Sustainability of Reductive Dechlorination at Chlorinated Solvent Contaminated Sites: Metrics for Assessing Potentially Bioavailable Natural Organic Carbon in Aquifer Sediments

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ABSTRACT

Groundwater remediation strategies have advanced toward more effective and economical remedial technologies. Monitored natural attenuation (MNA) has become accepted by federal regulatory agencies as a viable remediation strategy for contaminants under site-specific conditions. At chloroethene contaminated sites where MNA is used as a remediation strategy, microbially-mediated reductive dechlorination is typically the dominant pathway for natural attenuation. The efficacy of reductive dechlorination at sites with no anthropogenic carbon sources is often influenced by the availability of readily-biodegradable natural organic carbon along with favorable geochemical conditions for supporting microbial dehalogenation. Recent research studies have suggested that the pool of labile natural organic carbon, operationally defined as potentially bioavailable organic carbon (PBOC), may be a critical component related to sustaining reductive dechlorination at MNA sites. The objective of this study was to evaluate PBOC as a quantitative measure of the labile organic carbon fraction of aquifer sediments in relation to microbial reductive dechlorination of chlorinated solvents.

In the first phase of this study, the variability of PBOC in aquifer sediments was examined among 15 chloroethene contaminated sites. Results showed that PBOC displayed considerable variability among the study sites, ranging over four orders of magnitude. Regression results demonstrated that a positive correlation existed between PBOC, solid phase total organic carbon (TOC_s), and reductive dechlorination activity at the sites. Results supported that greater levels of PBOC and TOC_s corresponded to higher reductive dechlorination activity at the sites. Composition results showed that 6-86% of PBOC consisted of proteins and amino acids. Results also suggested a positive relationship existed between PBOC, concentrations of potentially bioavailable organic compounds present in the aquifer system, expressed as hydrolyzable amino acids (HAA), and the natural attenuation capacity (NAC) at the sites. Higher PBOC levels were consistently observed at sites with greater NAC and levels of HAA. The results of this study suggested that the variability of PBOC in the aquifer sediments exhibited a reasonable correlation with TOC_s, hydrolyzable amino acids, and chloroethene transformation among the selected sites.

In the second phase of this study, the relationship between PBOC in aquifer sediments and site specific performance data was evaluated among 12 chloroethene contaminated sites. Results demonstrated that PBOC in aquifer sediments was directly correlated to independent field metrics associated with reductive dechlorination. Levels of PBOC demonstrated direct relationships with hydrogen (H_2) and dissolved oxygen (DO) concentrations within the groundwater system at the selected study sites. Results also indicated that PBOC demonstrated positive relationships with reductive dechlorination activity and the natural attenuation capacity of the sites. The findings of this study suggested that the level of PBOC in aquifer sediments may be a key factor in sustaining conditions favorable for microbial reductive dechlorination.

In the third phase of this study, the distribution of PBOC was investigated at a chloroethene contaminated site. PBOC was measured in surficial aquifer sediment samples collected at varying depths in the vicinity of a chloroethene plume. Results demonstrated that levels of PBOC were consistently higher in aquifer sediments with minimal chloroethene exposure relative to samples collected in the PCE-contaminated source zone. Regression results demonstrated that a statistically significant inverse correlation existed between PBOC levels and chloroethene concentrations for selected temporary wells in the contaminated source zone at the study site. Consistent with these findings, results also indicated a similar trend of increased PBOC in aquifer sediments outside the chloroethene plume relative to aquifer sediments inside the plume. Results from this study further suggested that differences in extracted carbon levels at the site for surficial aquifer sediment samples in the PCE-contaminated source zone could impact the extent of reductive dechlorination within the hydrographic unit.

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Chapter 1. Introduction

The presence of chloroethenes in soil and groundwater has become a widespread environmental concern (Hopkins et al. 1993). Recognized by U.S. EPA (1999) as being a common class of groundwater contaminants, chlorinated solvents, such as perchloroethylene (PCE) and trichloroethylene (TCE), represent a significant source of pollution in groundwater systems (Grandel et al. 2004). Remediation of these contaminants poses a difficult challenge due to their recalcitrant and persistent behavior (Lee et al. 2007). Monitored natural attenuation (MNA) can be a viable remedial strategy for chlorinated solvents under favorable site conditions (Weidemeier et al. 1999; Chapelle et al. 2001). The effectiveness of MNA is dependent upon naturally attenuating processes to reduce contaminants to less harmful forms or immobilize them in the subsurface (U.S. National Research Council, 2000).

In anaerobic groundwater systems, microbial reductive dechlorination is considered the primary biological degradation pathway for chloroethenes (Chapelle et al. 1996). At chlorinated solvent sites, contaminants have been well-documented to undergo reductive dehalogenation in subsurface environments (Vogel, 1994; Odum et al. 1995). In reductive dechlorination, chloroethenes, such PCE and TCE, are sequentially reduced, such that a hydrogen atom and two electrons replace one chlorine ion for each degradation reaction, resulting in less-chlorinated transformation products such as dichloroethenes (DCEs), vinyl chloride (VC), ethene, and ethane (Morrill et al. 2005). Under highly reducing conditions and with a sufficient supply of organic carbon, several dechlorinating microorganisms have demonstrated the capability of mediating chloroethene biotransformation (Maymo-Gatell et al. 1997; Bradley et al. 2000; Duhamel et al. 2004; Schmidt et al. 2004).

The sustainability of reductive dechlorination at chloroethene-contaminated sites with no anthropogenic carbon sources is often dependent on the fermentation of natural organic carbon (Wiedemeier et al. 1997). During microbial fermentation of natural organic carbon, hydrogen is produced and used as the electron donor. Wiedemeier et al. (1997) demonstrated that a continuous source of hydrogen is necessary to sustaining contaminant biotransformation over the time duration of the remediation system. Thus, at chloroethene-contaminated sites where the main source of fermentable organics is derived from natural sources, the supply of

biodegradable natural organic carbon becomes an important factor in assessing the sustainability of reductive dechlorination (Newell et al. 2004).

Microbial and plant residues represent significant sources of natural organic carbon (NOC) in soils and sediments (Kogel-Knabner, 2002). Microbial remains provide organic carbon in the form of proteins, lipids, and polysaccharide-based components (Stevenson et al. 1994). Other natural organic compounds, such as lignin and cellulose, form the basis for plant residues (Sarkanen et al. 1971). The bioavailability of NOC in soils and groundwater is often dependent on its chemical composition, growth conditions, and diagenetic state. Research has supported that during early diagenesis of natural organic carbon various biomolecules are readily degraded, while other more recalcitrant biomolecules are selectively preserved (Cowie and Hedges, 1994). Since the bioavailable fraction of natural organic carbon is an important source of energy that supports reductive dehalogenation, quantification of metabolizable organic carbon becomes an important component in assessing sustainable MNA (Chapelle et al. 2007).

The research questions that guide this study are focused toward addressing the variation, nature, and composition of labile organic carbon that support microbially-mediated reductive dechlorination at chloroethene-contaminated sites. In particular, questions as to whether labile organic carbon varies among sites representing various environmental and hydrogeological conditions. Rectanus (2006) examined the variability of labile organic carbon fractions, termed potentially bioavailable organic carbon (PBOC), among 6 chloroethene-contaminated sites, which poses the question as to whether a similar variation in carbon levels would be observed among sites exhibiting a wider range of geology and geography. Related to the nature and composition of extracted carbon, what compounds are present in the extractions, how does composition vary among sites, and does a relationship exist between the measureable compounds and the potential for biodegradability? Rectanus et al. (2007) demonstrated that extracted carbon fractions were readily-biodegraded in laboratory microcosms; however, the question still remains as to whether composition impacts the potential for microbial reductive dechlorination. Finally, is there a measureable relationship between PBOC and site-specific conditions at chloroethene-contaminated sites? Rectanus (2006) employed a qualitative approach to evaluate the relationship between PBOC and

reductive dechlorination at field sites; however, a more robust relationship between PBOC and quantitative measures of reductive dechlorination has yet to be explored.

The aim of this research is to evaluate PBOC as a quantitative measure of the labile organic carbon fraction of aquifer sediments in relation to microbial reductive dechlorination of chlorinated solvents. To achieve this objective, this research investigation encompassed three main components. The first component of this study examined the variability of PBOC in aquifer sediments among chloroethene-contaminated sites. PBOC was measured from a wide range of chlorinated solvent sites exhibiting various levels of natural organic carbon and reductive dechlorination potential. In the second portion of this study, the relationship between PBOC in aquifer sediments and site-specific performance data was evaluated among chloroethene-contaminated sites displaying diverse environmental conditions. Relationships were examined between PBOC and three independent field metrics. The third component of this research study investigated the distribution of PBOC at a chlorethene-contaminated site. PBOC was measured at varying locations and depths within a chloroethene plume to assess the effect of chloroethene exposure on carbon levels.

Dissertation Outline

Chapter 1. Introduction

This introductory chapter provides background information, states research components, and briefly summarizes each chapter presented in this research dissertation.

Chapter 2. Literature Review

Chapter 2 provides a literature review of the background and framework for this research investigation. This chapter presents an overview of monitored natural attenuation (MNA) and key components that should be evaluated when assessing the sustainability of MNA at chloroethene-contaminated sites. This chapter also provides a discussion of the environmental conditions needed for sustaining reductive dechlorination at MNA sites and current methodologies used to assess these conditions.

Chapter 3. Evaluation of Potentially Bioavailable Natural Organic Carbon and Biodegradable Organic Carbon Compounds in Aquifer Sediments

Chapter 3 examines the variability of potentially bioavailable organic carbon (PBOC) among chloroethene-contaminated sites representing a diverse range of hydrogeological settings and reductive dechlorination activity. Carbon content present in aquifer sediments is also characterized through analysis of amino acids present in site samples of aquifer sediment, expressed as hydrolyzable amino acids (HAA). PBOC is measured using aquifer sediments collected at 15 study sites. The composition of PBOC is examined through the analysis of proteins and amino acids. Relationships are evaluated between PBOC, solid-phase total organic carbon (TOC_s), and reductive dechlorination activity at the selected study sites. Correlations between PBOC are also examined between HAA present in the aquifer sediments and the natural attenuation capacity of the site.

Chapter 4. Evaluation of Field Metrics for Assessing Potentially Bioavailable Natural Organic Carbon in Contaminated Aquifer Sediments

In Chapter 4, relationships are evaluated between levels of potentially bioavailable organic carbon (PBOC) in aquifer sediments and site specific contaminant and geochemical data at 12 chloroethene-contaminated sites. Using performance well data collected from historical reports, direct correlations are evaluated between PBOC in aquifer sediments and independent field metrics associated with reductive dechlorination. Specifically, relationships are evaluated between PBOC in aquifer sediments and (1) dissolved oxygen concentrations, (2) hydrogen concentrations, and (3) the natural attenuation capacity at the selected study sites.

Chapter 5. Distribution of Potentially Bioavailable Natural Organic Carbon in Aquifer Sediments at a Chloroethene Contaminated Site

In this chapter, the distribution of potentially bioavailable organic carbon (PBOC) is investigated at a chloroethene-contaminated site. PBOC is measured using surficial aquifer sediment samples collected at varying depths in the vicinity of a chloroethene plume. The effect of long-term chloroethene exposure on PBOC in aquifer sediments is examined by evaluating the differences in extractable carbon in aquifer sediments with minimal chloroethene exposure

relative to samples collected in the contaminated source zone. Using performance monitoring data, correlations are evaluated between PBOC levels and groundwater chloroethene concentrations. Laboratory assays using aquifer sediments and amended with PCE are constructed to assess carbon depletion due to chloroethene exposure. As an additional assessment, anaerobic bioassays using an enrichment culture and amended with PCE are also constructed to determine the extent of reductive dechlorination supported by PBOC in surficial aquifer sediments.

Chapter 6. Engineering Significance

This chapter discusses the engineering significance of this research, and proposes recommendations for future work.

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Chapter 2. Literature Review

Chloroethenes

The extensive use of chloroethenes at industrial and dry cleaning facilities has resulted in widespread groundwater and soil contamination (Jendrzejewski et al. 2001). Chlorinated solvents vary in the number of chlorine substituents from the mono-chlorinated vinyl chloride (VC) to the most chlorinated, tetrachloroethene (PCE). VC and PCE are both classified as priority pollutants by the U.S. EPA (2001) and are known or potential carcinogens. Other chlorinated solvents include, trichloroethene (TCE), *cis*-1,2-dichloroethene (*cis*-1,2-DCE), and *trans*-1,2-dichloroethene (*trans*-1,2-DCE). Both PCE and TCE are detected with the greatest frequency and highest concentration in groundwater systems (Hopkins et al. 1993; Canter and Sabatini, 1994; McCarty, 1997). However, *cis*-1,2-DCE, *trans*-1,2-DCE, and VC typically occur in groundwater systems as a direct result of biological degradation (Bradley, 2000). Due to their toxicity and persistence in the environment, the fate of these contaminants poses a significant regulatory and public concern.

Monitored Natural Attenuation (MNA)

Monitored Natural Attenuation (MNA) has emerged as an effective remediation strategy at chloroethene-contaminated sites (Weidemeier et al. 1999; Chapelle et al. 2001). MNA relies on natural attenuation processes to achieve site-specific remedial objectives within a time frame that is reasonable when compared to other remedial alternatives. At sites where MNA is employed as the remediation strategy, contaminants remain onsite and the naturally occurring processes are left to attenuate the site.

The implementation of MNA requires a monitoring strategy and a site characterization to demonstrate the efficacy of contaminant attenuation (U.S. EPA, 1999). MNA involves data collection to estimate both the rate of attenuation processes and the anticipated time required to achieve remediation objectives. Documentation of contaminant reduction is an important piece of evidence that natural attenuation is suitable for a site. However, additional documentation is often required to demonstrate that natural attenuation mechanisms are active. A ‘‘lines of evidence’’ approach has been adopted by the U.S. EPA (1999) as a method of

documenting natural attenuation. This approach involves providing three tiers of site-specific information to demonstrate that natural attenuation is occurring. These steps include:

1. Demonstration of a reduction in contaminant mass and/or concentration levels observed over time in historical groundwater and /or soil chemistry performance data

2. Assessment and characterization of geochemical and hydrogeological data that supports natural attenuation mechanisms are active at the site and significantly influence contaminant reduction

3. Evaluation of laboratory or field studies that assess the potential of a site to undergo specific natural processes and its ability to degrade contaminants

In prior years, chloroethenes were generally thought to be resistant to biological transformation. Today, chloroethenes have been observed at several sites to undergo biotransformation under favorable microbial and hydrogeochemical conditions (Weidemeier et al. 1999; Vogel, 1994; Bradley, 2000). Several methods have been commonly used to assess the effectiveness the biological and other natural attenuation processes. Alvarez and Illman et al. (2006) examined performance assessment methods for evaluating natural attenuation at a site. Table 2-1 shows a comparison of established and emerging techniques used to assess the efficacy of natural attenuating processes.

Table 2-1. Comparison of established and emerging techniques to assess natural attenuation (adapted from Alvarez and Illman, 2006)

Method of investigation	Applicability	Comments
Graphical and statistical analyses, analytical and numerical models	Established	Determine whether plume is stabilizing, increasing or decreasing in size
Geochemical parameters	Established	Observe contaminant trends; daughter product formation; presence of geochemical footprints to indicate biogeochemical process responsible for biodegradation
Push-pull tests	Established	Single-well tracer tests used to evaluate in situ degradation rates and metabolite formation.
Microcosm studies	Established	Observe presence of microorganisms, protozoan predators, cellular biomarkers, nucleic acids, and phospholipids; determination of cellular contents of ribosomes, intracellular energy reserves, nutritional status; measure cell growth and uptake of physiological substrates, respiratory activity from the reduction of dyes; determination of novel organisms from DNA sequencing; compute relative abundances of each phylogenetic group; observe unique intermediary metabolites indicative of biodegradation
Stable isotope analysis	Emerging but in greater use	Demonstrate biodegradation of organic compounds; quantify microbial activities in situ; quantify relative extent of biodegradation between zones of contaminant plume
Chemical fingerprinting	Emerging but in greater use	Identification of fuel types, determination of contaminant sources that will assist in the mapping of subsurface contamination and backward in time contaminant transport simulations
Molecular techniques	Emerging	Used to increase understanding of biochemical reactions and their mechanisms; techniques allow one to obtain answers to questions such as, “What microorganisms are there?”, “What are microorganisms doing?”, and “When are the microorganisms active?” It also will likely provide a comprehensive mechanistic understanding of processes that regulate the genes that encode the proteins that actually degrade the contaminants.

Natural Attenuation Capacity (NAC)

For groundwater systems, the natural attenuation capacity (NAC) is described as its ability to lower contaminant concentrations along aquifer flowpaths; and it is defined as the slope of the steady state concentration profile along a groundwater flow path (Chapelle and Bradley, 1998). The NAC is the sum of all physical, chemical, and biological processes that contribute to the attenuation of a contaminant plume. Equation 1 shows the four attenuation processes acting on a solute in the one-dimensional solute transport equation. These processes include dispersion, advection, sorption, and biodegradation.

$$\frac{\partial C}{\partial t} = -v\frac{\partial C}{\partial x} + D\frac{\partial^2 C}{\partial x^2} - \frac{K_d \rho_b}{n}\frac{\partial C}{\partial t} - kC \qquad (1)$$

In Equation 1, C is concentration, t is time, D is the coefficient of hydrodynamic dispersion (m^2/d), v is velocity of groundwater flow (m/d), ρ_b is bulk density, K_d is a linear sorption distribution coefficient, n is porosity, and k is a first-order biodegradation rate constant (d^{-1}) (Freeze and Cherry, 1979).

If steady-state conditions are obtained within the contaminant plume, sorption processes become insignificant and the solute-transport equation can be further represented by the following ordinary differential equation:

$$D\frac{d^2 C}{dx^2} - v\frac{dC}{dx} - kC = 0 \qquad (2)$$

The concentration profile along the centerline of the plume may be represented by the solution to the one-dimensional solute transport equation for boundary conditions of $C = C_o$ at $x = 0$ and $C = 0$ as $x \to \infty$.

$$C(x) = C_o e^{-\left[\frac{-v+\sqrt{v^2+4Dk}}{2D}\right]x} \qquad (3)$$

Assuming uniform groundwater flow and first order decay, the NAC can be expressed in relationship to contaminant concentration and distance along the plume centerline. Equation 4 illustrates the mathematical definition for NAC (Chapelle and Bradley, 1998). This equation demonstrates that the steady-state solute concentration is a function of hydrodynamic dispersion (D), the biodegradation rate constant (k), and groundwater velocity (v) and is expressed in units of m^{-1}.

$$NAC = \left[\frac{-v + \sqrt{v^2 + 4Dk}}{2D}\right] \quad (4)$$

NAC is particularly useful when assessing the effectiveness of a MNA at chloroethene-contaminated sites because it provides a quantitative measure of reductive dechlorination by incorporating rates of contaminant biotransformation. Therefore, an evaluation of the NAC of a site is important when determining the potential of a groundwater system to attenuate contaminants.

Biotransformation of Chlororethenes

Studies have suggested that biodegradation is the dominant natural attenuation mechanism for chloroethenes (Chapelle et al. 1996). Therefore, an understanding of the transformation pathways is important for determining the potential of natural attenuation at a given site. During microbial degradation, three modes of contaminant biotransformation are possible, including aerobic oxidation, anaerobic, and co-metabolism degradation.

Aerobic Oxidation

Chlorinated ethenes such as PCE and TCE are well-known to undergo biological degradation in subsurface anaerobic zones at contaminated sites, resulting in less-chlorinated daughter products such as DCEs, VC, ethene, and ethane (Vogel et al. 1987; McCarty and Semprini, 1994; Bouwer, 1994). These highly chlorinated ethenes are not generally subject to oxidative biodegradation precesses (Vogel et al. 1987); however, daughter products, such as DCEs, VC, ethene, and ethane can be oxidized to carbon dioxide (CO_2) under aerobic reducing

conditions (Bradley and Chapelle, 1996). During direct oxidation, microorganisms obtain energy and organic carbon from the chlorinated ethenes that undergo oxidative biodegradation.

Davis et al. (1990) were among the first to report the aerobic oxidation of VC in groundwater samples. Results from this study demonstrated the oxidation of VC in the absence of co-substrates and without a lag phase; and thus was the first indication that microorganisms that grow on VC were present at chloroethene-contaminated sites. In 1994, McCarty and Semprini demonstrated that VC can serve as a primary substrate for some microorganisms under aerobic conditions. In another study, the complete mineralization of VC by direct oxidation was observed using used iron-reducing aquifer sediments in the presence of Fe (III) (Bradley and Chapelle, 1996).

Furthermore, studies have also demonstrated that DCE can be microbially oxidized without co-subtrates to CO_2 in aerobic conditions (Bradley et al. 1998a). In a study conducted by Bradley et al. (1998a), the mineralization of DCE was reported in the absence of a co-substrate in stream-bed sediments. If aerobic microorganisms capable of biotransforming chlorinated ethenes are present and active at contaminated sites, this may be a significant factor influencing the natural attenuation of chloroethenes.

Co-metabolism

Co-metabolism is another potential process for the biological degradation of chloroethenes in groundwater systems (Vogel et al. 1987). A wide range of chlorinated solvents can be microbially degraded under aerobic conditions by means of co-metabolic transformation reactions (McCarty and Semprini, 1994). Co-metabolic transformations are reactions that are catalyzed by existing microbial enzymes and that yield no carbon or energy benefits to the transforming cells (Horvath, 1972). Therefore, a growth substrate must be available to grow new cells, provide an energy source, and induce production of the co-metabolic enzymes.

