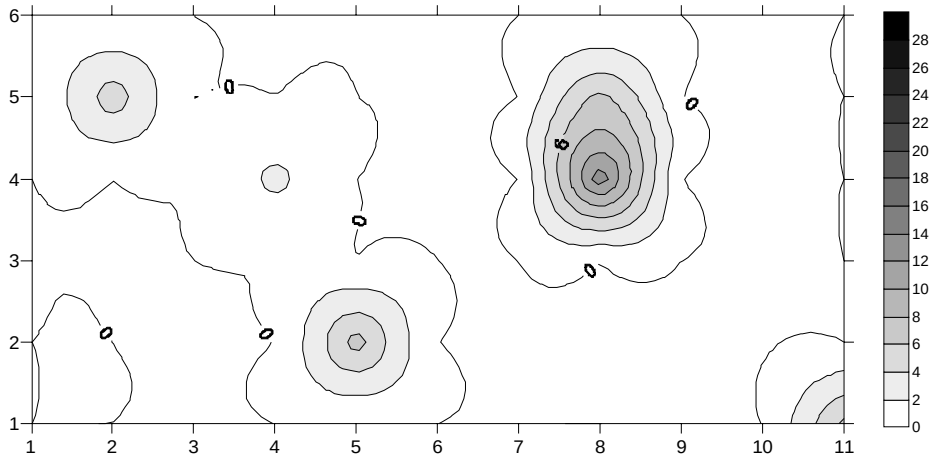


TCE Layer C

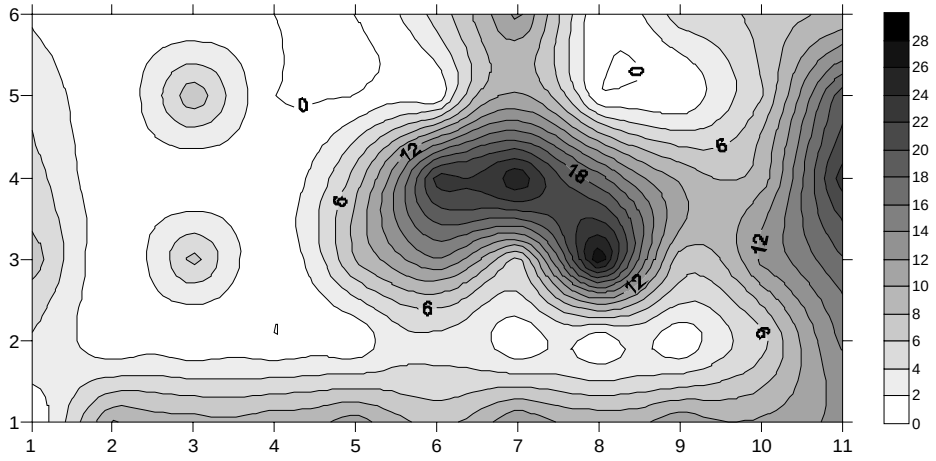
Appendix H: *cis*-DCE Contour Plots (Oct-Nov 03)