Since co-metabolic oxidation of chloroethenes does not supply energy for microbial growth or metabolism, the biotransformation of chlorinated solvents requires molecular oxygen, a source of reducing equivalents, typically NADH, and a primary substrate to initiate the production of a suitable oxygenase. The oxygenase reaction generates chlorinated solvent

oxidation products that may react with cellular macromolecules or may be hydrolyzed spontaneously into carbon dioxide, chloride, or other non-volatile products that are easily mineralized by microorganisms (Little et al. 1988).

Chloroethenes can be oxidized by a wide range of oxygenase-expressing microorganisms including those that utilize methane (Wilson et al. 1985), propane (Fliermans et al. 1988), propene (Ensign et al. 1992), isoprene (Ewers et al. 1990), isopropylbenzene (Dabrock et al. 1992), phenol (Folsom et al. 1990), butane (Wilson et al. 1988), and ammonia (Arciero et al. 1989) as energy and/or carbon sources. In 1985, Wilson et al. reported that methanotrophic bacteria were capable of oxidizing TCE to CO_2 under aerobic conditions. In recent studies, a number of aerobic microorganisms, capable of oxidizing TCE, DCE, and VC to CO_2 without accumulation of toxic intermediates, have been identified (Fan and Scow, 1993; Hopkins and McCarty, 1995). Results from these studies suggest that co-metabolism may be significant contributing factor in the biotransformation of chloroethenes.

Anaerobic Degradation

Anaerobic degradation involves the passing of electrons to an acceptor other than oxygen, such as nitrate (denitrification), Mn (IV), Fe (III), sulfate (sulfate reduction) and ultimately carbon dioxide (methanogenesis). Microorganisms preferentially utilize electron acceptors that provide the maximum free energy during respiration (Vogel et al. 1987). Biotransformation of highly chlorinated organics, such as PCE and TCE are not easily oxidized since there is less energy to be gained by the microorganism. Instead, they are used as electron acceptors and are reduced to less oxidized forms, daughter products.

Reductive dechlorination is thought to be the primary degradation pathway for highly chlorinated solvents in anaerobic environments (Chapelle et al. 1996). The sequence of reductive dechlorination yields less chlorinated compounds, such that an H^+ and two electrons replace one chlorine ion for each degradation reaction. For chlorinated solvents, the potential for reductive dechlorination increases with increasing number of chlorine substituents (Vogel et al. 1987; Bouwer, 1994; McCarty and Semprini 1994; Vogel, 1994).

In reductive dechlorination, chloroethenes serve as electron acceptors for *Dehalococchoides sp.* and other dechlorinating bacteria (Maymo-Gatell et al. 1997). Many

dechlorinating bacteria, for example Dehalobacter restrictus (Holliger et al. 1998), and Dehalospirillum multivorans (Neumann et al. 1994), can reduce PCE and TCE to cDCE, but only members of the genus Dehalococcoides (Maymo-Gatell et al. 1997) have been reported to reduce PCE to ethane. Since these dechlorinating microorganisms are able to grow using chloroethenes as sole terminal electron acceptors, several studies have reported that under strongly reducing conditions PCE could be micorbially degraded to CO_2 (Bradley, 2000). These results indicate that anaerobic degradation of chloroethenes is a key component in the effectiveness of natural attenuation.

Sustainability of Reductive Dechlorination

Sustainability is an important factor that often dictates the long term effectiveness of natural attenuation. The U.S. National Research Council (2000) describes sustainability as occurring when the mechanisms that destroy or immobilize contaminants are sustainable for the operational lifetime of the system. Furthermore, U.S. EPA (1999) also concludes that the effectiveness of long term MNA should be demonstrated at a site through its ability to naturally attenuate contaminants and achieve remedial goals in a reasonable time frame when compared to other remedial alternatives.

Reductive dechlorination at solvent sites is controlled by the availability of electron donors from either man-made (Type I site) or naturally occurring (Type II site) (Wiedemeier et al. 1997). At Type II sites, natural organic carbon is the key factor supporting reductive dechlorination which provides a supply of hydrogen to be used as the electron donor. Thus, in order to address the sustainability of reductive dechlorination at sites without anthropogenic carbon sources, the nature and bioavailability of natural organic carbon along with geochemical conditions must be evaluated.

Bioavailability of Natural Organic Carbon (NOC)

The availability of natural organic carbon often influences the effectiveness of microbially mediated processes in groundwater systems (Bradley, 2000). Natural organic carbon (NOC) is mainly derived from two major sources, which include microbial and plant residues (Kogel-Knabner, 2002). Microbial remains provide organic carbon in the form of

proteins and lipids (Stevenson, 1994). Proteins consist of polypeptides, which contain long chains of various amino acids (Kogel-Knabner, 2002). Microbial remains also contribute polysaccharide-based components such as peptidoglucan, which is comprised of carbohydrates and amino acids (Koch, 1990). Plant residues are composed of a mixture of organic compounds, such as lignin, aliphatic biopolymers, and tannins (Kogel-Knabner, 2002). Lignin is a high macromolecular organic compound and is more resist to biological degradation, when compared to organic compounds in microbial residues (Kogel-Knabner, 2002).

Because soil organic carbon consists of a complex mixture of organic materials, a thorough understanding of the nature of organic carbon is necessary to assess the quantity and quality of natural organic compounds present in the aquifer system. Factors such as chemical composition, diagenetic state, and bioavailability affect the nature of organic carbon (Boyer et al. 1996). The influence of chemical composition on the reactivity of organic carbon is most apparent during early stages of decomposition, when organic carbon is more susceptible to biotransformation (Opsahl and Benner, 1995). Increased knowledge of the factors that affect degradation and reactivity of natural organic matter is therefore important in understanding its bioavailability.

Terminal Electron Acceptor Process (TEAP) and H_2 Utilization

Spatial and temporal heterogeneity of the subsurface can influence the concentrations of predominant terminal electron acceptors at any particular site (Vogel et al. 1987). Because of the complexity associated with each site, defining these variabilities is important in assessing the sustainability of MNA. At many chlorinated contaminated sites, microorganisms will pair oxidation and reduction reactions that yield the most energy (Vogel et al. 1987). With the same organic substrate, microorganisms will preferentially use O_2, NO_3^-, Fe^{3+}, SO_4^{2-}, and CO_2 (Fe^{2+} and CH_4 are products of the reduction of Fe^{3+} and CO_2). The presence and concentrations of these constituents aid in the determination of the dominant terminal electron acceptor process (TEAP). In anaerobic environments, hydrogen serves as the electron donor for dechlorinating microorganisms. The energy derived from the TEAP utilizing H_2 as an electron donor has been shown to establish a minimum H_2 threshold concentration (Chapelle et al. 1995; Loffler et al.

1999). As a result, the ranges for hydrogen utilization as an electron donor with respect to the TEAPs is indicative of dominant geochemical processes present within the aquifer system. Table 2-2 illustrates the ranges of hydrogen concentrations for different TEAPs.

Table 2-2. Ranges of Hydrogen Concentrations for TEAPs (adapted from Chapelle et al. 1995)

Hydrogen Concentration (nM)	TEAP
<0.1	Denitrification
0.1 to 0.8	Iron (III) Reduction
1 to 4	Sulfate Reduction
5 to 25	Methanogenesis

Bioavailable Organic Carbon Analysis

Analytical techniques, consisting of chemolytic, thermolytic, and spectroscopic methods, are currently used to determine labile fractions of organic carbon in soils sediments (Goni and Hedges, 1990; Benner, 2002; Rectanus et al. 2007). Both chemolytic and thermolytic techniques provide quantitative concentration data on organic carbon components, while spectroscopic techniques provide insight on its chemical structure. This information provides additional insight on the nature and bioavailability of the organic carbon. This section will provide a brief overview of chemolytic, thermolytic, and spectroscopic analytical techniques used for organic carbon analysis.

Chemolytic Techniques

Chemolytic techniques have been commonly used in soil science to determine labile fractions of plant and microbial organic biopolymers (Kogel-Knabner, 1995). In this section, general chemolytic techniques for organic carbon measurement will be discussed. This section

will provide an overview of the techniques and potential limitations for organic carbon measurement.

Protein Hydrolysis

Protein hydrolysis is a chemolytic technique in which amino acid fractions are obtained from sample media. The determination of proteins consists of an acid hydrolysis, usually HCL or H2SO4, followed by followed either by a chromatographic separation of the individual amino acids usually by high performance liquid chromatography, or gas chromatography (Stevenson and Cheng, 1970; Kogel-Knabner, 1995). Fatty acids, proteins, and polysaccharides are susceptible to acid hydrolysis treatment, while long-chain alkyls, lignin, and other aromatics are resistant to hydrolysis.

Cowie et al. 1992 demonstrated that carbohydrates and amino acids could be used as maturity indicators of natural organic carbon; and further studies indicated that they could be identifiable at the molecular level (Benner et al. 2003). The bioreactive fraction of the natural organic carbon has been characterized based on the amount of total hydrolyzable amino acids and neutral sugars (Ittekot et al. 1988; Cowie and Hedges, 1994). In a study conducted by Kaiser and Benner (2005), concentrations of total hydrolyzable amino acids were analyzed using high-performance liquid chromatography (HPLC) and pre-column derivatization with *o*-phthaldialdehyde (OPA) and *N*-isobutyrylcysteine (IBC).

From concentration data obtained from hydrolyzable amino acids, Cowie and Hedges (1994) demonstrated that the diagenetic state of the organic matter could also be negatively related to the carbon-normalized (%OC) yields of the natural organic carbon. The carbon-normalized yield expresses the moles of total hydrolyzable amino acid per mole of natural organic carbon for a particular soil sediment sample. These normalized yields are calculated as a percentage of organic carbon by dividing fraction of organic carbon present in hydrolyzable amino acids (AA-C) by the total organic carbon in the sediment sample. Therefore, low % OC yields of bioreactive components are indicative of a greater degraded organic material. Since natural organic carbon is more susceptible to biotransformation during early stages of decomposition, a low % OC yield would suggest organic carbon that is less available for

microbial degradation. One main criticism of this technique is that acid pre-treatment is required on media samples before analysis. Despite this shortcoming, concentrations of these organic carbon fractions could serve as indicators of the bioavailability of organic carbon in soil sediments.

Chemical Extraction Methods

Chemical extraction methods have been commonly used to determine the bioavailable organic carbon fraction in soil and sediment samples (Marschner and Kalbitz, 2003; Rectanus et al. 2007). Schnabel et al. (2002) demonstrated that organic carbon chemically extracted from upper horizon soils in agricultural and forest regions were bioavailable components of organic carbon. In this study, a relationship was developed between extractable organic carbon and its biodegradability by soil microorganisms. Other studies have also reported that extractable organic carbon techniques in upper soils could be microbially utilized under favorable conditions (Marschner and Kalbitz, 2003).

Rectanus et al. (2007) developed a method for quantifying the fraction of labile organic carbon present in the aquifer sediment through a multi-step chemical extraction process. Termed "potentially bioavailable organic carbon (PBOC)", the method operationally defines the fraction of natural organic carbon most loosely-associated with sediment. Rectanus et al. (2007) demonstrated that the extracted carbon was biodegraded in aerobic and anaerobic bioassays and served as an electron donor for reductive dechlorination in laboratory microcosms.

Cu Oxidation

Hedges et al. (1982) demonstrated that other chemolytic techniques such as CuO oxidation, could also be used to characterize the lignin component of soil organic carbon. The combination of gas chromatography with mass spectrometry provides results that aid in the identification of the phenols. Oxidation with CuO transforms the lignin network to phenol units with aldehyde, carboxylic acid and ketone functionality. The ratio of the amount of more oxidized lignin monomers (carboxylic acids) to that of the corresponding aldehyde monomer released during CuO oxidation provides an index of lignin alteration during biodegradation

(acid-to-aldehyde ratio). This ratio has been shown to systematically increase as the lignin is degraded in soils (Kogel-Knabner, 2000). However, one disadvantage of this method is the negative impact of additional derivatization of phenols following the CuO oxidation.

Pyrolysis Techniques

Pyrolysis is an alternative analytical method for the analysis of natural organic carbon that has shown an acceptable degree of success (Kogel-Knabner, 2000). Various pyrolysis techniques combined with modern analytical instruments, such as gas chromatographs and/or mass spectrometers (Py-GC/MS) and pyrolysis-field ionization mass spectrometry (Py-FIMS) have made considerable advancements in assessing soil organic carbon composition (Saiz-Jimenez, 1994; Leinweber and Schulten, 1998). Py-GC MS involves chromatographic separation of pyrolysis products into single components and mass spectral data is obtained for each component; while, Py-FIMS does not involve separation of the pyrolysis products, but uses soft ionization to produce predominantly molecular ions of the pyrolysis products. In general, analytical pyrolysis has been used in most studies environmental studies as a qualitative method; however, it has been recently used as a quantitative technique (Parsi et al. 2005). One of the main disadvantages for this technique is the formation of secondary reactions during pyrolysis (Simon and Giacobbo, 1965).

Spectroscopic Techniques

Spectroscopic techniques are alternative non-destructive methods for the examination of natural organic carbon (Kogel-Knabner, 2000). With spectroscopic techniques, structural information on natural organic carbon at the molecular level can be obtained on diverse structural units that are amenable to degradation techniques. Sediment samples can be analyzed without major pretreatment, while avoiding secondary reactions. These techniques can give good results concerning the gross chemical composition, although specific compounds are not identified. However, the combination of spectroscopic techniques with chemolytic and pyrolysis methods could add substantially to understanding the nature and bioavailability of natural organic carbon.

^{13}C NMR Spectroscopy

Nuclear magnetic resonance (NMR) spectroscopy is valuable tool for the characterization of soil organic carbon and humification processes in soils. The first attempt to use NMR spectroscopy for structural characterization of soil humic substances was reported by Barton and Schnitzer (1963). The major advantage of this technique is the ability to obtain structural information on SOM in bulk soils or solid fractions without major pretreatment and extraction (Preston et al. 1996).

NMR spectra of soil or humic substances are generally divided into four main chemical-shift regions, representing alkyl C (0-45 ppm), O-alkyl C (45-110 ppm), aromatic C (110-160 ppm), and carboxyl C (160-210 ppm) (Wilson et al. 1983; Preston and Trofymov, 2000). Amino acids generate signal intensities between 17 and 50 ppm (alkyl C). Aliphatic alcohol and ether structures (O-alkyl-C), such as those found in carbohydrates, have resonances between 50 and 110 ppm. The signal intensity in lignin typically ranges from at 148-153 ppm (Gil and Pascoal Neto, 1999). Generally, the relative amount of alkyl C increases during biodegradation, whereas the amount of O-alkyl C shows a relative decrease. Baldock and Preston (1995) suggested the use of the ratio of alkyl to O-alkyl C as an index of the extent of decomposition. This ratio has been shown to systematically increase as the lignin is degraded in soils. Kogel-Knabner (1993) and Baldock et al. (1997) demonstrated this concept in studies of forest soil profiles, peats and of plant residue components.

One major concern associated with this technique derives from the presence of paramagnetic compounds, which may complicate the interpretation of ^{13}C and NMR spectra of soils and its fractions (Kogel-Knabner, 1997). Such compounds are well-known to broaden NMR signals, leading to spectra with strongly overlapping resonance lines. Since various forms of the organic carbon bound to paramagnetic compounds, some forms of organic carbon present in the sediment sample may not be detected by solid-state ^{13}C NMR spectroscopy (Wilson et al. 1990). Quantitative interpretation of such spectra becomes difficult and could lead to under-estimations of the total organic carbon fraction and quantity of specific functional groups.

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Chapter 3. Evaluation of Potentially Bioavailable Natural Organic Carbon and Biodegradable Organic Carbon Compounds in Aquifer Sediments

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Abstract

This study examined the variability of potentially bioavailable organic carbon (PBOC) among chloroethene contaminated sites representing a wide range of environmental conditions. Using a novel approach, this study also characterized the carbon content through analysis of amino acids present in site samples of aquifer sediment. The composition of PBOC was also examined through the analysis of proteins and humic acids. PBOC was measured using aquifer sediment samples collected at 15 study sites. Results demonstrated a positive correlation between PBOC, solid-phase total organic carbon (TOC_s), and reductive dechlorination activity at the sites. Regression analysis showed 25% of TOC_s was extracted using the PBOC method. Results indicated that pyrophosphate and alkali extracted carbon comprised 52.4% and 47.6% of PBOC, respectively. Composition results showed that 6-86% of PBOC consisted of proteins and amino acids. Regression results indicated that 8% of pyrophosphate extracted carbon consisted of proteins, while alkali extracted carbon represented 17% of humic acids. Results also demonstrated a positive relationship between PBOC and potentially bioavailable organic compounds present in the aquifer system, expressed as hydrolyzable amino acids (HAA). Results from data regressions suggested pyrophosphate extracted carbon exhibited more reliable fractions of total HAA when compared to alkali extractions. Consistent with these findings, lower concentrations of total HAA were representative of study sites that exhibited minimal levels of reductive dechlorination activity. Higher levels of HAA were consistently observed at sites with greater natural attenuation capacity (NAC) and reductive dechlorination activity. Overall, these results emphasize the need to evaluate the PBOC method for quantifying labile natural organic carbon that draws on measurements of TOC_s, biodegradable natural organic compounds, and extent of chloroethene transformation at a site.

Introduction

Monitored natural attenuation (MNA) has emerged as an effective and economical remedial strategy at many chloroethene-contaminated sites (Weidemeier et al. 1999; Chapelle et al. 2001). MNA relies on natural attenuation processes to achieve site-specific remedial objectives within a time frame that is reasonable when compared to other remedial methods. Successful implementation of MNA requires a monitoring strategy that not only demonstrates diminishing contaminant concentrations, but also provides insight into the processes responsible for contaminant attenuation (U.S. EPA, 1999). Natural attenuation capacity (NAC) provides a quantitative measure of the capacity of a groundwater system to attenuate contaminant plumes. The NAC of groundwater systems incorporates hydrologic (dispersive and advective), sorptive, and biological (biodegradative) processes within groundwater flow. It can be described for groundwater systems as its ability to lower contaminant concentrations along aquifer flowpaths; and it is defined as the slope of the steady state concentration profile along a groundwater flow path (Chapelle and Bradley, 1998).

For chlorinated ethenes, biodegradation is typically the dominant natural attenuation mechanism (Chapelle et al. 1996). Under anaerobic environments, biodegradable natural organic carbon is fermented to produce hydrogen, which serves as the electron donor for microorganisms. Reductive dechlorination is the primary degradation pathway for highly chlorinated solvents, in which chloroethenes serve as electron acceptors for *Dehalococchoides sp.* and other dechlorinating bacteria (Maymo-Gatell et al. 1997; Duhamel et al. 2004; Schmidt et al. 2004). The sequence of reductive dechlorination of tetrachloroethylene (PCE) produces trichloroethylene (TCE), 1,2-dichloroethene (*trans* and *cis*-1,2-DCE), vinyl chloride (VC) and ultimately ethane, ethene and methane, such that an H^+ and two electrons replace one chlorine ion for each degradation reaction. The less chlorinated daughter products are, in turn, more reduced and resistant to further reduction. For chloroethene contaminants, the tendency to undergo reductive dechlorination decreases with decreasing number of chlorine substituents (Vogel et al. 1987; Bouwer et al. 1994; McCarty and Semprini, 1994; Vogel et al. 1994).

At chloroethene-contaminated sites with no anthropogenic carbon sources, the fermentation of natural organic carbon is the key factor supporting reductive dechlorination.

Thus, the pool of bioavailable natural organic carbon along with geochemical conditions favoring reductive dechlorination often dictate the long-term effectiveness of MNA-based strategies at chlorinated solvent sites (Chapelle et al. 2007). Because the bioavailable fraction of natural organic carbon is an important source of energy that supports microbially-mediated reductive dechlorination, methods to quantify metabolizable organic carbon must be developed for assessing the long-term sustainability MNA.

A vast majority of natural organic carbon is comprised of humic substances, which consist of humified biopolymer organic material (Weber et al. 2001). Humic substances are categorized based on their solubility in an acid and base, which include: fulvic acids, humic acids, and humin. Fulvic acids are water soluble in all pH ranges. Humic acids are only water soluble at alkaline pH ranges; while, humins are insoluble at all pH ranges (Stevenson et al. 1994). The supply of biopolymers that provide the basis for natural organic carbon consists of both microbial and plant residues (Kogel-Knabner et al. 2002). Microbial by-products provide organic carbon in the form of proteins and lipids (Stevenson et al. 1994). These microbial remains also contribute polysaccharide-based components such as peptidoglucan, which is comprised of carbohydrates and amino acids. Other natural organic compounds such as lignin are attributed to plant residues (Kogel-Knabner et al. 2002). Lignin is unique to vascular land plants (Sarkanen et al. 1971) and plays a key role in the formation of soil because of its abundance and stability against biological and chemical degradation (Hedges et al. 1997).

Natural organic carbon can be generalized into two pools of carbon ranging from labile, which is commonly used by microbial ecologists to describe organic carbon that is potentially biodegradable, to recalcitrant, which is more resistant to biodegradation (McLauchlan et al. 2004). Research studies have supported that during early microbial transformation of organic matter, various biomolecules, such as carbohydrates, are preferentially degraded while other biomolecules are selectively preserved, such as lignin (Hatcher et. al 1983; Benner et. al 2002). Carbohydrates and amino acids have been commonly used as maturity indicators of natural organic carbon (Cowie et. al 1992; Hedges and Prahl, 1993). They have been generally determined to be biochemical components of natural organic carbon at the molecular level (Benner et al. 2003). The bioreactive fractions of natural organic carbon have been

characterized based on the amount of total hydrolyzable amino acids and neutral sugars (Ittekot et al. 1988; Cowie and Hedges, 1994).

Several analytical techniques, consisting of pyrolytic, spectroscopic and chemolytic methods, have been commonly used for the determination of organic carbon fractions in soil and aquifer sediments (Dinel et al. 1990; Goni and Hedges et al. 1990; Dauwe and Middleburg et al. 1998). Pyrolytic methods provide both quantitative and structural information on humic fractions of organic carbon in the sediment through high temperature chromatographic separation. Pyrolytic techniques involve the separation of organic carbon fractions into single components, for which mass spectral data is obtained for each component. However, the major criticism of this technique is the formation of secondary reactions during pyrolysis, which can considerable modification to organic carbon fractions (Simon and Giacobbo et al. 1965).

Spectroscopic techniques, such as ^{13}C nuclear magnetic resonance (^{13}C NMR), provide structural information on natural organic carbon at the molecular level. In ^{13}C NMR, the intensity of a NMR signal is proportional to the concentration of the nuclei creating the signal (Knicker and Nanny et al. 1997). NMR spectra of soil or humic substances are generally divided into four main chemical-shift regions, representing alkyl C (0-45 ppm), O-alkyl C (45-110 ppm), aromatic C (110-160 ppm), and carboxyl C (160-210 ppm). The chemical composition of bulk soil organic carbon can vary widely, from highly aliphatic to highly aromatic (Wilson, 1987; Piccolo and Conte, 1997). Generally, the relative amount of alkyl C increases during biodegradation, whereas the amount of O-alkyl C shows a relative decrease. Baldock and Preston (1995) suggested the use of the ratio of alkyl to O-alkyl C as an index of the extent of decomposition. One of the limitations of the NMR method is the low resolution of soil organic matter characterized by low organic carbon content (Mahieu et al. 1999). For soil sediments with low C contents (5 g/kg), it becomes difficult to acquire a ^{13}C NMR spectrum with an acceptable signal-to-noise ratio (Kogel- Knabner et al. 1997).

Chemolytic methods, such as protein hydrolysis and other chemical extraction techniques, have been successfully applied to soil sediments to analyze both plant and microbial biopolymers (Kogel-Knabner, 1995). In protein hydrolysis, amino acids and neutral sugars are obtained from soil and sediment samples. Samples are hydrolyzed with a

concentrated acid, such as HCl or H_2SO_4, over a specified time. Organic carbon fractions such as fatty acids, proteins, and polysaccharides are susceptible to acid hydrolysis treatment, while long-chain alkyls, lignin, and other aromatics are more resistant to hydrolysis. In soil science, these organic carbon fractions have been used as biochemical indicators of natural organic matter (Benner et al. 2003). The main disadvantage of this technique is that acid pre-treatment is required on soil and sediment samples before analysis, which can alter the structure of the natural organic carbon. Despite this shortcoming, protein hydrolysis provides a quantitative measure of more readily, biodegradable organic carbon fractions in sediment samples.

In addition to protein hydrolysis, other chemical extraction methods have also been used to determine bioavailable organic carbon fractions in soil and sediment samples (Boyer et al. 1996, DeLuca and Keeney, 1993). Chemical extraction techniques for quantifying labile carbon fractions in soil and sediment samples have used a variety of extracting solutions, ranging from mild solvents such as water, pyrophosphate, and salt solutions to more aggressive solvents such as sodium hydroxide (Nelson et al. 1994; You et al. 2006). Mild solvents extract loosely-bound organic carbon fractions, which have been demonstrated to be labile components of organic carbon (Qualls and Haines, 1992). With the use of mild extracting solutions, biodegradable fractions of organic carbon are quantified without exposing them to extreme conditions. Low-molecular organic compounds have been commonly extracted using mild extracting agents, such as sodium pyrophosphate. In a study conducted by Bonmati et al. (1998), greater quantities of fulvic acids were extracted from upper horizon soils using sodium pyrophosphate (140mM), when compared to humic acids.

More aggressive extracting solutions have been used to remove humic fractions of organic carbon. Generally, humic substances have been extracted from organic carbon under alkaline conditions using sodium hydroxide. Hayes et al. (1978) demonstrated that a significant fraction of humic acids could be extracted from upper soils using sodium hydroxide as the extracting agent. Since humic substances are more resistant to microbial and chemical alteration, they are thought to be more recalcitrant forms of organic carbon.

Schnabel et al. (2002) demonstrated that water extractable organic carbon (WEOC) from horizon soils in agricultural and forest regions were bioavailable components of organic carbon.

In this study (Schnabel et al. 2002), a relationship was developed between WEOC and its biodegradability by soil microorganisms. Other studies have quantified humic fractions of organic carbon through alkali extraction procedures (You et al. 2006). In the study conducted by You et al. (2006), humic fractions of organic carbon were identified and measured by exposing soil sediments to sodium hydroxide.

Rectanus et al. (2007) developed a method for quantifying the fraction of labile organic carbon present in the aquifer sediment through a multi-step chemical extraction process. Termed "potentially bioavailable organic carbon (PBOC)", the method operationally defines the fraction of natural organic carbon loosely-associated with sediment. Rectanus et al. (2007) demonstrated that the extracted carbon was biodegraded in aerobic and anaerobic bioassays and served as an electron donor for reductive dechlorination in laboratory microcosms.

The PBOC extraction method consisted of a five-step chemical extraction process where three sequential 24- hour extractions with 0.1% pyrophosphate are followed by a 24-hour 0.5 N sodium hydroxide extraction and a final 24-hour 0.1% pyrophosphate extraction (Rectanus et al. 2007). The pyrophosphate extractions (Extractions 1-3) were thought to represent the mildly-extractable organic carbon, while alkali extractions (Extractions 4-5) were assumed to account for the more strongly-adsorbed and humic fractions of organic carbon associated with the sediment. PBOC was defined as the total mass of organic carbon extracted by the pyrophosphate and alkali extractions. Rectanus et al. (2007) showed that some extractable carbon remained after the sequential step, indicating the PBOC defined through this method is a conservative estimate of the available pool of carbon. Although the study did not address the underlying chemistry of organic carbon present in aquifer sediments, it provided a methodology for evaluating the carbon present in aquifer sediments.

This study examines the variability of PBOC among chloroethene-contaminated sites representing a diverse range of hydrogeological settings, natural organic carbon distribution, and reductive dechlorination potential. Using a novel technique, this study also seeks to characterize the carbon content through analysis of amino acids present in site samples of aquifer sediment, expressed as hydrolyzable amino acids (HAA). To evaluate the nature of PBOC, the composition of extracted carbon was examined through analysis of proteins and

humic acids. PBOC was measured using aquifer sediments that were collected at 15 study sites. Correlations were established between PBOC, total organic carbon associated with the solid-phase of the aquifer sediment (TOC_s) and reductive dechlorination activity at the study sites. Direct relationships with PBOC were also developed between HAA and the natural attenuation capacity (NAC) of each site where sufficient monitoring well data was available.

Materials and Methods

Site Description

Aquifer sediment samples were obtained from 15 chloroethene-contaminated sites chosen from various hydrogeological settings and representing a range of levels in microbially-mediated reductive dechlorination. The latter was based on a review of historical monitoring data and specificially, the presence or absence of reductive dechlorination daughter products (*cis*-DCE and VC) and redox indicator data. Aquifer sediment samples were typically collected at depths associated with the contaminant plume in the saturated zone using either direct push technology or a hand auger and placed in sealed containers. After collection, samples were transported and stored at 4°C until analysis.

A listing of the study sites is shown in Table 3-1. Sediment samples were collected from various geographic regions of the United States. The sampling locations included four sites in the Pacific region (NUWC Keyport, WA; Beale AFB, CA; NAS North Island, CA; Fort Lewis, WA), one site in the Mountain region (Hill AFB, UT), one site in the north-central Glaciated region (ACRP, MN); one site in the Gulf Coastal Plain region (NWIRP, TX); and eight sites in the Atlantic Coastal Plain region (NABL Little Creek, VA; NAES Lakehurst, NJ; NSB Kings Bay, GA; MCRD Parris Island, SC; NTC Orlando (OU2/OU4), FL; NAS Pensacola, FL; NAS Jacksonville, FL).

Table 3-1 summarizes the site information, sediment characteristics, and a qualitative description of reductive dechlorination activity for each site where samples were obtained. For each study site, the assessment of reductive dechlorination activity was evaluated using the Wiedemeier et al. (1998) framework for classifying chlorinated ethene plumes. The Type I and Type II environments were denoted as moderate to high dechlorination sites, while Type III environments were indicated as sites with minimal reductive dechlorination activity. Sites with

minimal reductive dechlorination activity exhibited no or limited production of DCE and VC. Sites displaying moderate reductive dechlorination activity demonstrated a greater production of DCE with limited conversion to VC or ethene, while sites exhibiting high reductive dechlorination activity displayed complete conversion of source compounds to DCE, VC, and ethene.

Table 3-1. Summary of site information, sediment characteristics and reductive dechlorination activity for aquifer sediment samples.

Facility Name	Site	Aquifer Sediment Sample ID	Sediment Characteristics	Contamination	Reductive Dechlorination
Beale AFB, CA	Site 10	10 CO 39 RW	Sandy	TCE	Minimal
		10 CO 41 RW	Sandy		
		10 CO 49 MW	Silty, sandy		
Anoka County Riverfront Park, MN (ACRP)	OU1	PESMW13A	Brown, Sandy	TCE	Minimal
		PESMW10B	Fine Sandy		
Naval Weapons Industrial Reserve Plant, TX (NWIRP)	SWMU 118	799E152I	Brown, Sandy	TCE	Minimal
		799E115I	Brown, Silty sandy		
		799E151U	Brown, Sandy		
Marine Corps Recruit Depot Parris Island, SC (MCRD)	Site 45	PAI-45-MW21-SU	Brown, Sandy gravel	PCE and TCE	Moderate
		PAI-45-MW22-SU	Brown, Sandy gravel		
		PAI-45-MW22-SL	Grey, Silty sandy		
		PAI-45-MW21-SL	Grey, Silty sandy		
Naval Amphibious Base Little Creek, VA (NABLC)	Site 12	MLS10	Shallow depths: Sandy	PCE	Moderate
		MLS12			
		MLS20			
		MLS22	Deep depths: Grey, Silty sand		
		MW06			
		MIP			
Naval Submarine Base Kings Bay, GA (NSB)	Site 11	Outcrop	Black, silty sandy	PCE	High
		KBA-13A	Sandy		
		5/11/2002	Grey Silty		
NAS Pensacola, FL	WWTP	ORC-1	Fine, Sandy	TCE	Moderate
Naval Undersea Warfare Center Keyport, WA (NUWC)	OU1	Keyport	Brown, Silty sandy	PCE and TCE	High
Naval Air Engineering Station Lakehurst, NJ (NAES)	Sites I & J	MW6	Brown, Silty sandy	PCE and TCE	Minimal

NAS Jacksonville, FL	OU3	NAS JAX	Fine, sandy	PCE and TCE	Minimal
Hill AFB, UT	OU2	U2 1000		PCE and TCE	Minimal
		U2 1001	Brown, Silty		
		U2 1002	sandy		
		U2 1003			
NAS North Island, CA	Site 5- Unit 2	158	Black, Silty Sandy	PCE and TCE	Moderate/High
		159	Tan, Silty Sandy		
		160	Tan, Silty Sandy		
Former Naval Training Center Orlando, FL (NTC)	OU2	SB1	Fine, sandy	PCE	Moderate/High
		SB2	Fine, sandy		
Former Naval Training Center Orlando, FL (NTC)	OU4	SB1	Fine, sandy	PCE	Moderate/High
Fort Lewis, WA	Qv Unit	BIOINJ3	Brown, sandy gravel	TCE	Minimal
		BIOINJ4	Brown, sandy gravel		

Type I Environment (Moderate to High): *An environment that occurs when the primary substrate is anthropogenic carbon, and microbial degradation of this anthropogenic carbon drives reductive dechlorination*

Type II Environment (Moderate to High): *An environment that occurs in areas that are characterized by relatively high concentrations of biologically available native organic carbon*

Type III Environment (Minimal): *An environment that occurs in areas that are characterized by low concentrations of native and/or anthropogenic carbon, and concentration of dissolved oxygen that are greater than 1.0 mg/L*

PBOC Extraction Method

For this study, the PBOC extraction method of Rectanus et al. (2007) was used to quantify the carbon concentration (i.e., PBOC) which is the mass of extracted carbon per mass of aquifer sediment. To begin the process, 10 grams of aquifer sediment dried at 70°C over-night and sieved through 2-mm openings were combined with 20 mL of an extracting solution in carbon-free 40-mL vials. The two extracting solutions used in this study were 0.1% sodium pyrophosphate (pH 8.5, Crystalline/Certified ACS; Fisher Scientific, Pittsburgh, Pennsylvania) and 0.5 N NaOH (pH 13, Pellets/Certified ACS; Fisher Scientific). Aquifer sediments were exposed to alternating sequential extractions using 0.1% pyrophosphate and 0.5 N NaOH. After a 24-h extraction cycle on the rotary tumbler, samples were centrifuged for 25 min at 2000 rpm for solids separation. The supernatant was decanted and stored at 4°C until analyzed for extracted organic carbon. The PBOC content for extraction samples was analyzed using a Shimadzu Total Organic Carbon Analyzer (TOC)-VCSN with a detection limit of 0.10 mg/L carbon. Calibration curves for PBOC content were confirmed by external standards.

For quality control, triplicate samples containing soil sediment were prepared for each extracting solution. Extracting solutions were autoclaved twice for 15 min at 14 PSI and 121 °C before combined with sediment samples. Control samples containing only the extracting solution were also prepared for each extraction. PBOC data was reported as the arithmetic mean for the triplicate extractions.

Proteins and Humic Acids

The protein and humic acid content in PBOC was determined by the modified Lowry method et al. (1951) described by Frølund et al. (1996) using bovine serum albumin (BSA) as the standard. Proteins and humic acids were measured using a Beckman DU640, ultraviolet-visible scanning spectrophotometer (UV/VIS) at a wavelength of 750 nm. Standard curves were obtained by plotting the protein and humic acid concentration vs. the absorbance. For quality assurance, duplicate samples containing PBOC samples were prepared for each sampling location. Protein and humic acid data were reported as arithmetic mean for each location.

Hydrolyzable Amino Acid (HAA) Analysis

For hydrolyzable amino acids (HAA), aquifer sediment samples were placed in ampoules and hydrolyzed with 6 M HCl at 110°C for 22 hours. After hydrolysis, samples were neutralized with 1 M Na_2CO_3. HAA concentrations were determined using the EZ:faast Amino Acid Analysis Kit (Phenomenex, Inc. Torrance, CA). A HP 5890 gas-chromatograph (GC) equipped with a HP 5972 quadrupole mass detector was used for sample analysis. A Zebron GC column was provided with the EZ:faast Amino Acids Analysis kit. The GC column gas flow was 1 ml min^{-1}; and HAA were quantified using selected ion monitoring. The limit of quantification was 1 nmol mL^{-1}.

For quality assurance, all glassware was acid-rinsed (2 M HCL), rinsed three times with Milli-UV +water (Millipore) and combusted at 500°C for 3 hours. All plasticware was soaked in acid for a minimum of 12 hours, rinsed with Milli-UV+ water, and dried. Duplicate samples containing aquifer sediment were prepared for each sampling location. Concentrations of HAA were confirmed by internal and external standards.

Solid-Phase Organic Carbon (TOC_s)

The solid-phase organic carbon (TOC_s) content of sediment samples (total mass of organic carbon per mass sediment) was determined by elemental analysis using flash combustion and chromatographic separation (Costech Instruments). An ECS 4010 carbon gas chromatograph (GC) configuration with a 3 meter column was used for sample analysis. The gas flow rate within the GC column was 100 ml min^{-1}. Solid-phase organic carbon content was quantified using the thermal conductivity detector with a detection limit of 10 mg/kg carbon. For quality control, duplicate samples were analyzed for each sampling location. TOC_s data was reported as arithmetic mean for each location.

Results and Discussion

PBOC Variability

In this study, concentrations of PBOC (sum of pyrophosphate and alkali extracted carbon) and TOC_s were measured and reported for each selected site (Figure 3-1). Results of PBOC showed considerable variability among the study sites (complete results tabulated in Appendix A), ranging over four orders of magnitude. Similar variability was observed in the TOC_s concentration in the aquifer sediment. Concentrations of PBOC and TOC_s ranged from 3.3 to 14,170 mg/kg and 22.9 to 40,724 mg/kg, respectively for aquifer sediment samples. The lowest concentrations of PBOC and TOC_s were observed in sediments collected from Beale AFB; while, the greatest concentrations of PBOC and TOC_s were present in sediment samples collected from sites such as NSB Kings Bay, NAS Pensacola, NUWC Keyport, NTC Orlando OU2/OU4, NAS North Island, and MCRD Parris Island. Average concentrations of PBOC and TOC_s were 361 mg/kg and 2,424 mg/kg, respectively, with standard deviations of 31.7 and 207, respectively (N = 134). On average, PBOC represented 25% of TOC_s and ranged from 1.7% to 91.6%.

In Figure 3-1, regression results show a positive correlation exists between TOC_s and PBOC among study sites. A similar trend with TOC_s and PBOC is displayed from all sites, except for NWIRP. Aquifer sediments collected at NWIRP demonstrated some of the highest levels of TOC_s while exhibiting moderate concentrations of PBOC. The moderate concentrations of PBOC at NWIRP could be attributed to insufficient labile fractions of organic carbon associated with the local geology. For the remaining study sites, the regression results demonstrate a favorable relationship between the relative variation of PBOC and TOC_s across selected aquifer samples. If the results from the NWIRP site are not included in the regression, 67% of the variance is similar for PBOC and TOC_s among the study sites. Regression results indicate the standard error associated with the exponent in the power function is 0.052.

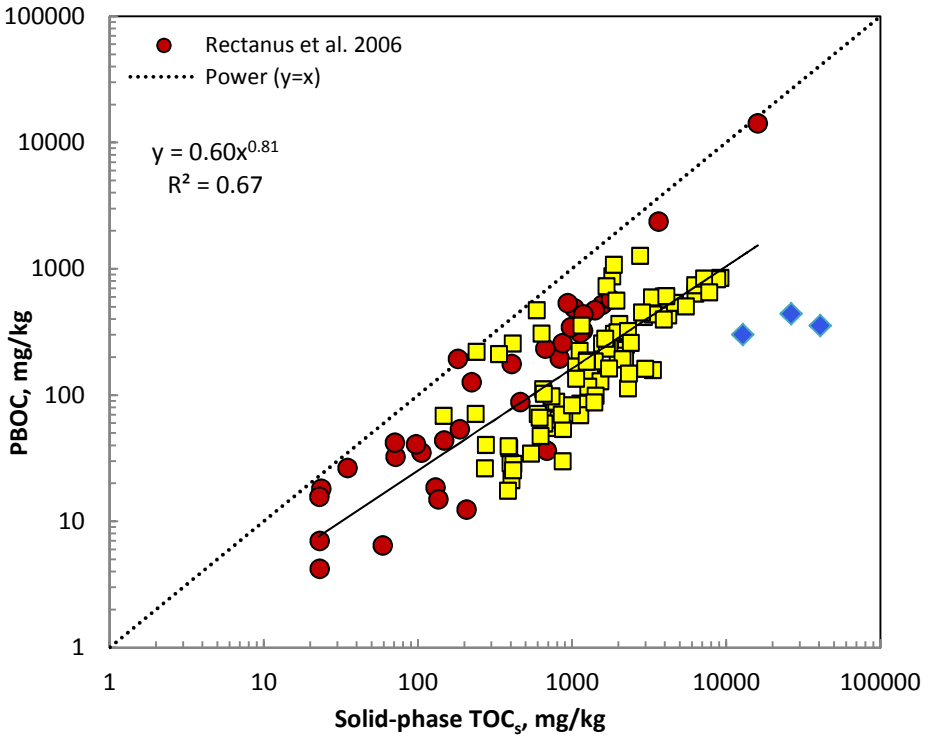

Figure 3-1. PBOC (mg/kg) versus TOC_s (mg/kg) for 15 selected study sites (NWIRP omitted from the regression –diamonds) including data from Rectanus (2006).

Results from regression analysis demonstrated that PBOC does not increase in a 1:1 ratio relative to increases in TOC_s. As the exponent of the power function indicates, at the higher end of the spectrum of TOC_s there is not necessarily a corresponding increase in PBOC. The results obtained from this analysis suggest that TOC_s alone does not provide a true indicator of the amount of readily-extractable organic carbon.

When mean values for PBOC and TOC_s at each site were analyzed, results yielded a slightly different regression. Figure 3-2 shows mean values for PBOC plotted versus TOC_s for each of the selected study sites. Because 70% of the aquifer sediment samples were collected at MCRD Site 45 and NAB Little Creek, regression results were subject to skew in the data among individual PBOC and TOC_s values. As shown in Figure 3-2, less variation existed for mean PBOC and TOC_s values about the 1:1 line, when compared to the entire sample set of PBOC and TOC_s values. Results further suggested that mean site values would yield greater quantities of PBOC for a given amount of TOC_s, relative to individual PBOC and TOC_s values for sediment samples collected at the sites. Regression results also indicated a greater standard error in the

slope between mean PBOC and TOC_s values, when compared to individual PBOC and TOC_s values. However, consistent with previous findings, these results supported that PBOC yield diminished with increasing TOC_s concentrations in aquifer sediments.