cis-DCE Layer A

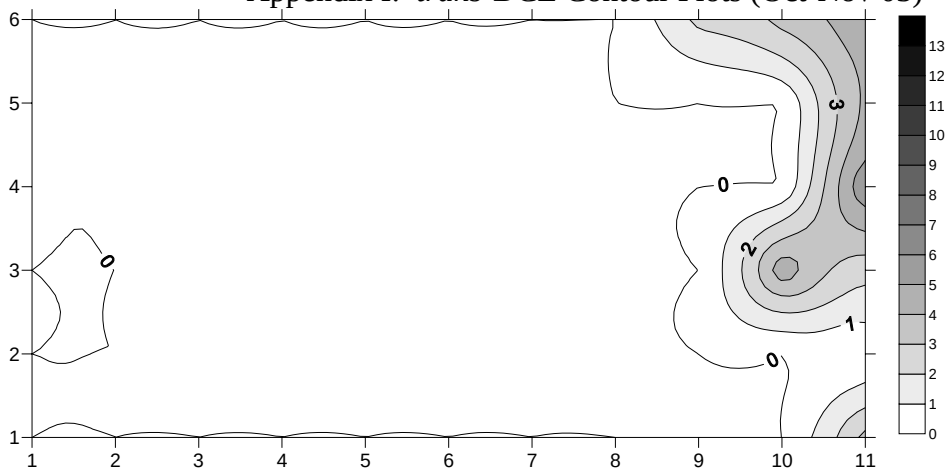


cis-DCE Layer B

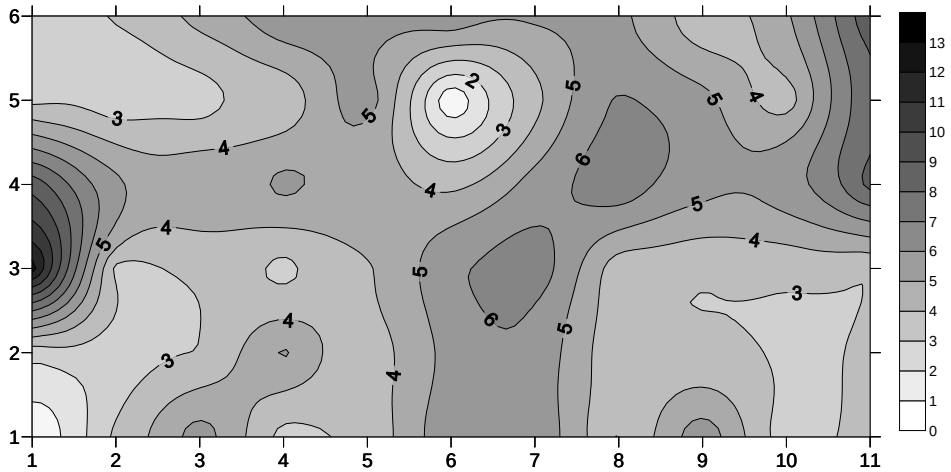


cis-DCE Layer C

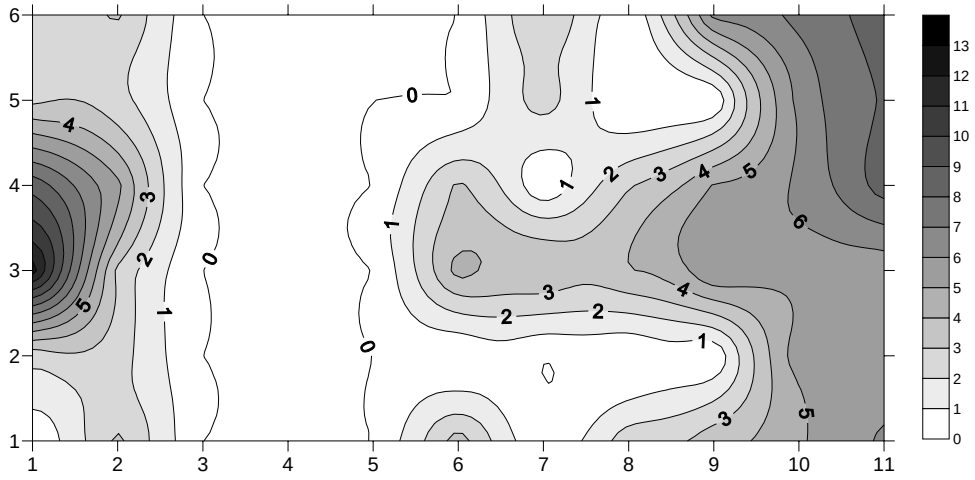
Appendix I: *trans*-DCE Contour Plots (Oct-Nov 03)