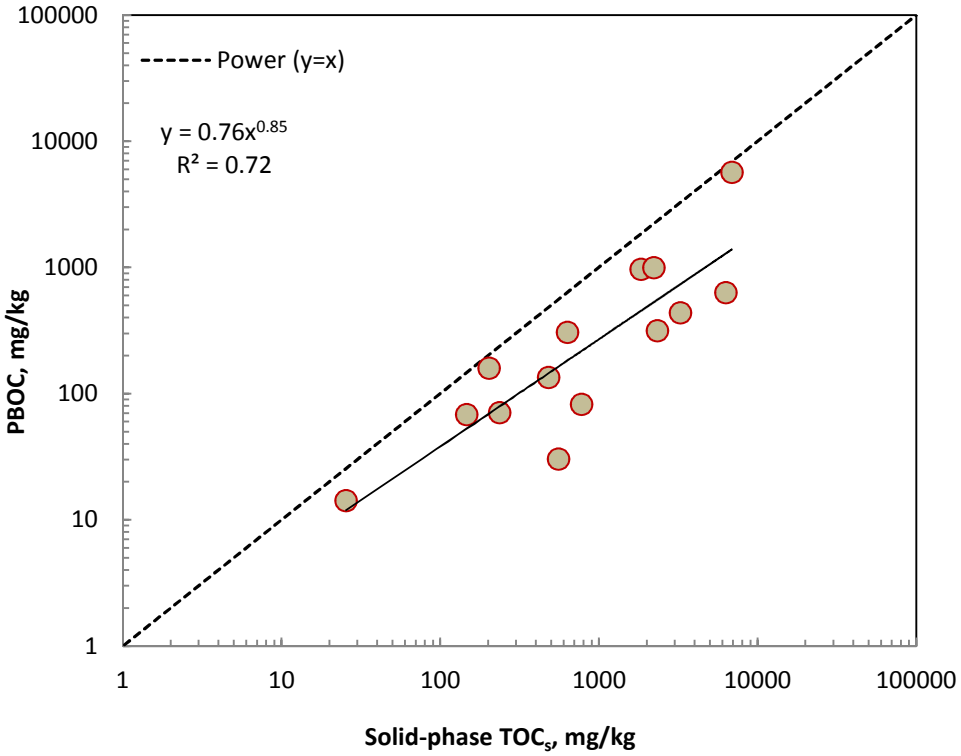

Figure 3-2. Mean site values for PBOC (mg/kg) versus TOC_s (mg/kg) for 15 selected study sites (NWIRP omitted from the regression) including data from Rectanus (2006).

The results from this study are consistent with other experimental studies for carbon extracted using aquifer sediments (Rectanus et al. 2006), which also suggested a correlation existed between PBOC and TOC_s. In the study conducted by Rectanus et al. (2006), PBOC in aquifer sediments was evaluated across 6 chloroethene-contaminated sites, which included Beale AFB, ACRP, NSB Kings Bay, NWIRP, NABL, and MCRD Parris Island. Results from the study demonstrated that 30% of TOC_s was extracted using the PBOC extraction method.

Results also show that concentrations of PBOC and TOC_s corresponded to reductive dechlorination activity in aquifer sediment samples (NWIRP omitted). Study sites that exhibited

minimal reductive dechlorination activity, such as NAES Lakehurst, Beale AFB, NAS Jacksonville, Hill AFB, and ACRP had consistently lower concentrations of PBOC and TOC_s. On the other hand, greater quantities of PBOC and TOC_s were reported from sites that exhibited moderate to high reductive dechlorination activity. The greatest concentrations of PBOC and TOC_s were observed from sediment samples collected at MCRD Parris Island, NAS Pensacola, NUWC Keyport, NTC Orlando OU2/OU4, NAS North Island, and NSB Kings Bay, which demonstrated greater reductive dechlorination activity. However, at the NWIRP site, higher reductive dechlorination activity did not correspond with greater concentrations of PBOC and TOC_s. The site displayed high levels of TOC_s; however, exhibited minimal levels of reductive dechlorination activity. As previously mentioned, the lack of chloroethene attenuation could be attributed to an insufficient supply of labile carbon, combined with microbial and geochemical conditions not conducive for reductive dechlorination. Overall, results from this study have provided an assessment of the variability of PBOC among sites displaying a variety of hydrogeological conditions and reductive dechlorination potential. Direct correlations between TOC_s and PBOC have allowed for a reasonable estimation of the quantity of PBOC present in aquifer sediments as a function of TOC_s.

To further investigate the relationship of PBOC with TOC_s, extracted carbon fractions were also examined. Figure 3-3 shows the carbon removed from pyrophosphate and alkali extractions and its relationship to TOC_s for each study site. Concentrations of extracted carbon from pyrophosphate and alkali extractions ranged from 1.72 to 1,145.4 and 0.86 to 554.6 mg/kg, respectively for aquifer sediment samples. Average concentrations of extracted carbon from pyrophosphate and alkali extractions were 125.6 mg/kg and 122.3 mg/kg, respectively for aquifer sediment samples. For the selected study sites, pyrophosphate and alkali extracted carbon comprised 52.4% and 47.6% of PBOC, respectively.

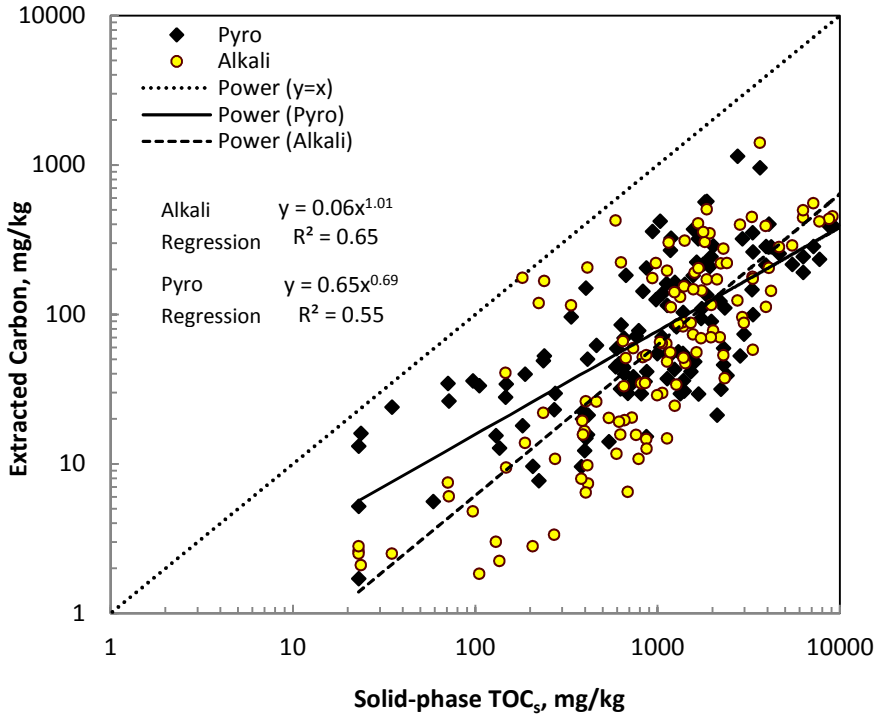

Figure 3-3. Extracted carbon (mg/kg) using pyrophosphate and alkali solutions versus TOC_s (mg/kg) for selected study sites (NWIRP omitted).

In Figure 3-3, results show that regressions for pyrophosphate and alkali extractions intersect at high concentrations of TOC_s. This suggests that at lower TOC_s concentrations greater fractions of pyrophosphate extracted carbon would be yielded. However, at higher TOC_s concentrations, alkali extractions would yield greater fractions of extracted carbon, while lower levels of extracted carbon would be observed from pyrophosphate extractions. Consistent with previous findings, lower concentrations of extracted carbon and TOC_s were observed at sites demonstrating minimal reductive dechlorination activity. Overall, study sites with greater levels of extracted carbon and TOC_s exhibited higher levels of reductive dechlorination activity.

PBOC Composition

To further examine the composition of PBOC in aquifer sediments, proteins and humic acids were evaluated for 8 study sites. Sampling locations included the following study sites: NAS Jacksonville, NAES Lakehurst, Hill AFB, NAS Pensacola, NAS North Island, NTC Orlando OU2, NTC Orlando OU4, and NUWC Keyport. Figure 3-4 shows concentrations of proteins and humic

acids in PBOC for sampling locations evaluated in this study. Concentrations of proteins and humic acids in PBOC ranged from non-detect to 28.6 mg/kg and 6.8 to 108.1 mg/kg, respectively for selected study sites. Greater concentrations of humic acids and proteins were observed for sediments collected at NTC Orlando OU2, NTC Orlando OU4, and NUWC Keyport; while lower concentrations of protein and humic acids were observed in samples collected from NAS Jacksonville, NAES Lakehurst, Hill AFB, and NAS Pensacola study sites.

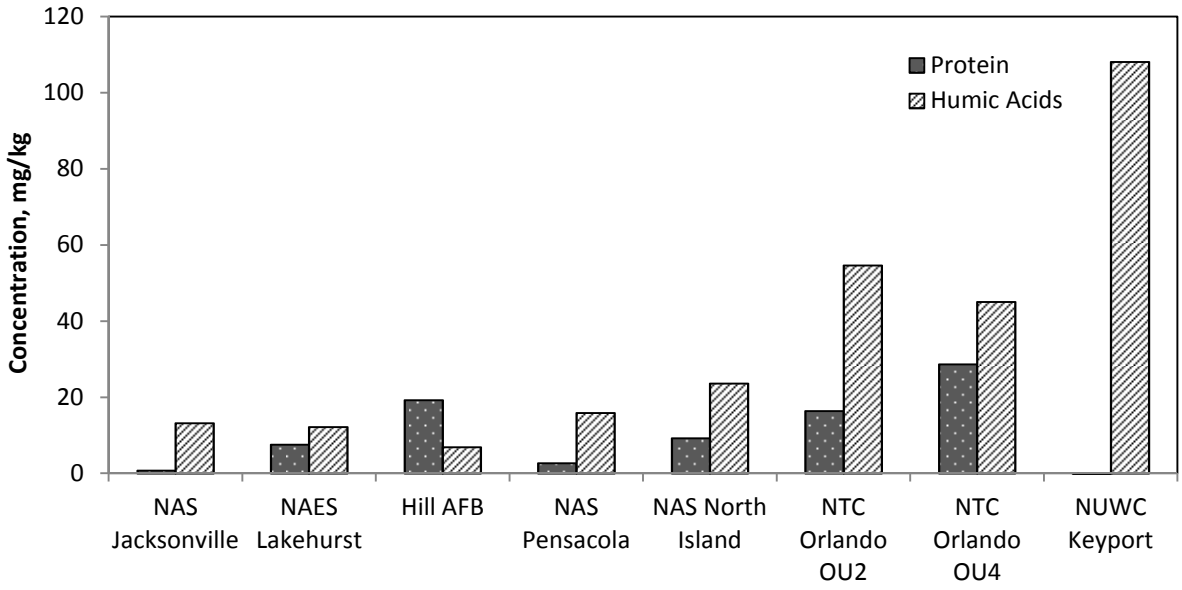

Figure 3-4. Proteins and humic acid concentration (mg/kg) for 8 selected study sites.

For the selected aquifer samples, results from this study showed that 6-86% of PBOC consisted of proteins and amino acids. Higher concentrations of proteins and humic acids in PBOC were characteristics of study sites displaying relatively efficient reductive dechlorination activity, when compared to sites with lower reductive dechlorination activity. The greatest concentrations of proteins and humic acids in PBOC were observed for sediments collected from NUWC Keyport, which exhibited high reductive dechlorination activity. For upper horizon soils, greater concentrations of humic acids have been reported for fractions of extracted carbon. Bonmati et al. (1998) reported humic acid concentrations ranging from 590 mg/kg to 3,400 mg/kg for sodium pyrophosphate (140mM) extracted organic carbon. Since proteins and humic acids are thought represent the mildly extractable and strongly-adsorbed fractions of

organic carbon, respectively, it was hypothesized that the levels of pyrophosphate and alkali extracted carbon would be directly proportional to the concentrations of proteins and humic acids.

Results from this study suggest a moderately weak correlation (R^2=0.47) exist between proteins and pyrophosphate extracted carbon; and, do not support that a relationship exist between proteins and alkali extracted carbon (Figure 3-5). Results from regression analysis indicated that the standard error in the slope and intercept between proteins and pyrophosphate extracted carbon was 0.011 and 3.64, respectively; while the error in the slope and intercept observed between proteins and alkali was 0.025 and 6.26, respectively.

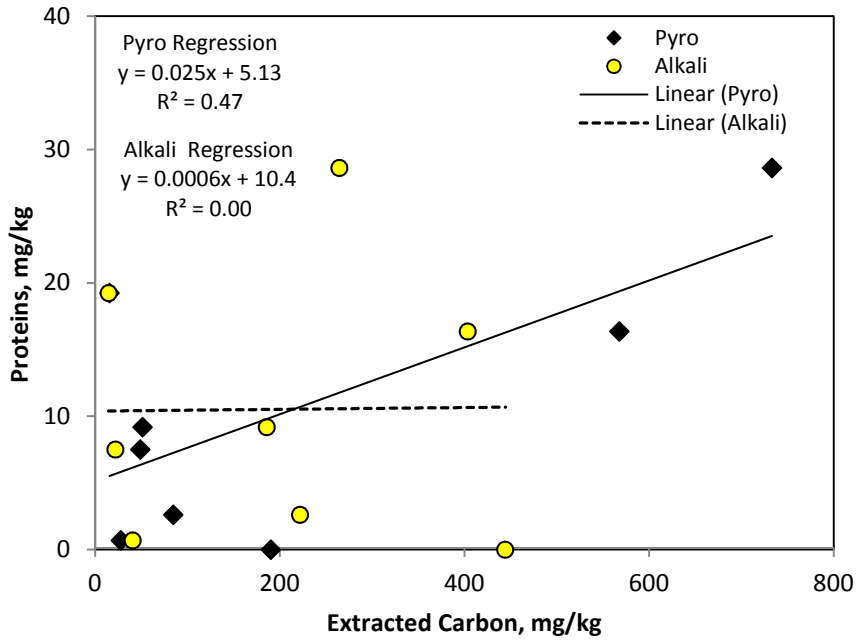

Figure 3-5. Protein concentration (mg/kg) versus extracted carbon (mg/kg) for 8 study sites.

Based on the regression, results indicate that 8% of pyrophosphate extracted carbon consists of proteins. The greatest concentrations for proteins were detected for sediment samples at collected from NTC Orlando OU4, which exhibited moderate to high reductive dechlorination activity. Although NUWC Keyport also displayed relatively high levels of reductive dechlorination, concentrations of proteins were not observed in the pyrophosphate extracted carbon fraction. For sediment samples collected at sites displaying minimal reductive

dechlorination activity, the fraction of pyrophosphate extracted carbon consistently displayed concentrations less than 200 mg/kg.

Results also demonstrate that a statistically significant positive correlation exist between humic acids and alkali extracted carbon (p=0.004) (Figure 3-6); however, results do not suggest that a correlation exist between humic acids and pyrophosphate extracted carbon. Based on regression analysis, standard error in the slope and intercept between humic acids and pyrophosphate extracted carbon was 0.045 and 15.3, respectively; while lower error in the slope and intercept was observed between humic acids and alkali extracted carbon.

Regression results indicated that 17% of alkali extracted carbon consists of humic acids (Figure 3-6). Consistent with the trend observed between proteins and pyrophosphate extracted organic carbon, moderate to high reductive activity was generally observed at sites with alkali extracted fractions of organic carbon greater than 200 mg/kg, with the exception being NAS North Island where alkali extracted carbon was 186 mg/kg. Overall, for the study sites selected in this study, regression results suggest that pyrophosphate extracted carbon represents a more reasonable measure of proteins when compared to alkali extracted carbon.

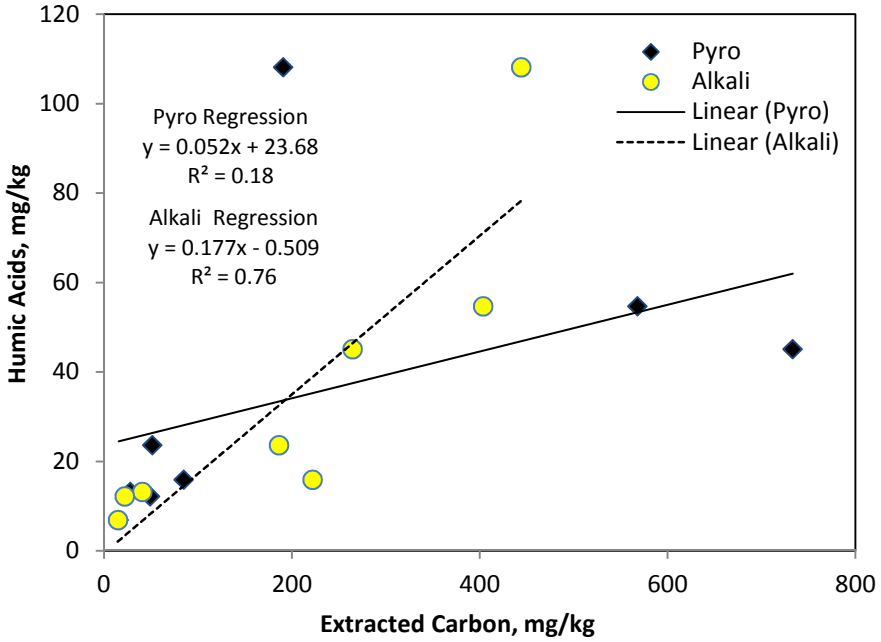

Figure 3-6. Humic acid concentration (mg/kg) versus extracted carbon (mg/kg) for 8 study sites.

Hydrolyzable Amino Acids (HAA)

Concentrations of hydrolyzable amino acids (HAA) were measured in aquifer sediments samples collected from 7 study sites (see Appendix A). Sampling locations included the following study sites: NABLC, Beale AFB, MCRD Parris Island, NTC Orlando OU2, NTC Orlando OU4, NAS Pensacola, and NAS North Island. The selected sites exhibited various levels of reductive dechlorination activity and TOC_s content. HAA consisted of protein amino acids with neutral, acidic, and basic functionalities. Concentrations of total HAA and PBOC ranged from 0.67 to 49.5 mg/kg and 4.21 to 1,269 mg/kg, respectively, for aquifer sediment samples. The lowest concentrations of total HAA were observed at Beale AFB and NTC Orlando OU2/OU4 study sites. For marine sediments, greater concentrations of total HAA have been reported for sampling locations. Dauwe and Middleburg (1998) reported total HAA concentrations ranging from 100 mg/kg to 9,000 mg/kg for marine sediments collected at 6 sampling locations in the North Sea. However, in this study, average concentrations of total HAA and PBOC for aquifer sediment samples were 9.54 mg/kg and 381.8 mg/kg, respectively. It was hypothesized that the level of PBOC would be directly proportional to the concentrations of HAA in the aquifer system.

Results from this study suggest a moderate positive correlation exist between total HAA and PBOC associated with aquifer sediments (Figure 3-7). Data from this study show that concentrations of total HAA and PBOC for NTC Orlando sites do not follow similar regression trends as the remaining sites. NTC Orlando sites have relatively high levels of PBOC and reductive dechlorination activity; however, lower concentrations of total HAA were observed at the sites. Generally, sites with greater concentrations of total HAA corresponded to higher levels of PBOC and reductive dechlorination activity. If the NTC Orlando sites are considered as data outliers, the lowest concentrations of total HAA and PBOC were observed in sediments collected from Beale AFB which exhibited minimal reductive dechlorination activity. Higher levels of PBOC and total HAA were detected from sediment samples collected from NAS North Island, CA, which exhibited moderate to high reductive dechlorination activity.

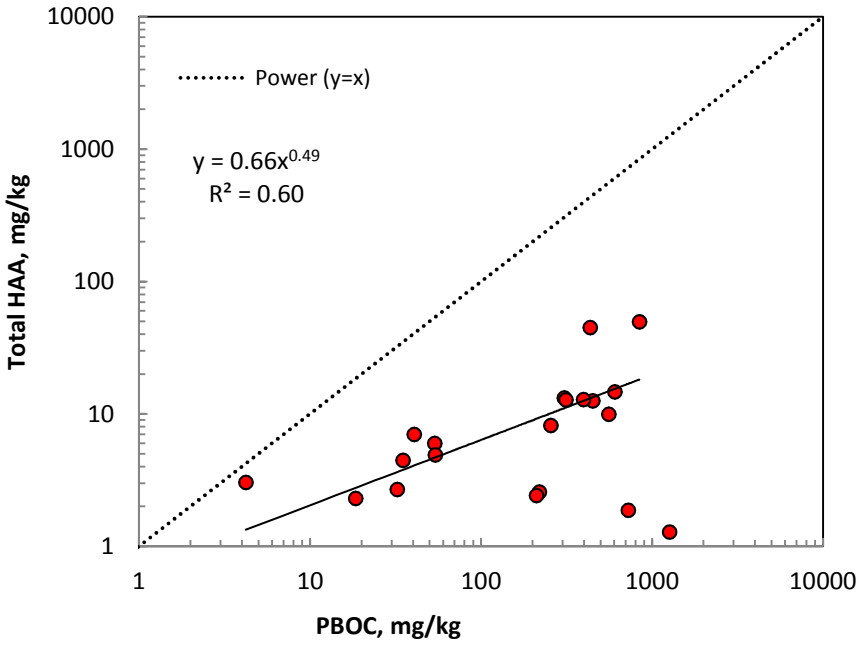

Figure 3-7. Total hydrolyzable amino acid concentration (mg/kg) versus PBOC (mg/kg) for selected study sites (NTC Orlando sites omitted from the regression).

Based on the regression, results indicate that 6.3% of PBOC consists of total HAA (Figure 3-7). This regression also supports that a separate trend may exist in the total HAA data with PBOC ranging from 200-500 mg/kg. The existence of two different data trends would be applicable to sites with that exhibited different levels of reductive dechlorination. Results suggest that sites with lower reductive dechlorination potential, such as Beale AFB, would fit one trend; while other sites with higher reductive dechlorination potential, such as MCRD Parris Island, would appear to fit a separate trend. Differences in the regression trend for sites exhibiting higher reductive dechlorination potential relative to sites displaying lower reductive dechlorination activity could be attributed to higher quantities of biomass from microbial remains present within the aquifer system. Overall, the correlation represented by the results show a reasonable positive relationship between PBOC, total HAA, and reductive dechlorination activity.

To further investigate the correlation between PBOC and its biodegradable components, fractions of extracted carbon and total HAA were also examined. Figure 3-8 shows extracted carbon from pyrophosphate and alkali extractions and its relationship to total HAA for each study site. Concentrations of extracted carbon from pyrophosphate and alkali extractions

ranged from 1.72 to 1,145.4 mg/kg and 1.39 to 503.2 mg/kg, respectively, for these aquifer sediment samples. Total HAA displayed a range of 48.8 mg/kg, with a minimum concentration of 0.67 mg/kg. Greater concentrations of total HAA and extracted carbon were observed in sediments collected from sites NAS North Island, NAS Pensacola, and MCRD Parris Island, which demonstrated moderate to high levels of reductive dechlorination activity.

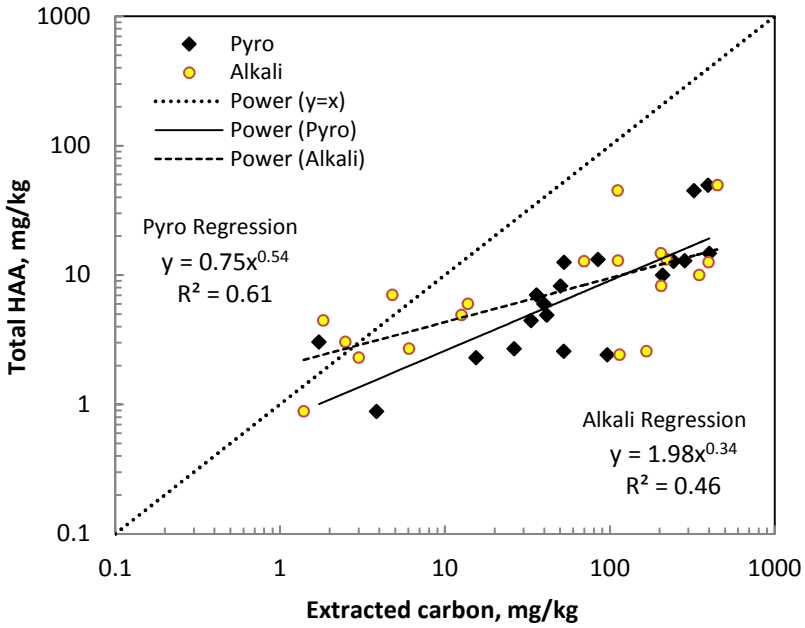

Figure 3-8. Total hydrolyzable amino acid concentration (mg/kg) versus extracted carbon (mg/kg) for selected study sites (NTC Orlando sites omitted).

Based on regression analysis, pyrophosphate extractions exhibited a stronger correlation with total HAA when compared to alkali extractions. Results from regression analysis indicated that lower standard errors in the slope and intercept were observed between total HAA and pyrophosphate extracted carbon, when compared to alkali extracted carbon. Figure 3-8 shows that regressions for pyrophosphate and alkali extractions intersect at high concentrations of extracted carbon. This would suggest that at greater levels of PBOC, alkali extractions would yield more total HAA than pyrophosphate extractions. However, the weak correlation between total HAA and alkali extracted carbon would suggest that the majority of the data does not follow this regression trend. For the selected study sites, regression results

suggest that pyrophosphate extracted carbon would yield more reliable measure of total HAA, when compared to alkali extracted carbon.

HAA Composition

To further evaluate the nature and composition of total HAA, protein amino acids were evaluated for each site based on functional groups. Protein amino acids were categorized into three groups based on their functionality. These functional groups included: acidic (aspartic acid and glutamic acid), neutral (alanine, valine, glycine, iso-leucine, leucine, proline, threonine, serine, phenylalanine, tyrosine, and methionine), and basic (histidine and lysine) amino acids. Figure 3-9 shows concentrations of HAA for the 3 protein amino acid functional groups for the aquifer samples evaluated in this study. Concentrations of acidic, neutral, and basic HAA ranged from 0.08 to 2.61 mg/kg, 0.98 to 10.39 mg/kg and 0.00 to 1.45 mg/kg, respectively, for aquifer sediment samples. Greater concentrations of HAA were observed in aquifer samples collected from NAS North Island, NAS Pensacola, and MCRD Parris Island; while lower levels of HAA were present in sediment samples from Beale AFB and NABLC.

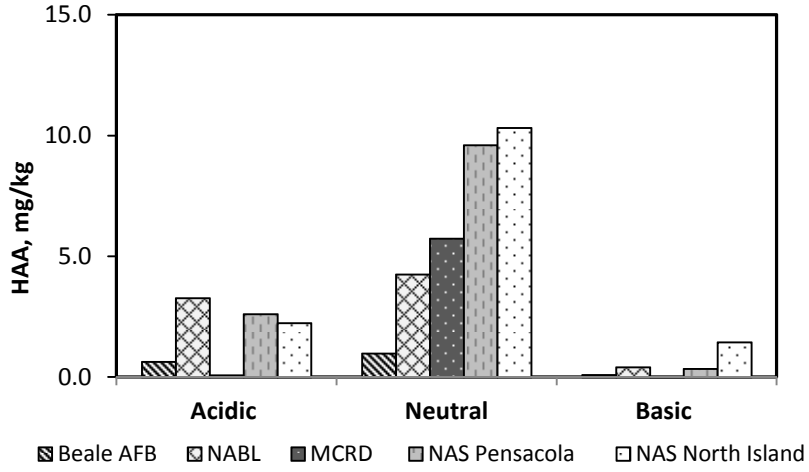

Figure 3-9. Functional groups of protein amino acids (mg/kg) for 5 of 7 study sites (NTC Orlando sites omitted). Acidic: aspartic acid and glutamic acid. Neutral: alanine, valine, glycine, iso-leucine, leucine, proline, threonine, serine, phenylalanine, tyrosine, and methionine. Basic: histidine and lysine.

Results reveal a dominance of neutral HAA, when compared to acidic and basic HAA. These findings are consistent with other experimental studies for total HAA analysis (Dauwe et al. 1998), suggesting that neutral amino acids accounted for the majority of total HAA. For the selected aquifer samples, results from this study showed that 50-93% of total HAA consisted of neutral HAA. Lower concentrations of neutral HAA were observed at sites with minimal reductive dechlorination activity, when compared to sites with greater reductive dechlorination activity. In sediments collected from NABL, which exhibited moderate reductive dechlorination activity, concentrations for neutral HAA were less by a factor of 3.4, when compared to NAS North Island, CA, which demonstrated higher reductive dechlorination activity. Based on the results obtained in this study, neutral HAA are the more dominant fraction in total HAA. Research studies (Jones et al. 1999) have also related neutral AA in soils sediments to microbial utilization. Biological uptake of neutral AA was observed at significant rates favorable for microbial respiration in soil sediments. These results further support that neutral HAA are not only the more dominant fraction in HAA, but they are also bioavailable organic carbon fractions.

NAC and Hydrolyzable Amino Acids (HAA) as a Measure of Sustainability

To evaluate the sustainability of reductive dechlorination, the natural attenuation capacity (NAC) was determined for each selected site (Chapelle and Bradley, 1998). In previous discussions, reductive dechlorination activity has been evaluated using a qualitative approach. However, because NAC incorporates rates of contaminant biotransformation, it provides a fully-quantitative measure of the rate of natural attenuation. Chapelle and Bradley (1998) demonstrated that a relatively large NAC value corresponds with a high level of reductive dechlorination. NAC values were determined for the 5 sites using the Natural Attenuation Software (Chapelle et al. 2003). Concentrations of total chloroethenes for each site were evaluated along the groundwater flowpath. Values for the NAC ranged from 0.00247 ft-1 to 0.0132 ft-1 for the selected study sites. The lowest NAC value was observed at Beale AFB, which exhibited minimal reductive dechlorination. The NAC observed at Beale AFB was lower by a

factor of 4, when compared to NAS North Island, CA, which demonstrated moderate to high reductive dechlorination activity.

Since NAC is a quantitative assessment of reductive dechlorination, it is hypothesized that NAC should be directly proportional to the concentrations of metabolizable carbon fractions in the aquifer system. In the previous discussion, neutral HAA demonstrated to be both the dominant and more readily bioavailable organic carbon fraction in total HAA. It is expected that neutral HAA will exhibit a direct correlation with the NAC at each site. Figure 3-10 shows the NAC of each site plotted versus neutral HAA for selected aquifer sediments. Study sites with higher NAC values generally displayed greater levels of neutral HAA. Overall, results from this study show that the NAC observed at each site positively correlate with concentrations of neutral HAA. Based on the regression, NAC exhibited a reasonable relationship with neutral HAA (R^2= 0.78). These results further support that NAC and neutral HAA can serve as quantitative tools for assessing the sustainability of reductive dechlorination.

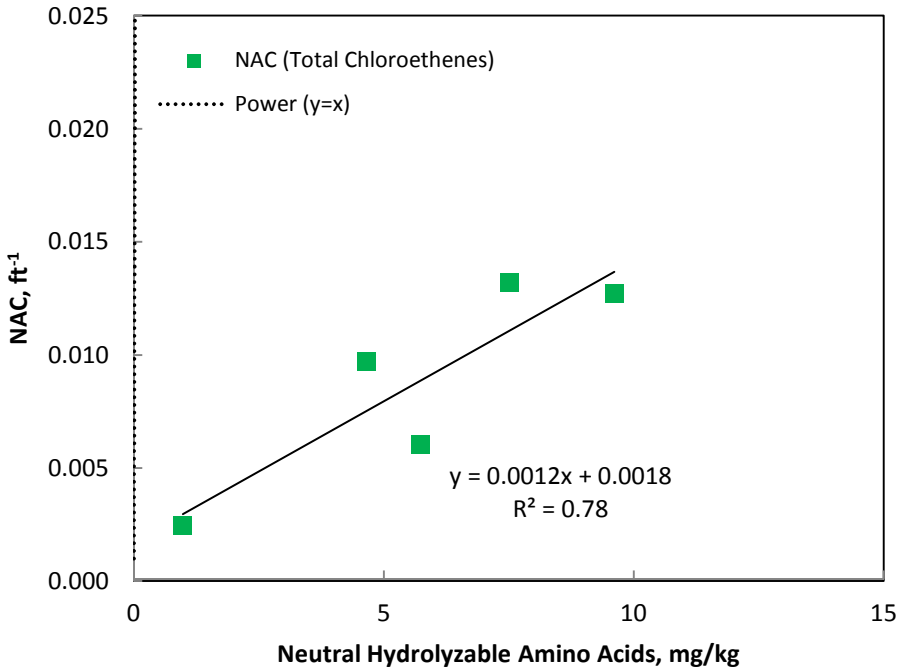

Figure 3-10. NAC (ft^{-1}) versus neutral hydrolyzable amino acid concentration (mg/kg) for 5 study sites.

Conclusions

In this study, the variability of potentially bioavailable organic carbon (PBOC) was examined among chlorinated solvent contaminated sites representing a wide range of environmental conditions. Using a novel approach, this study also characterized the carbon content through analysis of amino acids present in site samples. PBOC was measured using aquifer sediment samples collected at 15 study sites. Relationships were established between PBOC, TOC_s, and reductive dechlorination activity at the study sites. The composition of PBOC was evaluated through the analysis of proteins and amino acids. Direct correlations with PBOC were also developed between potentially bioavailable organic compounds present in the aquifer system, expressed as hydrolyzable amino acids (HAA), and the natural attenuation capacity of the site.

Results demonstrated that a positive correlation existed between PBOC, TOC_s, and reductive dechlorination activity at the sites. In this study, PBOC was expressed as the sum of pyrophosphate and alkali extracted fractions of organic carbon. Since pyrophosphate extractions were thought to represent loosely-bound organic carbon, it was expected that pyrophosphate extracted carbon would yield more reliable fractions of readily bioavailable organic carbon. Results supported that pyrophosphate extracted carbon comprised of greater than 50% of PBOC for sediment samples. Results demonstrated that pyrophosphate extracted carbon represented a more reasonable measure of proteins when compared to alkali extracted carbon. Results also demonstrated that greater levels of PBOC and TOC_s corresponded to higher reductive dechlorination activity at the sites.

Regression results also exhibited a similar relationship between PBOC, concentrations of natural organic carbon compounds, and the natural attenuation capacity (NAC) at the sites. Higher PBOC levels were consistently observed at sites greater NAC and levels of HAA. Overall, results from this study demonstrated the variability of PBOC in the aquifer sediments exhibited a reasonable correlation with TOC_s, hydrolyzable amino acids, and chloroethene transformation among the selected sites.

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Chapter 4. Evaluation of Field Metrics for Assessing Potentially Bioavailable Natural Organic Carbon in Contaminated Aquifer Sediments

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Abstract

This study evaluated the relationship between potentially bioavailable organic carbon (PBOC) and site specific performance data among chloroethene-contaminated sites representing a diverse range of hydrogeological conditions and reductive dechlorination activity. PBOC was measured using aquifer sediment samples collected at 12 study sites. Correlations with PBOC in the aquifer sediments were established between three independent field metrics associated with reductive dechlorination. Specifically, correlations with PBOC were established between geochemical conditions, including dissolved oxygen (DO) concentrations within the groundwater system. Direct relationships with PBOC were also developed between reductive dechlorination activity and the natural attenuation capacity (NAC) of the sites. Results indicated that the relationship between PBOC in aquifer sediments and DO concentrations follow a pattern similar to the hyperbolic relationship observed at petroleum-contaminated sites. Results indicated that PBOC in aquifer sediments was consistently lower in samples collected from sites with weakly reducing anaerobic to aerobic geochemical conditions, when compared to aquifer sediments collected from sites with more strongly reducing conditions. Higher DO concentrations in groundwater samples corresponded with lower PBOC levels in aquifer samples. Results from this study also demonstrated a positive relationship between hydrogen (H_2) concentrations in groundwater samples and PBOC levels in aquifer samples. Regression results suggested a moderate linear relationship between PBOC in aquifer sediments and hydrogen levels for the selected study sites. Consistent with these results, higher NAC values obtained from groundwater chloroethene data and reductive dechlorination activity corresponded with greater PBOC levels in aquifer samples from selected study sites. Overall, results from this study demonstrated that the level of PBOC in aquifer sediments may be a useful metric for sustaining conditions favorable for reductive dechlorination.

Introduction

Chloroethenes have extensively been used as degreasing agents and solvents for many military and industrial applications (Jendrezejewski et al. 2001). As a result of improper use and storage, they have become a significant source of soil and groundwater pollution (Grandel et al. 2004). Chloroethenes vary in the number of chlorine substituents, from the most chlorinated, tetrachloroethene (PCE), to the mono-chlorinated vinyl chloride (VC). Because of their use, PCE and trichloroethene (TCE) appear in groundwater systems with the greatest frequency and highest concentration levels (Stroo et al. 2003). Both PCE and TCE have potential health risks and EPA maximum contaminant levels (MCL) for drinking water of 5 µg/L. The less chlorinated ethenes, dichloroethene (DCE) and VC, are typically introduced to the groundwater system as secondary contaminants due to reductive transformations of PCE and TCE (Lorah and Olsen, 1999). However, increased levels of VC have been reported in groundwater as the result of accidental discharges from polyvinyl chloride industrial facilities (Hartmans et al. 1992). *Cis*-1,2-DCE, the most predominant isomer of DCE, and VC both have EPA drinking-water MCLs of 70 and 2 ug/L, respectively. VC is the only known carcinogen and is considered to be the greatest potential risk to human health.

Remediation of chloroethenes is quite difficult due to their persistent and mobile nature. Because of their physico-chemical properties they can dissolve in groundwater, volatilize in the unsaturated zone, sorb onto soil matrix or organic material, or exist as dense non-aqueous-phase-liquids (DNAPLs). Although chloroethenes tend to be more resistant to natural attenuation processes, significant evidence now exist that these contaminants can be transformed through biological degradation (Bradley and Chapelle, 1999). According to the U.S. EPA (1999), natural attenuation processes refers to naturally occurring processes that act without human intervention to reduce the mass, toxicity, mobility, volume, or concentration of contaminants in soil or groundwater. In this case, the contaminants remain onsite and the naturally occurring processes are left to attenuate the site. Monitored natural attenuation (MNA) has been an effective and economical remedial strategy at many chloroethene-contaminated sites (Weidemeier et al. 1999; Chapelle et al. 2001). MNA relies on natural

attenuation processes to achieve site-specific remedial objectives within a time frame that is reasonable when compared to other remedial alternatives.

When assessing the sustainability of a MNA at chloroethene impacted sites, it is often useful to evaluate the potential of the system to attenuate contaminants. Natural attenuation capacity (NAC) provides a quantitative measure of the capacity of a groundwater system to attenuate contaminant plumes. It can be described for a groundwater system as its ability to lower contaminant concentrations along the aquifer flowpath; and it is defined as the slope of the steady state concentration profile along a groundwater flow path (Chapelle and Bradley, 1998). The natural attenuation capacity of groundwater systems incorporates hydrologic (dispersive and advective), sorptive, and biological (biodegradative) processes within groundwater flow. The processes acting on a solute are shown below in the one-dimensional solute-transport equation (Freeze and Cherry, 1979)

$$\frac{\partial C}{\partial t} = -v\frac{\partial C}{\partial x} + D\frac{\partial^2 C}{\partial x^2} - \frac{K_d \rho_b}{n}\frac{\partial C}{\partial t} - kC \qquad (1)$$

where C is concentration, t is time, D is the coefficient of hydrodynamic dispersion (m^2/d), v is velocity of groundwater flow (m/d), ρ_b is bulk density, K_d is a linear sorption distribution coefficient, n is porosity, and k is a first-order biodegradation rate constant (d^{-1})

If steady-state conditions are achieved within the contaminant plume, the concentration profile along the centerline of the plume may be represented by the solution to the one-dimensional solute transport equation. Assuming uniform groundwater flow and first order decay, the NAC can be expressed in relationship to contaminant concentration and distance along the plume centerline. Equation 2 illustrates the mathematical definition for NAC (Chapelle and Bradley, 1998) where NAC is expressed in units of m^{-1}.

$$NAC = \left[\frac{-v + \sqrt{v^2 + 4Dk}}{2D}\right] \qquad (2)$$

For chlorinated ethenes, biodegradation is typically the critical natural attenuation mechanism (Chapelle et al. 1996). Reductive dechlorination is often the primary anaerobic degradation pathway for highly chlorinated ethenes. The sequence of reductive dechlorination

of PCE produces TCE, *trans*- and *cis*-1,2-DCE, VC and ultimately ethane, ethene and methane, such that an H^+ and two electrons replace one chlorine ion for each degradation reaction (Morrill et al. 2005). During anaerobic degradation of chloroethenes, electrons are transferred to an acceptor other than oxygen, such as nitrate (denitrification), Mn (IV), Fe (III), sulfate (sulfate reduction) and ultimately carbon dioxide (methanogenesis). Under such conditions, microorganisms will pair with oxidation and reduction reactions that yield the most energy (Vogel et al. 1987); thus, with the same organic substrate, microorganisms will preferentially use O_2, NO_3^-, Fe^{3+}, SO_4^{2-} and CO_2 (Fe^{2+} and CH_4 are products of the reduction of Fe^{3+} and CO_2). Biotransformation of highly chlorinated organics, such as PCE and TCE are not easily oxidized since there is less energy to be gained by the microorganism. Instead, they are used as electron acceptors and are reduced to less oxidized forms, daughter products.

In anaerobic environments, dissolved hydrogen (H_2) serves as the electron donor for dehalogenating microorganisms (Holliger et al. 1994). Minimum threshold concentrations have been established for hydrogen utilization as an electron donor with respect to the terminal electron acceptor process (TEAP) present in the aquifer system (Chapelle et al. 1995; Loffler et al. 1999). Chapelle et al. (1995) demonstrated that several dominant geochemical processes were associated with characteristic H_2 ranges, including methanogenesis (5–25 nM), sulfate reduction (1–4 nM), iron reduction (0.1–0.8 nM), and denitrification (<0.1 nM). Yang and McCarty (1998) showed that reductive dehalogenating bacteria compete best when H_2levels are maintained between 2 and 11 nM.

Theoretically, PCE and TCE can act as an electron acceptor under iron reducing conditions; however, there is evidence that dechlorination of these compounds occurs at a significant rate only under methanogenic conditions; and stronger reducing conditions are required for the dechlorination of the daughter products DCE and VC (Freedman and Gossett, 1989; DeBrunin et al. 1992). Many microbiologists refer exclusively to highly reducing sulfate and/or methanogenic conditions as strictly anaerobic, but not to the more oxidized nitrate-reducing, manganese-reducing, or iron-reducing conditions, which also occur in the absence of air (Bradley et al. 2008). Even though iron-reducing and methanogenic conditions also occur in the absence of oxygen, methanogenic conditions are substantially more reducing than iron-

reducing conditions. Hence, anoxic conditions are commonly referred to those highly reducing conditions under which sulfidogenesis and methanogenesis provide evidence for the absence of oxygen (Bradley et al. 2008). Under anoxic conditions, the threshold DO concentrations for groundwater has been considered below 0.1 to 0.5 mg/L (Bradley et al. 2008).

The efficacy of reductive dechlorination is often dictated by the availability of electron donors from two potential sources: (1) man-made or anthropogenic sources (Type I site), or (2) naturally-occurring organics (Type II site) (Wiedemeier et al. 1997). At chloroethene-contaminated sites with no anthropogenic carbon sources, the fermentation of natural organic carbon is the key factor supporting the dehalogenation process. Thus, the pool of bioavailable natural organic carbon along with geochemical conditions favoring reductive dechlorination often dictate the long-term effectiveness of MNA-based strategies at chlorinated solvent sites (Chapelle et al. 2007).