trans-DCE Layer A



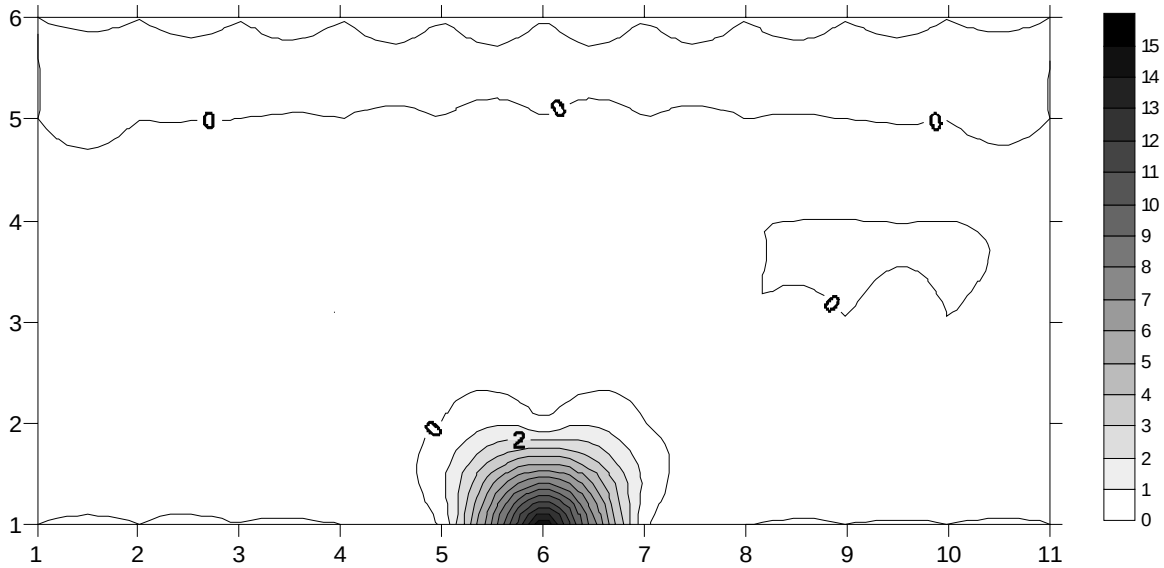
trans-DCE Layer B

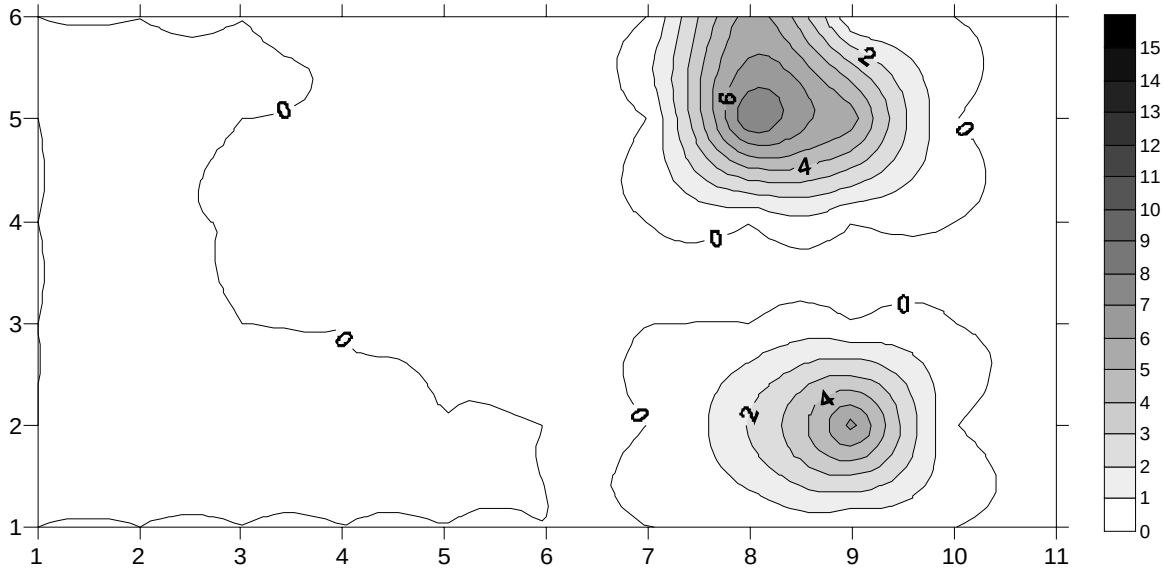


trans-DCE Layer C

Appendix J: *1,1*-DCE Contour Plots (Oct-Nov 03)

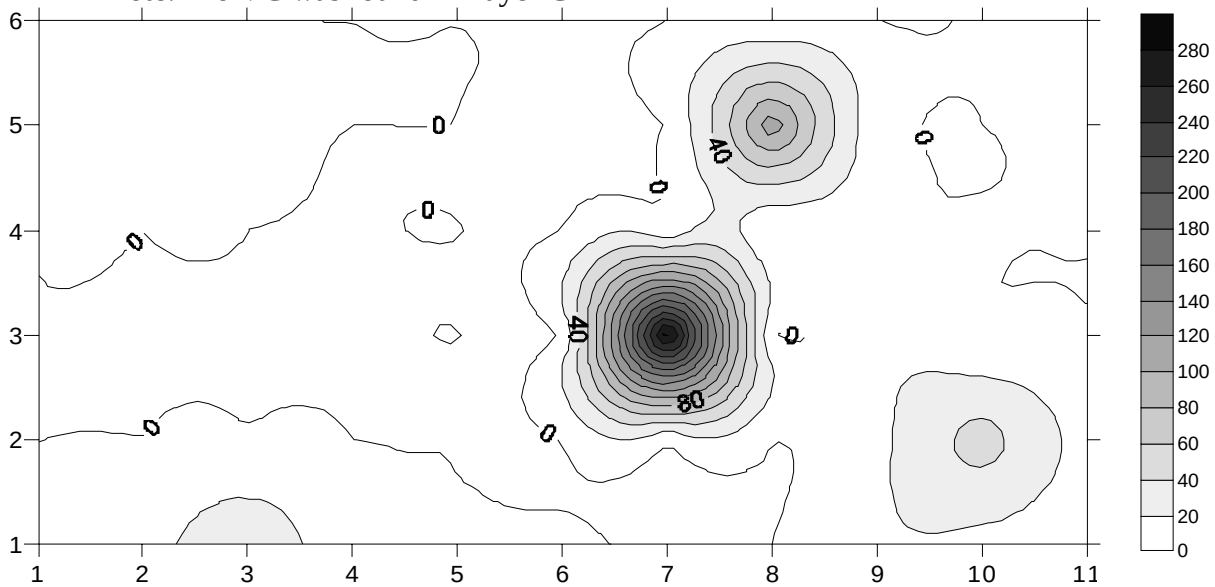
Note: No *1,1*-DCE was found in Layer A



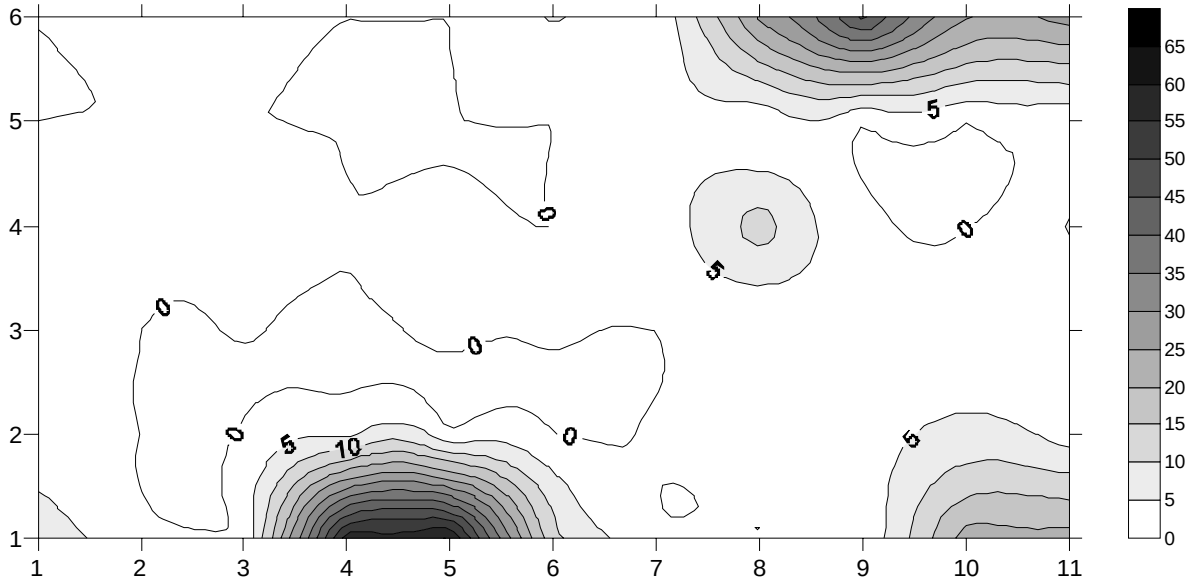


Appendix K: VC Contour Plots (Oct-Nov 03)