In the previous chapter, the variability of potentially bioavailable organic carbon (PBOC) was examined among chloroethene-contaminated sites representing a range of environmental conditions. Using the method developed by Rectanus et al. (2007), operationally defined fractions of labile organic carbon present in the aquifer sediment were quantified through a multi-step chemical extraction process. Concentrations of PBOC ranged from 3.3 to 14,170 mg/kg and represented on average 25% of solid-phase total organic carbon (TOC_s) associated with the aquifer sediments. Results from this study demonstrated that PBOC demonstrated a positive correlation between solid-phase TOC_s, reductive dechlorination activity, and potentially bioavailable organic compounds present in the aquifer system, expressed as hydrolyzable amino acids (HAA). Overall, the initial study examined the variability of PBOC among sites; however, it did not address the relationship between PBOC and field parameters associated with reductive dechlorination.

In this study, the relationship between PBOC and site specific performance data were evaluated among chloroethene-contaminated sites representing a diverse range of hydrogeological settings, natural organic carbon distribution, and reductive dechlorination potential. The objective of this study was to determine whether a relationship existed between the level of PBOC in aquifer sediments and quantitative field metrics associated with reductive

dechlorination. Using performance well data collected from historical monitoring reports, direct correlations were established with PBOC in aquifer sediments and three independent field metrics. Correlations with PBOC in the aquifer sediments are established between dissolved oxygen concentrations and geochemical conditions within the groundwater system at the selected study sites. Direct relationships with PBOC are also developed between reductive dechlorination activity and the NAC of the sites.

Materials and Methods

Aquifer Sediment Samples

Sediment samples were collected and stored following the procedure described in Chapter 3. The sampling locations included three sites in the Pacific region (NUWC Keyport, WA; Beale AFB, CA; NAS North Island, CA), one site in the Mountain region (Hill AFB, UT), one site in the Central Atlantic Coastal Plain region (NAES Lakehurst, NJ), and 7 sites in the Lower Atlantic Coastal Plain region (NAB Little Creek, VA; NSB Kings Bay, GA; MCRD Parris Island, SC; NTC Orlando (OU2/OU4), FL; NAS Pensacola, FL; NAS Jacksonville, FL). Table 4-1 summarizes the site information, reductive dechlorination activity, geochemical conditions, and hydrogeology for each site used in this study.

Table 4-1. Summary of site information for study sites.

Facility Name	Site	Type I, II, III Environment	Geochemistry	Physiographic Province
NAS Pensacola, FL	WWTP	Type II	Anaerobic (F/S)	Gulf Coastal Plain
Naval Air Engineering Station Lakehurst, NJ (NAES)	Sites I & J	Type III	Anaerobic (F) to Aerobic	Atlantic Coastal Plain
Marine Corps Recruit Depot Parris Island, SC (MCRD)	Site 45	Type II	Anaerobic (F/S/M)	Atlantic Coastal Plain
Hill AFB, UT	OU2	Type III	Aerobic	Basin and Range
NAS Jacksonville, FL	OU3	Type III	Anaerobic	Atlantic Coastal Plain
Former Naval Training Center Orlando, FL (NTC)	OU2	Type II	Anaerobic (F/S/M)	Atlantic Coastal Plain
Former Naval Training Center Orlando, FL (NTC)	OU4	Type II	Anaerobic (F/S/M)	Atlantic Coastal Plain
Naval Undersea Warfare Center Keyport, WA (NUWC)	OU1	Type I	Anaerobic (F/S/M)	Pacific Border
NAS North Island, CA	Site 5-Unit 2	Type I	Anaerobic (F/S/M)	Basin and Range
Naval Submarine Base Kings Bay, GA (NSB)	Site 11	Type I	Anaerobic (F/S/M)	Atlantic Coastal Plain
Beale AFB, CA	Site 10	Type III	Aerobic	Basin and Range
Naval Amphibious Base Little Creek, VA (NABLC)	Site 12	Type II	Anaerobic (S/M)	Atlantic Coastal Plain
F = Fe-reducing S = Sulfate-reducing M = Methanogenesis				

Type I Environment: *An environment that occurs when the primary substrate is anthropogenic carbon, and microbial degradation of this anthropogenic carbon drives reductive dechlorination*

Type II Environment: *An environment that occurs in areas that are characterized by relatively high concentrations of biologically available native organic carbon*

Type III Environment: *An environment that occurs in areas that are characterized by low concentrations of native and/or anthropogenic carbon, and concentration of dissolved oxygen that are greater than 1.0 mg/L*

PBOC Extraction Method

For this study, the PBOC extraction method in Rectanus et al. (2007) was used to quantify the carbon concentration (i.e., PBOC) which is the mass of extracted carbon per mass of aquifer sediment. To begin the process, 10 grams of aquifer sediment dried at 70°C over-night and sieved through 2-mm openings were combined with 20 mL of an extracting solution in carbon-free 40-mL vials. The two extracting solutions used in this study were 0.1% sodium pyrophosphate (pH 8.5, Crystalline/Certified ACS; Fisher Scientific, Pittsburgh, Pennsylvania) and 0.5 N NaOH (pH 13, Pellets/Certified ACS; Fisher Scientific). Aquifer sediments were exposed to alternating sequential extractions using 0.1% pyrophosphate and 0.5 N NaOH. After a 24-h extraction cycle on the rotary tumbler, samples were centrifuged for 25 min at 2000 rpm for solids separation. The supernatant was decanted and stored at 4°C until analyzed for extracted organic carbon. The carbon content of the supernatant was analyzed using a Shimadzu Total Organic Carbon Analyzer (TOC)- VCSN with a detection limit of 0.10 mg/L carbon. Calibration curves for PBOC content were confirmed by external standards.

For quality control, triplicate samples containing soil sediment were prepared for each extracting solution. Extracting solutions were autoclaved twice for 15 min at 14 PSI and 121 °C before combined with sediment samples. Control samples containing only the extracting solution were also prepared for each extraction. PBOC was reported as the total mass of organic carbon extracted by the pyrophosphate and alkali extractions and expressed as the arithmetic mean for the triplicate extractions.

Groundwater Parameter Analyses

Groundwater parameters were evaluated using historical performance data collected from monitoring reports (Appendix B). Performance monitoring well data collected from each sampling location was used to define the geochemical and dissolved oxygen distribution at each site. In addition, contaminant concentration data obtained from monitoring reports was used to evaluate the NAC of chloroethenes along the groundwater flowpath for each sampling location. Dissolved oxygen concentrations were measured using field calibrated meters for each sampling location. Hydrogen levels were determined using the bubble-strip method described

by Chapelle et al. (1997). For each site, pre-remediation performance data was used from monitoring wells screened at various depths within the contaminant plume and saturated zone. Groundwater parameter values were reported as the arithmetic mean for each sampling location. Data variability for each site was analyzed using standard deviations.

Results and Discussion

PBOC Extractions

Results of PBOC in aquifer sediments are shown in Table 4-2. Concentrations of PBOC ranged from 12.5 to 998.0 mg/kg for aquifer sediment samples. The lowest concentrations of PBOC were observed in sediments collected from Beale AFB. Higher concentrations of PBOC were present in sediment samples collected from sites such as NSB Kings Bay, NAS Pensacola, NUWC Keyport, NTC Orlando OU2/OU4, NAS North Island, and MCRD Parris Island. Average concentrations of PBOC reported for aquifer sediment samples were 361.9 mg/kg for the selected sites. PBOC levels in aquifer sediments were consistently lower in samples collected from sites with weakly reducing anaerobic to aerobic geochemical conditions, which displayed minimal reductive dechlorination activity (Table 4-2), while greater quantities of PBOC were present in aquifer sediments collected from sites with more strongly reducing conditions, exhibiting moderate to high reductive dechlorination activity. Because bioavailable organic carbon is a key factor supporting reductive dechlorination (Bradley, 2000), these results are consistent with observations from controlled laboratory experiments.

It has been suggested that the long term sustainability of reductive dehalogenation requires a flux of bioavailable organic carbon available for reducing microorganisms to consume dissolved oxygen (DO) and to provide a source of hydrogen (H_2) (Chapelle et al. 2007). Thus, it is expected that potentially bioavailable organic carbon in aquifer sediments will directly correlate with groundwater dissolved oxygen, geochemical, and contaminant parameters at the selected study sites. To examine the relationship between PBOC in aquifer sediments and site specific data at chloroethene-contaminated sites, correlations were developed between PBOC data and (1) DO concentrations, (2) H_2 levels, and the (3) NAC values.

Table 4-2. Summary of PBOC (mg/kg) in aquifer sediments for selected study sites.

Facility Name	Site	PBOC, mg/kg
NAES Lakehurst, NJ	Sites I & J	70.9
MCRD Parris Island, SC	Site 45	276.1±195.1
Hill AFB, UT	OU2	30.4±2.7
NAS Jacksonville, FL	OU3	68.4
NAS Pensacola, FL	WWTP	306.8
NTC Orlando, FL	OU2	971.7±143.7
NTC Orlando, FL	OU4	998.0±383.3
NUWC Keyport, WA	OU1	635.0
NAS North Island, CA	Site 5-Unit 2	237.6±25.7
NSB Kings Bay, GA	Site 11	534.1
Beale AFB, CA	Site 10	12.5±9.4
NAB Little Creek, VA	Site 12	201 (all wells)/266 (lower wells)

Groundwater Parameter Analyses

Dissolved Oxygen (DO)

DO concentrations were evaluated in groundwater samples using pre-remediation performance well data collected from all 12 study sites. Table 4-3 shows the number of sampling events, DO concentrations, and corresponding standard deviation values for each study site. DO levels for the selected sites ranged from 0.00 $\pm$ 0.00 mg/L to 6.42 $\pm$ 0.83 mg/L. The lowest concentrations of DO were observed in samples collected from NSB Kings Bay, GA, which exhibited relatively efficient reductive dechlorination of chlorothenes (Chapelle et al. 2005). In contrast, the highest concentrations of DO were observed at Beale AFB, CA, which exhibited relatively inefficient reductive dechlorination.

Table 4-3. Summary of dissolved oxygen and hydrogen concentrations with corresponding standard deviations for selected sampling events.

Facility Name	Number of Sampling Events	Dissolved Oxygen (mg/L)	Standard Deviation	Number of Sampling Events	Hydrogen (nM)	Standard Deviation
NAS Pensacola, FL	4	0.03	0.09	3	0.95	1.64
NAES Lakehurst, NJ	4	1.98	1.86			
MCRD Parris Island, SC	4	0.07	0.03	1	2.07	0.91
Hill AFB, UT	5	3.79	1.55			
NAS Jacksonville, FL	1	0.86	0.92			
NTC Orlando, FL	1	0.50	0.22	1	5.67	1.76
NTC Orlando, FL	4	0.43	0.46			
NUWC Keyport, WA	2	0.36	0.33	2	2.03	1.81
NAS North Island, CA	3	0.33	0.28			
NSB Kings Bay, GA	2	0.00	0.00	2	0.98	0.36
Beale AFB, CA	3	6.42	0.83			
NAB Little Creek, VA	2	0.51	0.19			

The range of DO concentrations is thought to be influenced by the presence of natural organic carbon (Chapelle et al. 2007). Since dissolved oxygen is microbially consumed in the presence of organic carbon, sites with small amounts of potentially bioavailable organic carbon were expected to have higher dissolved oxygen levels; while, sites with more PBOC were expected to have lower dissolved oxygen levels, which is a prerequisite for reductive dechlorination. It was hypothesized that dissolved oxygen concentrations would be inversely related to the level of PBOC in aquifer sediments.

Figure 4-1 shows the average concentrations of DO plotted versus PBOC for each site analyzed. At 8 of the selected sites (NAB Little Creek; NAS Pensacola; MCRD Parris Island; NTC Orlando OU2/OU4; NUWC Keyport; NAS North Island; NSB Kings Bay), which exhibited moderate to high reductive dechlorination activity, the average dissolved oxygen concentrations were less than or equal to 0.5 mg/L, while PBOC concentrations were greater than 200 mg/kg. Average DO concentrations for the remaining sites, which demonstrated

minimal reductive dechlorination activity, were greater than 0.5 mg/L with PBOC concentrations lower than 75 mg/kg. Higher DO concentrations in groundwater samples corresponded with lower PBOC levels in aquifer samples from selected study sites.

Regression results for PBOC and DO levels for the study sites are shown in Table 4-4. Based on trend analysis, the relationship between PBOC and dissolved oxygen concentrations at the study sites demonstrated statistically significant ($p<0.0001$) inverse correlations that fit a hyperbolic regression (Table 4-4). These results suggest that a threshold exists between DO concentrations in the groundwater system and PBOC in aquifer sediments. At sites with greater levels of PBOC and reductive dechlorination activity, DO was readily consumed by microorganisms. As a result, lower DO levels were observed at sites exhibiting stronger reducing or anoxic conditions. Under anoxic conditions, the minimum threshold for dissolved oxygen concentrations for groundwater has been considered below 0.1 to 0.5 mg/L (Bradley et al. 2008).

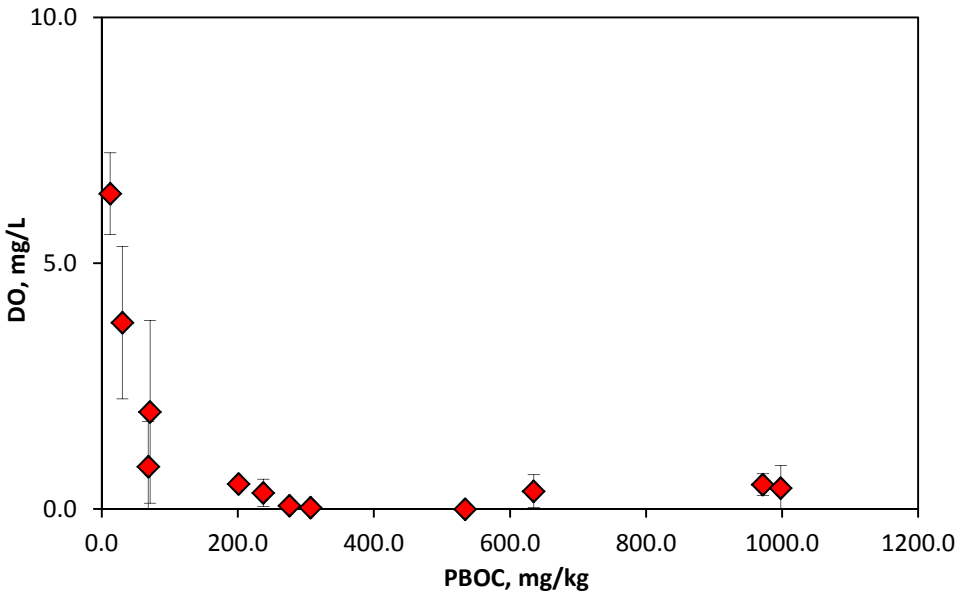

Figure 4-1. Dissolved Oxygen (DO) versus PBOC for selected study sites. Standard deviations for dissolved oxygen concentrations are shown with error bars.

Table 4-4. Hyperbolic and linear regression fit data for PBOC in aquifer sediments and groundwater parameters for selected study sites.

Groundwater Parameter	Fit	Parameters	P-Value	R^2
Dissolved Oxygen (DO)	y=(a*b)/(b+x)	a = 19.78 b = 6.12	<0.0001	0.96
Hydrogen (H_2)	y=mx+b	m = 0.005 b = -0.379	0.113	0.62

Hydrogen (H_2)

In addition to assessing DO concentrations, site monitoring data where H_2 concentrations in groundwater were collected was also evaluated. Hydrogen concentrations were evaluated in groundwater samples using pre-remediation performance well data collected from 5 study sites. Sampling locations included the following study sites: MCRD Parris Island, NAS Pensacola, NTC Orlando OU2, NUWC Keyport, NSB Kings Bay. Table 4-3 shows the number of sampling events, hydrogen concentrations, and corresponding standard deviation values for each selected site. Hydrogen levels for the selected sites ranged from 0.95 ± 1.64 to 5.67 ± 1.76 nM. The lowest concentrations of hydrogen were observed in samples collected from NAS Pensacola, while hydrogen levels greater than 5 nM were observed at NTC Orlando OU2.

Chapelle et al. (1995) demonstrated that H_2 levels were indicative of dominant geochemical processes and redox conditions within the aquifer groundwater system. For chloroethenes, stronger reducing conditions and greater H_2 levels are favorable for reductive dechlorination. Since H_2 is produced by microorganisms in the presence of organic carbon, sites with higher levels of bioavailable organic carbon were expected to have higher hydrogen levels, while, sites with lower amounts of bioavailable organic carbon were expected to have lower dissolved oxygen levels. It was hypothesized that H_2 concentrations in the groundwater system would be positively correlated with PBOC in the aquifer sediments.

Figure 4-2 shows the average concentrations of hydrogen plotted versus PBOC for each selected study site. At 4 of the selected 5 sites which exhibited moderate to high reductive dechlorination activity (the exception being NTC Orlando OU2), the average H_2 concentrations were greater than or equal to 0.95 nM, with PBOC concentrations ranging from 211.0 to 635.0

mg/kg. For these sites, H_2 levels were characteristic of sulfate-reducing conditions; however, greater levels of hydrogen and PBOC were reported at NTC Orlando, FL (OU2), which exhibited methanogenic conditions. Results in this study generally suggest that higher H_2 concentrations in groundwater samples corresponded with greater PBOC levels in aquifer samples from selected study sites.

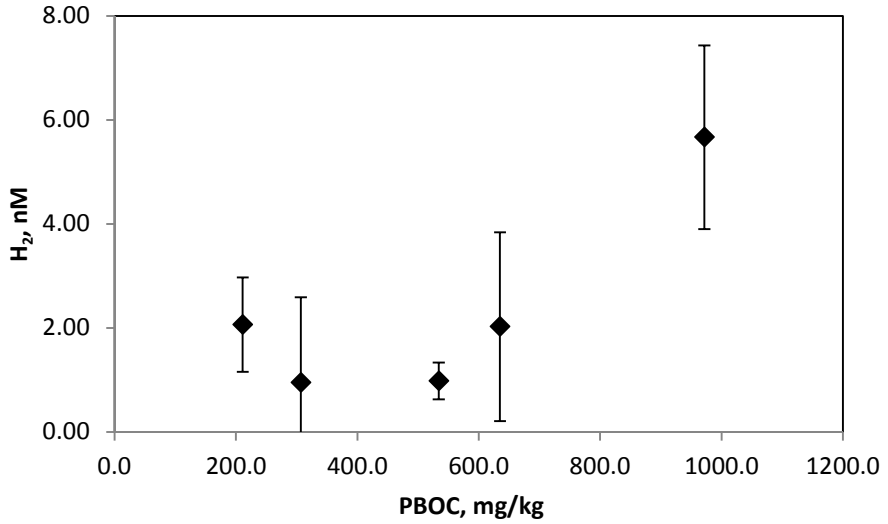

Figure 4-2. Hydrogen (H_2) versus PBOC for selected study sites. Standard deviations for hydrogen concentrations are shown with error bars.

Regression data for PBOC and hydrogen levels for the selected study sites are shown in Table 4-4. Results from this study suggest a moderate linear relationship exist between hydrogen concentrations and PBOC levels. Although the slope of the correlation between PBOC and hydrogen is not statistically significant ($p>0.05$), linear regression results suggest that PBOC accounted for 62% ($R^2= 0.62$; $p=0.113$) of the variation within hydrogen levels.

Natural Attenuation Capacity (NAC)

Concentrations of total chloroethenes were evaluated along the groundwater flowpath using pre-remediation performance well data collected from 10 of the 12 study sites. The natural attenuation capacity (NAC) for each site was determined using natural attenuation software. Sampling locations included the following sites: NAB Little Creek, NAES Lakehurst, MCRD Parris Island, Hill AFB, NAS Pensacola, NTC Orlando OU4, NUWC Keyport, NAS North

Island, NSB Kings Bay, and Beale AFB. For each study site, the NAC provided a quantitative measure of chloroethene attenuation by incorporating rates of contaminant biotransformation in the groundwater system.

Figure 4-3 shows boxplots of the NAC for selected study sites, displaying minimal, moderate, and high reductive dechlorination activity. The level of reductive dechlorination was assessed using geochemical and redox data from historical performance monitoring reports for selected study sites. In Figure 4-3, boxes represent the 25-75% quartile range for NAC values; while center lines and whiskers indicate median values and data ranges for the NAC, respectively. Values for the NAC ranged from 0.00095 to 0.00247 ft^{-1}, 0.00604 to 0.0132 ft^{-1}, and 0.00861 to 0.0227 ft^{-1} at sites that exhibited minimal, moderate, and high reductive dechlorination activity ranges, respectively. The lowest NAC value was observed at NAES Lakehurst, which exhibited minimal reductive dechlorination potential. The NAC observed at NAES Lakehurst was lower by a factor of 24, when compared to NTC Orlando OU4, which demonstrated higher reductive dechlorination activity.

Average NAC values were 0.00159ft^{-1}, 0.01065 ft^{-1}, and 0.01325 ft^{-1} for sites displaying minimal, moderate, and high reductive dechlorination activity, respectively. Results from statistical analysis suggested that a significant difference existed between the average NAC values for sites exhibiting minimal reductive dechlorination potential relative to sites displaying high levels of activity ($p=0.038$). These results further support that higher NAC values corresponded to greater contaminant attenuation at the selected study sites.

At chloroethene-contaminated sites, the capability of a groundwater system to attenuate contaminants is not only influenced by geochemical and redox conditions, but also by the presence of natural organic carbon. Because greater concentrations of bioavailable organic carbon will more adequately support reductive dechlorination of source compounds (e.g., PCE or TCE) and intermediates (e.g., DCE, VC, and ethene), the level of PBOC in aquifer sediments would be expected to be positively correlated with the natural attenuation capacity of a site.