Note: No VC was found in Layer C

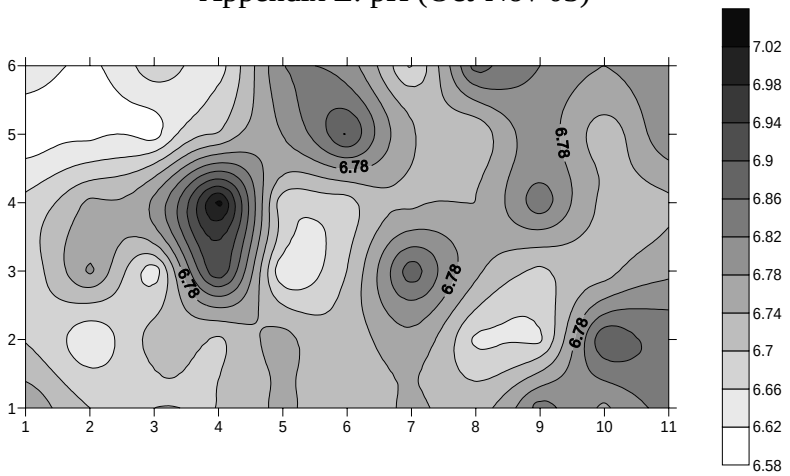


VC Layer A

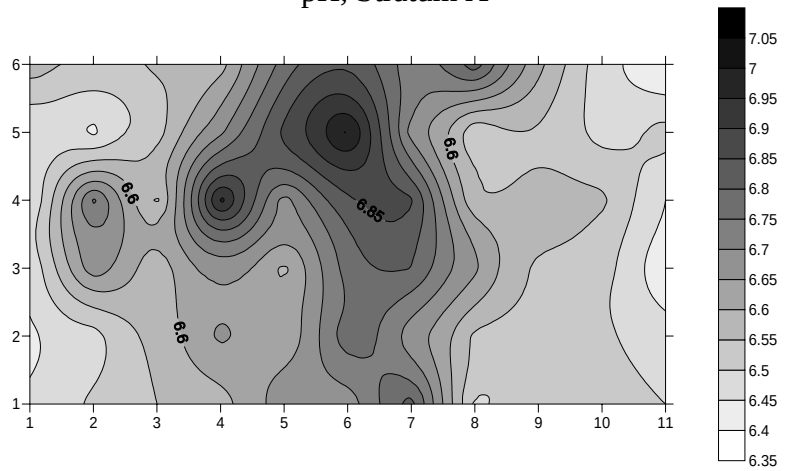


VC Layer B

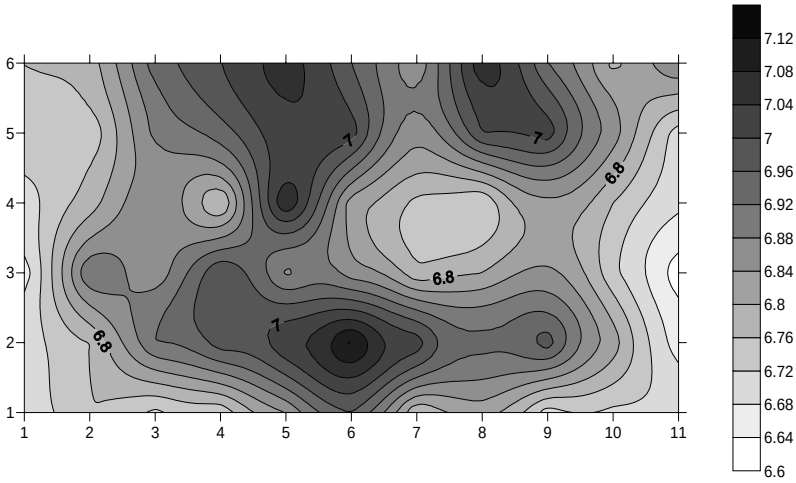
Appendix L: pH (Oct-Nov 03)