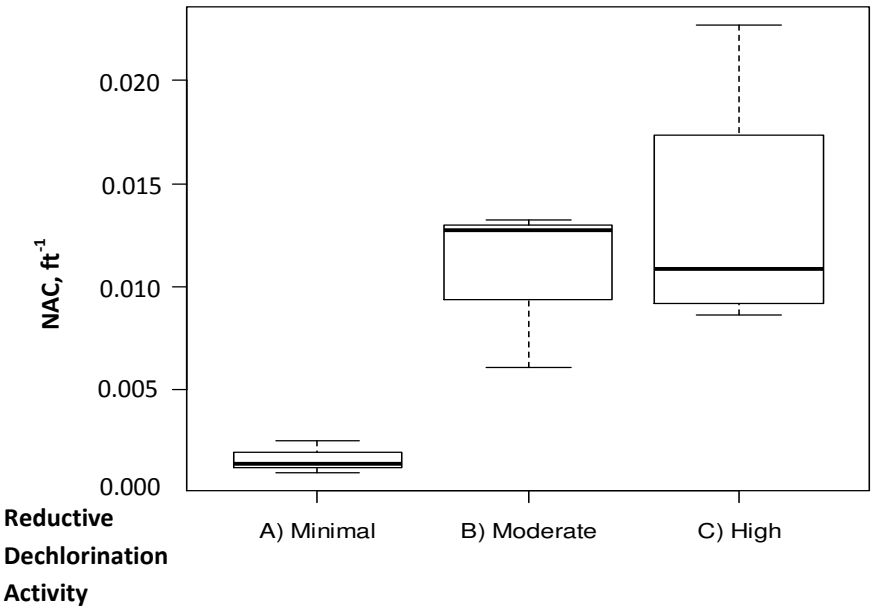

Figure 4-3. Boxplots of the natural attenuation capacity (NAC) for selected study sites displaying minimal, moderate, and high reductive dechlorination activity. Boxes represent the 25-75% quartile range for NAC values. Center lines and whiskers indicate median values and data ranges for the NAC, respectively.

Figure 4-4 shows the NAC of each site plotted versus PBOC for each selected study site. At 7 of the selected sites (NAB Little Creek; NAS Pensacola; MCRD Parris Island; NTC Orlando OU2; NUWC Keyport; NAS North Island; NSB Kings Bay), which exhibited moderate to high reductive dechlorination activity, NAC values were greater than or equal to 0.0060 ft^{-1}, with PBOC concentrations ranging from 211.0 to 1269.1 mg/kg. Lower NAC values and concentrations of PBOC were obtained for sites demonstrating minimal reductive dechlorination activity. Results in this study suggest that higher NAC values obtained from groundwater chloroethene data corresponded with greater PBOC levels in aquifer samples from selected study sites.

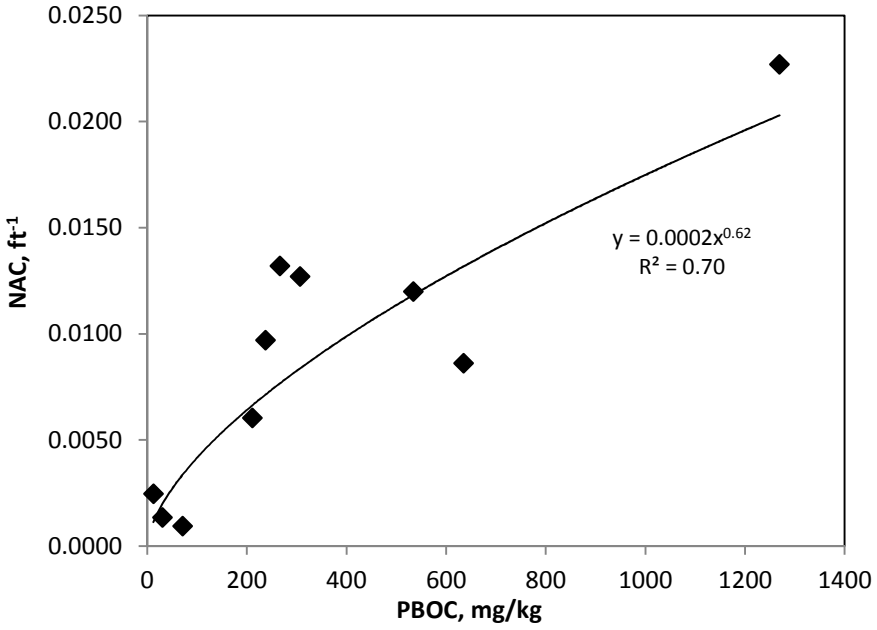

Figure 4-4. NAC for total chloroethenes (ft^{-1}) versus PBOC (mg/kg) for selected study sites.

Conclusions

Relationships between potentially bioavailable organic carbon (PBOC) and site specific contaminant and geochemical data were evaluated among 12 chloroethene-contaminated sites from various hydrogeological settings. Using performance well data from conventional monitoring wells collected from historical reports, this study established direct correlations with PBOC in aquifer sediments and independent field metrics associated with reductive dechlorination. Specifically, correlations were developed between PBOC data and (1) DO concentrations, (2) H_2 concentrations, and the (3) NAC values at selected study sites.

Results from this study demonstrated a statistically significant inverse relationship between PBOC in aquifer sediments and DO concentrations in groundwater samples. Because DO is microbially consumed in the presence of organic carbon, the finding that lower DO concentrations were consistently representative of sites with higher PBOC levels is consistent with observations at petroleum hydrocarbon contaminated sites. . In this study, PBOC levels greater than 200 mg/kg were characteristics of sites displaying maximum levels of DO (0.5 mg/L) associated with efficient reductive dechlorination. It is interesting to note that in Chapter

3, the level of PBOC associated with an increasing trend in total hydrolyzable amino acids also was within the range of 200-500 mg/kg.

Results also suggested a positive relationship between PBOC in aquifer sediments and dissolved H_2 concentrations in the groundwater system. Using data collected at 5 of the 12 study sites, higher hydrogen concentrations in the groundwater system corresponded with greater PBOC levels in aquifer sediments. In addition, results suggested a direct relationship between PBOC in aquifers sediments and quantitative measures of a groundwater system to attenuate a chloroethene plume. Higher NAC values corresponded with greater PBOC levels in aquifer samples and reductive dechlorination activity for the selected study sites. Similarly to results obtained for DO, higher NAC values were characteristic of sites with PBOC levels greater than 200 mg/kg. Overall, the results of this study have demonstrated that PBOC in aquifer sediments is a potentially useful metric and possibly a necessary component for sustaining conditions favorable for microbial reductive dechlorination at chloroethene-contaminated sites.

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Chapter 5. Distribution of Potentially Bioavailable Natural Organic Carbon in Aquifer Sediments at a Chloroethene Contaminated Site

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Abstract

The distribution of potentially bioavailable organic carbon (PBOC) was investigated at a chloroethene-contaminated site. PBOC was measured in surficial aquifer sediment samples collected at varying depths and locations in the vicinity of a chloroethene plume. The effect of long-term chloroethene exposure on PBOC levels was examined by comparing differences in extractable organic carbon in aquifer sediments with minimal chloroethene exposure relative to samples collected in the PCE-contaminated source zone. Using performance monitoring data, direct correlations with PBOC were also developed with groundwater chloroethene concentrations. Results suggested a natural logarithm-normal distribution for PBOC in aquifer sediments at the site, with PBOC ranging from 17.5 to 3,321 mg/kg. PBOC levels in sediments obtained from the confining unit were generally greater when compared to sediments collected in the surficial aquifer. Regression analysis demonstrated a statistically significant inverse correlation between PBOC levels in aquifer sediments and chloroethene concentrations for selected temporary wells where chloroethene exposure was the highest. Consistent with these findings, results also demonstrated a similar trend of increased PBOC in aquifer sediments outside the chloroethene plume relative to aquifer sediments inside the plume. Results from laboratory exposure assays also demonstrated that sediment samples exhibited a reduction in PBOC levels of 35% and 73%, respectively, after a 72-hour exposure period to PCE (20,000 µg/L). Differences in carbon from pyrophosphate and alkali extractions were observed in surficial upper and lower aquifer sediments. These findings further suggested the variability in extracted carbon levels at the site for surficial aquifer sediment samples in the PCE-contaminated source zone could impact the extent of reductive dechlorination within the hydrographic unit.

Introduction

The improper use and storage of chloroethenes in industrial processes have resulted in perchloroethylene (PCE) and trichloroethylene (TCE) being a major source of pollution in sediment and aquifer environments (Grandel et. al 2004). Although chloroethenes tend to be more resistant to natural attenuation processes, significant evidence now exist that these contaminants can be transformed through biological degradation (Bradley and Chapelle, 1999). Monitored natural attenuation (MNA) has emerged as an effective and economical remedial strategy at many chloroethene-contaminated sites (Weidemeier et al. 1999; Chapelle et al. 2001). MNA relies on natural attenuation processes to achieve site-specific remedial objectives within a time frame that is reasonable when compared to other remedial alternatives.

For chlorinated ethenes, microbial degradation is often the principal component of natural attenuation where MNA is employed as a remediation strategy. Under anaerobic environments, biodegradable natural organic carbon is fermented to produce hydrogen, which serves as the electron donor for *Dehalococchoides* sp. and other dechlorinating bacteria (Duhamel et al. 2004; Schmidt et al. 2004; He et al. 2005; Sung et al. 2006). These microorganisms, collectively termed halorespirers, are able to grow using chloroethenes as sole terminal electron acceptors. It has been demonstrated in several research studies that with a sufficient supply of natural organic carbon under strongly reducing conditions, halorespiring aquifer microorganisms can reductively dechlorinate PCE and TCE to less chlorinated daughter products, DCE, vinyl chloride (VC), ethene, and further to ethane and CO_2 (Bradley, 2000). In reductive dechlorination reactions, chlorinated solvents, such as PCE and TCE, are sequentially reduced to *trans* and *cis* 1,2-DCE, VC and ultimately ethene, ethane and methane, such that an H^+ and two electrons replace one chlorine ion for each degradation reaction.

The sustainability of microbially-mediated reductive dechlorination is thought to be dictated by the availability of electron donors from two potential sources: (1) anthropogenic sources (Type I site), or (2) naturally- occurring organics (Type II site) (Wiedemeier et al. 1997). At Type II sites, the fermentation of natural organic carbon is the key factor supporting the dehalogenation process. Bioavailable natural organic carbon is an important source of energy that supports microbially-mediated reductive dechlorination and is thought to be derived from

soil and aquifer sediment sources (Chapelle et al. 2007). Thus, at sites with no anthropogenic carbon sources, the ability to quantify the bioavailable fraction of organic carbon is needed to assess the potential for the long-term sustainability of MNA (Chapelle et al. 2007).

In earlier chapters, potentially bioavailable organic carbon (PBOC) was examined among chloroethene-contaminated sites representing a range of environmental conditions. Using the method developed by Rectanus et al. 2007, operationally defined fractions of labile organic carbon present in the aquifer sediment were quantified through a multi-step chemical extraction process. Initial results revealed that total PBOC demonstrated a positive correlation between solid-phase TOC_s, reductive dechlorination activity, and potentially bioavailable organic compounds present in the aquifer system, expressed as hydrolyzable amino acids (HAA). Additional results confirmed that a direct relationship also existed between PBOC and site specific performance data at chloroethene sites. Using performance monitoring well data collected from historical monitoring reports, correlations with PBOC in the aquifer sediments were established between dissolved oxygen (DO) concentrations and geochemical conditions within the groundwater system at chloroethene-contaminated sites. These results examined the variability of PBOC among chloroethene sites and its relationship to site specific performance data; however, the distribution of PBOC at a chloroethene-contaminated site within a single hydrographic unit remains largely unknown.

Previous studies conducted by Rectanus (2006) evaluated the variation of PBOC in aquifer sediments collected at a chloroethene-contaminated site (Site 12, Naval Amphibious Base, Little Creek, VA). Results suggested that aquifer sediments collected inside of a chloroethene plume contained less PBOC relative to sediments collected outside of the contaminant plume. Aerobic bioassays also showed decreased PBOC biodegradability in aquifer sediments collected inside of a chlororethene plume when compared to samples collected outside of the contaminant plume. Although these results suggested that long-term reductive dechlorination activity within a chloroethene plume contributed in PBOC depletion and decreased biodegradability relative to background carbon levels, Rectanus (2006) did not address whether chloroethene exposure also influences PBOC depletion.

In this study, the distribution of PBOC was investigated at a chloroethene-contaminated site. PBOC was measured in surficial aquifer sediment samples collected at varying depths both inside and outside of a chloroethene plume. The objective of the study was to examine the effect of long-term chloroethene exposure on PBOC levels in aquifer sediments by comparing differences in extractable organic carbon in aquifer sediments with minimal chloroethene exposure relative to samples collected in the PCE-contaminated source zone. Using performance monitoring data, correlations were established between PBOC levels and groundwater chloroethene concentrations. Laboratory assays were constructed using aquifer sediments and amended with PCE to assess carbon depletion due to chloroethene exposure. As an additional assessment, anaerobic bioassays were also constructed using an enrichment culture and amended with PCE to determine the extent of reductive dechlorination supported by PBOC in surficial upper (SU) and lower (SL) aquifer sediments. Levels of PCE and daughter products were monitored over time, and the results were used to compare differences in the reductive dechlorination activity for aquifer sediments with minimal chloroethene exposure.

Materials and Methods

Site Description

Marine Corps Recruit Depot, Parris Island (MCRD) Site 45 is a former dry-cleaning facility located along the southern coast of South Carolina in Beaufort County. The former dry-cleaning facility began its operations in the 1950s. On March 11, 1994, one of the above-ground tanks was overfilled with PCE; and PCE was released from the catch basin to the surrounding soil and storm drains (S&ME, Inc., 1994). Following the spill, the dry-cleaning operations were moved to a new facility, approximately 130 ft west of the former dry-cleaning facility. With the move to a new facility, the dry-cleaning operation switched from using PCE as the cleaning solvent to using a non-hazardous hydrocarbon-based cleaner (ExxonMobil DF-2000). Later in 2001, the former dry-cleaning facility was demolished, and related structures were removed from the site (Tetra Tech NUS, Inc., 2004).

Groundwater contamination at MCRD Site 45 consists primarily of PCE and its dechlorination products, TCE, *cis* DCE, and VC. Chlorinated solvents are present at the site

within two groundwater plumes with some probable overlap at down-gradient locations. As shown in Figure 5-1, the northern plume extends southeastward from the northern part of the former dry-cleaning facility, while the southern plume extends from the southeastern corner of the new dry-cleaning (Tetra Tech NUS, Inc., 2005). Although the southern plume spatially originates from the new dry-cleaning facility, an investigation conducted by Vroblesky et al. (2007) revealed that the contaminant source in the southern plume was from a leaky sanitary sewer in the vicinity of the new dry-cleaning facility.

At MCRD Site 45, the surficial aquifer consists of a heterogeneous mixture of Pliocene- to Holocene-age sediments of the Pamplico and Waccamaw Formations (Bechtel Environmental Inc., 1997), consisting primarily of fine sand, silty sand, and clayey sand and extends to a depth of about 18 ft below land surface (BLS) (Tetra Tech NUS, Inc., 2004). In general, the water table is shallow and typically encountered at a depth of 3 to 4 feet at the site; however, it has been observed to be as great as 6.5 feet at some locations (Vroblesky et al. 2009).

Monitoring wells at the site are referred to as surficial upper (SU) wells if they are screened predominantly shallower than about 11 ft BLS and are referred to as surficial lower (SL) wells if they are screened deeper than about 10 ft BLS. An organic rich layer (peat) has been reported at the site at depths of about 17 to 27 ft BLS overlying a clay layer that functions as a confining bed (Tetra Tech NUS, Inc., 2004). Samples collected within the peat layer are characteristic of a complex mixture of sand, silt, and clay with a substantial amount of black to brown organic material. Beneath the organic rich confining unit is a deep layer (D) of the surficial aquifer consisting of unconsolidated deposits primarily of sand, clayey sand, and silty fine sand with traces of shell fragments. Based on temporary monitoring well data near the new dry-cleaning facility, greater contamination is present at depths of 11-15 ft BLS, when compared to wells screened at depths of 7-11 ft BLS (Vroblesky et al. 2007).

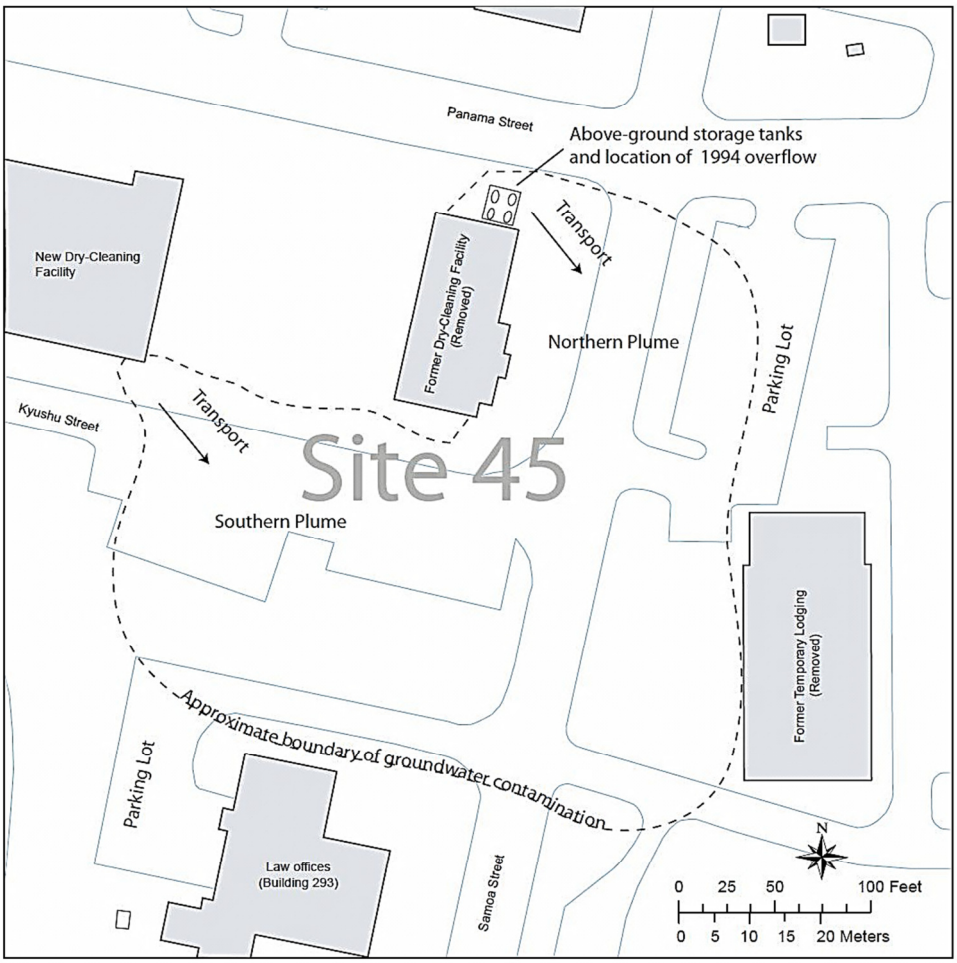

Figure 5-1. Approximate boundary of groundwater chloroethene contamination for MCRD Site 45, Parris Island, South Carolina (Vroblesky et al. 2009).

At MCRD Site 45, groundwater in the surficial upper and lower monitoring wells flows in the direction of northwest to the southeast, with typical groundwater seepage velocities ranging from 14.6 to 48.7 ft/yr. In the deeper aquifer, the overall groundwater flow direction is to the south-southwest with an estimated groundwater seepage velocity approximately 4.9 ft/yr. The actual groundwater velocity varies across the site with aquifer heterogeneity. However, based on aquifer tests, the average groundwater velocity in the southern plume ranged from 9 to 97 ft/yr, with discharges to the sewer systems (Tetra Tech NUS, Inc., 2004).

Sediment Samples

Aquifer sediment samples were obtained from 12 sampling locations within and in the vicinity of the southern plume at MCRD Site 45 (Figure 5-2). Sampling locations were selected to provide aquifer sediments from locations both outside and inside the contaminant source and plume. Each sampling location was adjacent to permanent and temporary monitoring wells with known groundwater parameters. A total of 114 sediment samples were collected at varying depths using a Geoprobe unit and placed in Mason jars. At each sampling location, 2-4 samples were collected from shallow depths (<5 ft BLS) within both unsaturated and saturated zones. An average of 2-3 aquifer sediment samples were collected within the surficial upper (5-10 ft BLS) and lower (10-15 ft BLS) units. For each location, a minimum of 1 sample was also collected at a depth within the organic rich confining unit (>15 ft BLS). After collection, samples were transported on ice and stored at 4°C until analysis.

PBOC Extraction Method

For this study, the PBOC extraction method in Rectanus et al. (2007) was used to quantify the carbon concentration (i.e., PBOC) which is the mass of extracted carbon per mass of aquifer sediment. To begin the process, 10 grams of aquifer sediment dried at 70°C over-night and sieved through 2-mm openings were combined with 20 mL of an extracting solution in carbon-free 40-mL vials. The two extracting solutions used in this study were 0.1% sodium pyrophosphate (pH 8.5, Crystalline/Certified ACS; Fisher Scientific, Pittsburgh, Pennsylvania) and 0.5 N NaOH (pH 13, Pellets/Certified ACS; Fisher Scientific). Aquifer sediments were exposed to alternating sequential extractions using 0.1% pyrophosphate and 0.5 N NaOH. After a 24-h extraction cycle on the rotary tumbler, samples were centrifuged for 25 min at 2000 rpm for solids separation. The supernatant was decanted and stored at 4°C until analyzed for extracted organic carbon. The carbon content of the supernatant was analyzed using a Shimadzu Total Organic Carbon (TOC) Analyzer-VCSN with a detection limit of 0.10 mg/L carbon. Calibration curves for TOC content were confirmed by external standards.