pH, Stratum A

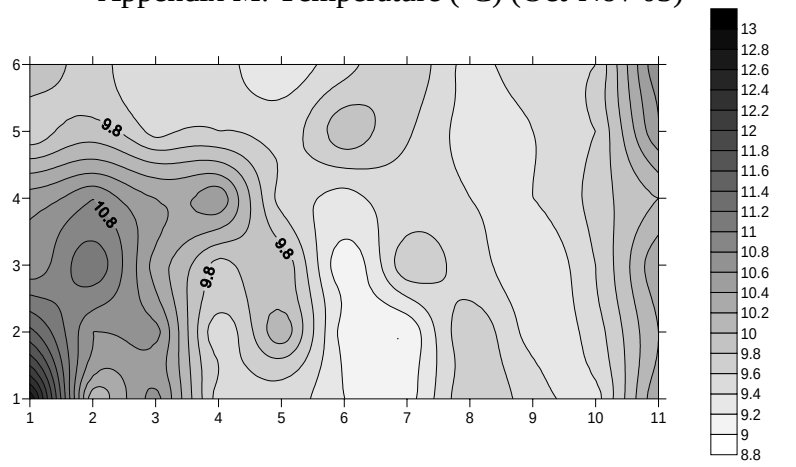


pH, Stratum B

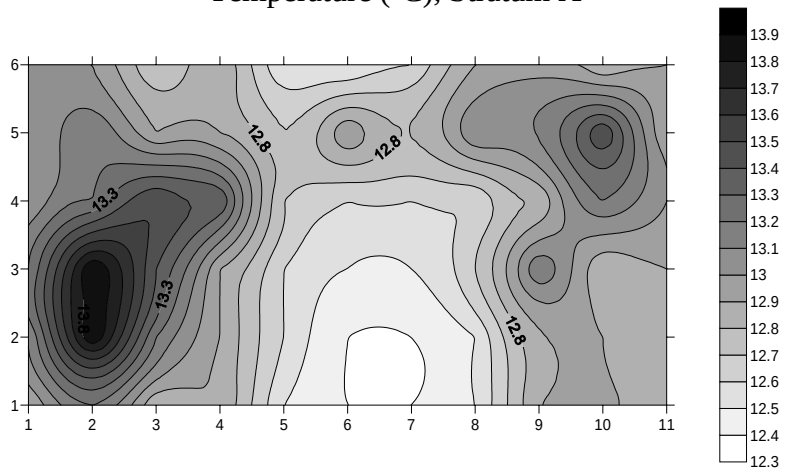


pH, Stratum C

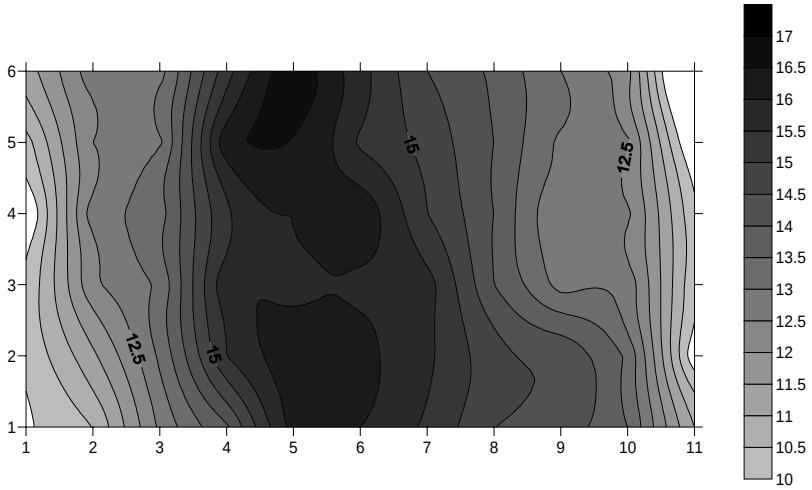
Appendix M: Temperature (˚C) (Oct-Nov 03)



Temperature (˚C), Stratum A



Temperature (˚C), Stratum B



Temperature (˚C), Stratum C

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Vita

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14. ABSTRACT

Perchloroethene (PCE) and its degradation products are among the most common organic groundwater contaminants in the United States. Constructed wetlands are a relatively new approach to dealing with this contamination problem. With their upward flow capability it is possible to introduce an aerobic and anaerobic environment with a consortium of microorganisms available to degrade the contaminants to within acceptable levels established by the Environmental Protection Agency (EPA). This study is a follow-up to the previous two years of research on PCE degradation in cell 1 at Wright-Patterson Air Force Base. This thesis was conducted in order to study the wetland and determine the mechanisms that exist to degrade the chlorinated solvent contamination that is present. It also provided additional evidence that the constructed wetland is degrading PCE to its innocuous byproducts. A purge-and-trap gas chromatograph was used to determine the concentrations of PCE, TCE, DCE isomers, and VC throughout the three layers of the constructed wetland. Inflow and outflow were also sampled and analyzed. In this year's data, PCE was detected at a level that was below the maximum contaminant level established by the EPA. However, it is clear that Cell 1 is still developing. This wetland cell has been in existence for three years and it is obvious that the development of a constructed wetland is a lengthy process. If a constructed wetland were to be used as a treatment process for contaminated water sources, time would have to be allowed for it to develop before it would reach maximum treatment efficiency.

15. SUBJECT TERMS
Constructed Wetlands, Chlorinated Ethenes, Reductive Dechlorination, Gas Chromatography, Perchloroethylene, Direct Oxidation, Cometabolic Oxidation

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