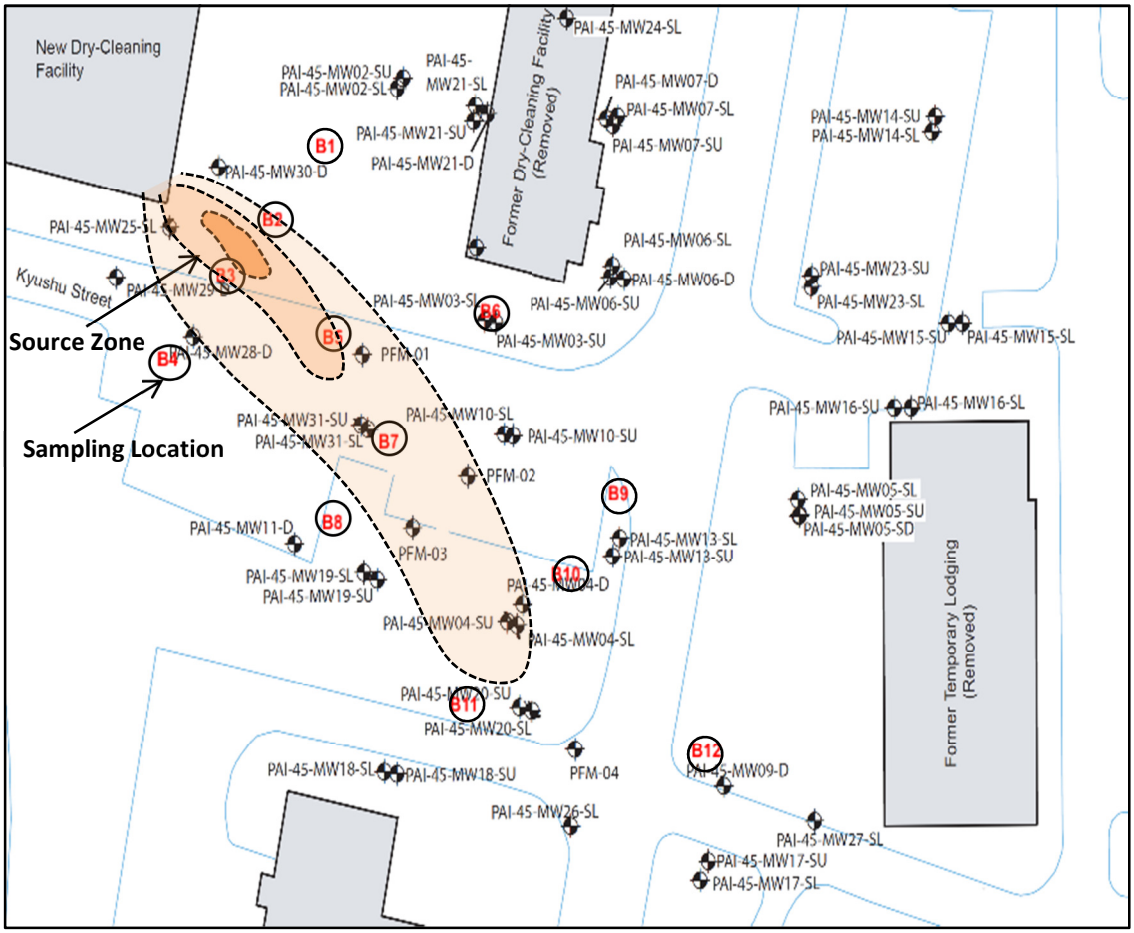

Figure 5-2. Sampling locations, groundwater monitoring wells, and approximate extent of tetrachloroethene (PCE) plume in the southern plume at MCRD Site 45, Parris Island, South Carolina (modified from Vroblesky et al. 2009).

For quality control, triplicate samples containing soil sediment were prepared for each extracting solution. Extracting solutions were autoclaved twice for 15 min at 14 PSI and 121 °C before combined with sediment samples. Control samples containing only the extracting solution were also prepared for each extraction. PBOC results were reported as the total mass of organic carbon extracted by the pyrophosphate and alkali extractions and expressed as the arithmetic mean for the triplicate extractions. Data variability for each extraction was assessed for using standard deviations.

Solid-Phase Organic Carbon (TOC_s)

The solid-phase organic carbon (TOC_s) content of sediment samples (total mass of organic carbon per mass sediment) was determined by elemental analysis using flash combustion and chromatographic separation (Costech Instruments). An ECS 4010 carbon gas chromatograph (GC) configuration with a 3 meter column was used for sample analysis. The gas flow rate within the GC column was 100 ml min^{-1}. Solid-phase organic carbon content was quantified using the thermal conductivity detector with a detection limit of 10 mg/kg carbon. For quality control, duplicate samples were analyzed for each sampling location. TOC_s data was reported as arithmetic mean for each location.

Groundwater Contaminant Parameter Analysis

Groundwater parameters were evaluated using historical performance data (Appendix C) collected from monitoring reports (Vroblesky et al. 2009). Performance monitoring well data collected from sampling locations was used to define the contaminant distribution at the site. Groundwater parameter values were reported as the arithmetic mean for each sampling location. Data variability for each site was analyzed using standard deviations.

PCE Exposure Assay

The PCE exposure assay experiments were constructed in carbon-free 15-mL glass vials using 5 grams of aquifer sediment. Sediment samples were dried at 70°C over-night and sieved through 2-mm openings. After construction of the assays, aquifer sediments were exposed to PCE (20,000 ug/L) for 24-hour and 72-hour incubation periods. The assay vials were prepared with minimal headspace and incubated under static conditions at 20°C without light until sampled. After incubation, the supernatant was decanted, and exposed sediments samples were rinsed with Nanopure water and dried under the flume hood for 24 hours. Exposed aquifer samples were measured for PBOC content as described in the previous section.

For quality assurance, all glassware was acid-rinsed, rinsed three times with Nanopure water and combusted at 350°C for 2 hours. Triplicate samples containing aquifer sediment and PCE were prepared for each sample. Aqueous PCE concentrations were confirmed by external

standards using the purge and trap method (Rectanus, 2000). Control samples containing only aquifer sediments and Nanopure water were also prepared for each sample. PBOC content from exposed aquifer sediments was reported as arithmetic mean for the triplicate samples.

Enrichment Culture

Enrichment cultures were developed under anaerobic conditions using sediment samples collected from chlororethene-contaminated sites with known reductive dechlorinating microbial cultures (Rectanus, 2006). For enrichment cultures, setup included 10 g sediment and 90 mL of minimal salts media, Wolin vitamins, and yeast extract. The minimal salts media (MSM) contained 3.4g KH_2PO_4, 4.35g K_2HPO_4, 1.0g NH_4Cl, 150mg $MgSO_4 \bullet H_2O$, 4.5mg $MnSO_4 \bullet 4H_2O$, 0.5mg $NaMoO_4 \bullet 2H_2O$, 0.15mg H_3BO_3, 20mg $CaCl_2$, 1.5mg $ZnCl_2$, 0.5mg $CuCl_2 \bullet 2H_2O$, 1.5mg $CoCl_2 \bullet 6H_2O$, and 11mg $FeCl_2 \bullet 4H_2O$ per 100 mL. The Wolin vitamin stock was adapted from Mattson et al. 2004, which consisted of a consortium of vitamins needed to promote microbial growth. Yeast extract (1%) and PCE (100 ug/L) was added to the enrichment cultures to provide electron donors and acceptors for microorganisms. After a four week period, aqueous PCE concentrations and daughter products were measured using the purge and trap method (Rectanus, 2000). The enrichment cultures were maintained every four weeks by transferring 10 mL of culture into 90 mL of sterile minimal salts media with Wolin vitamins, yeast extract, and PCE.

Anaerobic Bioassay

Anaerobic bioassay experiments were constructed in carbon-free 40-mL glass vials using 10 grams of aquifer sediment, 10 mL minimal salts media, 5 mL of enrichment culture, and 75 µL Wolin vitamins purged with sterile N_2 to remove O_2 and facilitate anaerobic microorganism growth. The anaerobic bioassays were incubated in an anaerobic chamber (Plas Labs Inc., Lansing, MI) under a N_2 atmosphere with negligible H_2. For each bioassay vial, a reducing agent (8.9 µM Na_2S) and color indicator (0.1% resazurin) were added to ensure anoxic conditions. PCE (1500ug/L) was added to the bioassays experiments last to minimize volatility. After a 30-day

incubation period, aqueous PCE, TCE, and *cis*DCE, was measured using the purge and trap method (Rectanus, 2000).

For quality assurance, all glassware was acid-rinsed, rinsed three times with Nanopure water and combusted at 350°C for 2 hours. Triplicate samples containing aquifer sediment, MSM, enrichment culture, Wolin vitamins, and PCE were prepared for each sample. Control bioassays were constructed without addition of the microbial culture and autoclaved twice for 15 min at 14 PSI and 121 °C.

Results and Discussion

PBOC Concentrations

Figure 5-3 shows the distribution of PBOC in aquifer sediments collected at the site (N=72). Aquifer samples were collected at various sampling locations at shallow depths and depths within the surficial aquifer and confining unit at the site. Surface soils samples and sediments collected from the confining unit were not included in the distribution. The results obtained for PBOC concentrations in aquifer sediments at the site suggest a natural logarithm-normal distribution. The average concentration for PBOC in aquifer sediments was 411.8 mg/kg with the greatest frequency for PBOC ranging from 157.6 to 397.0 mg/kg. The lowest concentrations for PBOC were reported for samples collected inside the chloroethene plume within the surficial lower aquifer at Boring 3; while the greatest concentration of PBOC was present in sediment samples collected within the confining unit on the periphery of the contaminant plume at Boring 12.

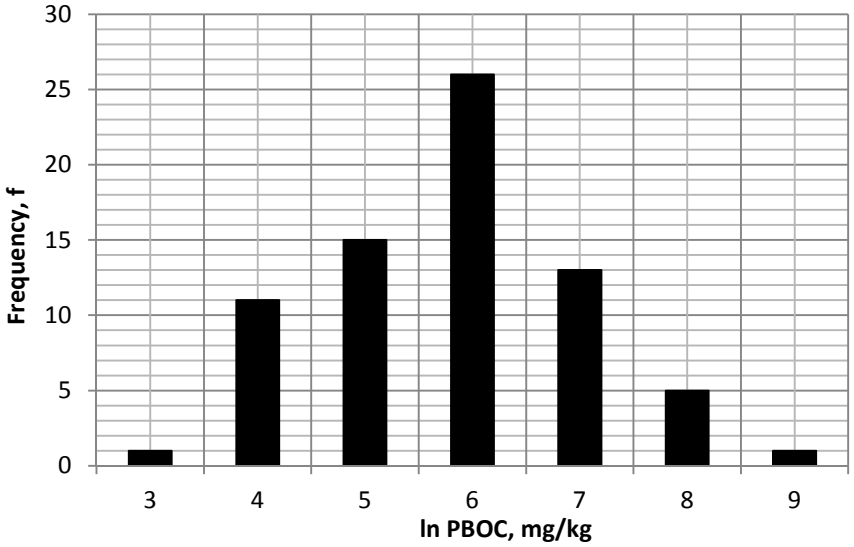

Figure 5-3. Frequency (f) vs ln PBOC for sampling locations at MCRD Site 45.

As shown in Figure 5-4, concentrations of PBOC and TOC_s ranged from 17.5 to 536.5 mg/kg and 271.0 to 5470.0 mg/kg, respectively, for surficial aquifer sediment samples. The power regressions shown in Figure 5-4 illustrate the difference in PBOC levels for sediment samples collected in the surficial aquifer (surficial upper and lower) and the confining unit. When PBOC levels are plotted against their TOC_s values for all depths and sampling locations at MCRD Site 45, the values obtained for the confining unit are generally larger than surficial aquifer values. Higher carbon levels for sediments collected in the confining unit could be attributed to larger quantities of adsorbed carbon relative to sediments collected in the surficial aquifer. Based on regression analysis, extractable carbon from samples collected in the surficial aquifer represented approximately 7% of the TOC_s concentration.

With the quantity of TOC_s and PBOC being lower in the surficial aquifer sediments in comparison to samples collected in the confining layer, the increase in carbon levels also corresponded to textural transitions for sediments. Sediment samples collected within the surficial aquifer contained light gray to brown fine sand, silty sand, and clayey sand, while sediment samples collected in the confining layer were comprised of complex mixture of sand, silt, and clay with a substantial amount of black to brown organic rich material dispersed throughout the sample.

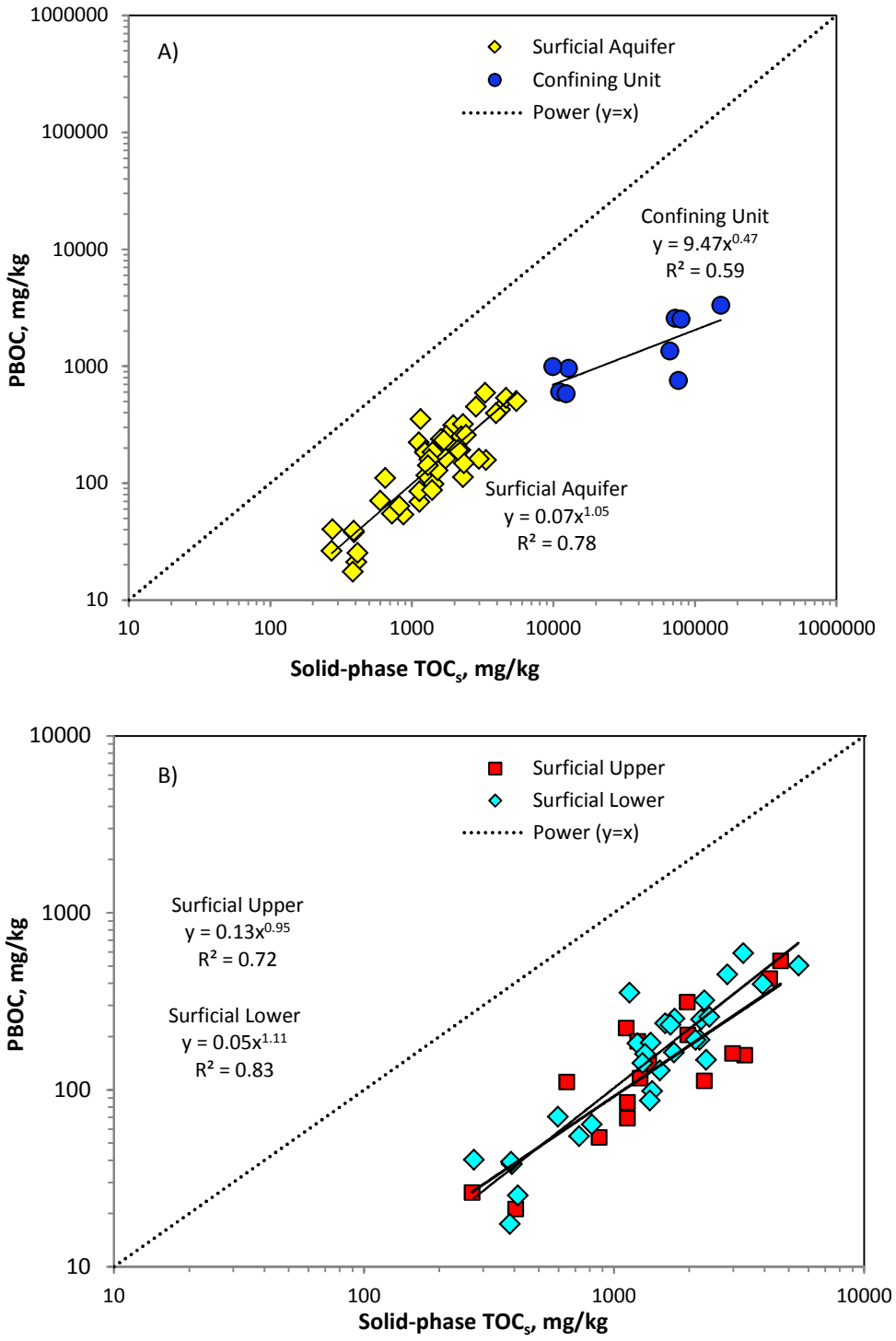

Figure 5-4. A) PBOC (mg/kg) versus solid-phase TOC_s (mg/kg) for selected samples collected within the surficial aquifer and confining unit at MCRD Site 45. B) PBOC (mg/kg) versus solid-phase TOC_s (mg/kg) for surficial upper and lower aquifer sediments at MCRD Site 45.

Based on regression results, PBOC levels in aquifer sediments demonstrated a consistent increase relative to TOC_s. As shown by the regression equation, the exponent of the power function suggests that the level of PBOC follows a similar trend with TOC_s. However, in Chapter 3 regression results did not yield the same conclusion; which showed that variation existed between PBOC and TOC_s about the 1:1 line. Specifically, results in Chapter 3 further demonstrated that in the proportion of PBOC yield through extraction diminished as TOC_s concentrations increased. In contrast, results from this site-specific study show that PBOC and TOC_s in surficial aquifer sediments collected at MCRD Site 45 followed a fairly uniform trend in term of the proportion of PBOC yielded relative to the base amount to TOC_s.

As shown in Figure 5-4, average PBOC and TOC_s concentrations were 173.6±139.0 and 1814.7±1285.3 mg/kg, respectively, for samples collected in the surficial upper aquifer. For samples collected in the surficial lower aquifer, average PBOC and TOC_s concentrations were 176.3±127.6 and 1649.1±1138.6 mg/kg, respectively. In the previous chapters, results revealed that PBOC levels were greater than 200 mg/kg at sites exhibiting moderate to high reductive dechlorination activity and where spatially-averaged site DO concentrations fell below the 0.5 mg/L. However, results from this site indicate that 54% of the sediment samples displayed PBOC levels below 200 mg/kg. The variability of PBOC within aquifer sediments suggests the question as to whether carbon levels are impacted by long-term chloroethene exposure relative to background carbon or whether this is a product of natural variation.

Groundwater Contaminant Parameter

To assess the impact of chloroethene exposure on PBOC levels in sediments within the surficial aquifer at MCRD Site 45, temporary wells were selected adjacent to a boring location with known carbon levels. Because the lowest carbon levels were observed in aquifer sediments collected within the source zone at Boring 3, this location was used to evaluate whether long-term contaminant exposure impacted carbon depletion, relative to samples in a less chloroethene-contaminated zone. Table 5-1 shows the temporary monitoring well, screen interval, and corresponding chloroethene concentrations for each monitoring well.

Table 5-1. Summary of groundwater chloroethene concentrations for selected temporary monitoring wells at MCRD Site 45 (provided by Vroblesky et al. 2009).

Temporary Monitoring Well (TW)	Screen Interval (ft)	Sampling Zone	Chloroethenes (ug/L)
PAI-45-USGS-TW94	7-11	Surficial Upper (SU)	1448.3
PAI-45-USGS-TW39	10-14	Surficial Lower (SL)	13518.0
PAI-45-USGS-TW83	11-15	Surficial Lower (SL)	499.5
PAI-45-USGS-TW70	11-15	Surficial Lower (SL)	5.21

The greatest concentration for chloroethenes was observed in groundwater samples collected from temporary well, PAI-45-USGS-TW39, screened within the 10-14 feet depth interval. Results from monitoring data show that less contamination was present at PAI-45-USGS-TW83, which would suggest that the monitoring well was screened below the major contamination depth.

Figure 5-5A shows the groundwater concentrations for chloroethenes at selected temporary monitoring wells plotted versus PBOC in aquifer sediments collected from Boring 3 at MCRD Site 45. For temporary well, PAI-45-USGS-TW94, which was screened within the surficial upper aquifer, the corresponding PBOC level in aquifer sediments within the 7-11 feet depth interval at Boring 3 was 159.4 mg/kg. Temporary wells screened in the surficial lower aquifer within the 11-15 feet depth interval were partially screened within the upper and lower intervals. Vroblesky et al. (2009) reported that the majority of groundwater sampled from the temporary wells screened in within the surficial lower aquifer was derived from the upper screen interval in the loose sand rather than from the underlying less permeable aquifer material. Thus, aquifer sediments collected within the upper screen interval within the vicinity of the temporary wells in the surficial lower would be most representative of PBOC levels within the depth interval.

Results show that significantly lower PBOC levels were observed within the upper 10-14 feet depth interval at Boring 3, which was exposed to higher chloroethene concentrations. It

was hypothesized that long-term chloroethene exposure would contribute to PBOC depletion in aquifer sediments over time at contaminated sites. Hence, sediment samples with minimal to moderate chloroethene exposure were expected to have higher PBOC levels, when compared to sediments located in a highly contaminated zone. Based on regression analysis, the relationship between PBOC levels in aquifer sediments at Boring 3 and chloroethene concentrations for selected temporary wells in the contaminated source zone at MCRD Site 45 demonstrated a statistically significant ($p=0.007$) inverse correlation (Figure 5-5A). When PBOC levels in aquifer sediments at Boring 3 were compared with adjacent sampling locations outside the chloroethene contaminant plume, greater concentrations were observed in aquifer sediment samples outside of the source. Figure 5-5B shows depth plotted versus PBOC in aquifer sediments collected from Boring 3 and Boring 4 at MCRD Site 45. Statistical results demonstrated that PBOC levels in aquifer sediments collected at Boring 3 were significantly lower ($p=0.04$) relative aquifer sediments collected at Boring 4.

Results also show that PBOC levels in aquifer sediments collected at Boring 4 followed a similar trend with depth relative to Boring 3. Specifically, the lowest concentrations for PBOC in aquifer sediments at Boring 4 were collected at the 10-11 feet depth interval, which was followed by an increase in PBOC levels at a depth of 11-12 ft. Because Boring 4 is known to be outside of the contaminant plume and is assumed to have minimal exposure to chloroethenes, the lower levels of PBOC in aquifer sediments observed at this sampling location may be attributed to natural heterogeneity within the site lithology.

However, results demonstrated that for temporary well, PAI-45-USGS-TW70, which was in the vicinity of Boring 4 and screened within the 11-15 feet depth interval, PBOC levels in aquifer sediments collected within the upper screen interval were greater by more than a factor of 2, when compared to sediment samples collected at Boring 3 within the same depth interval (Figure 5-5A).

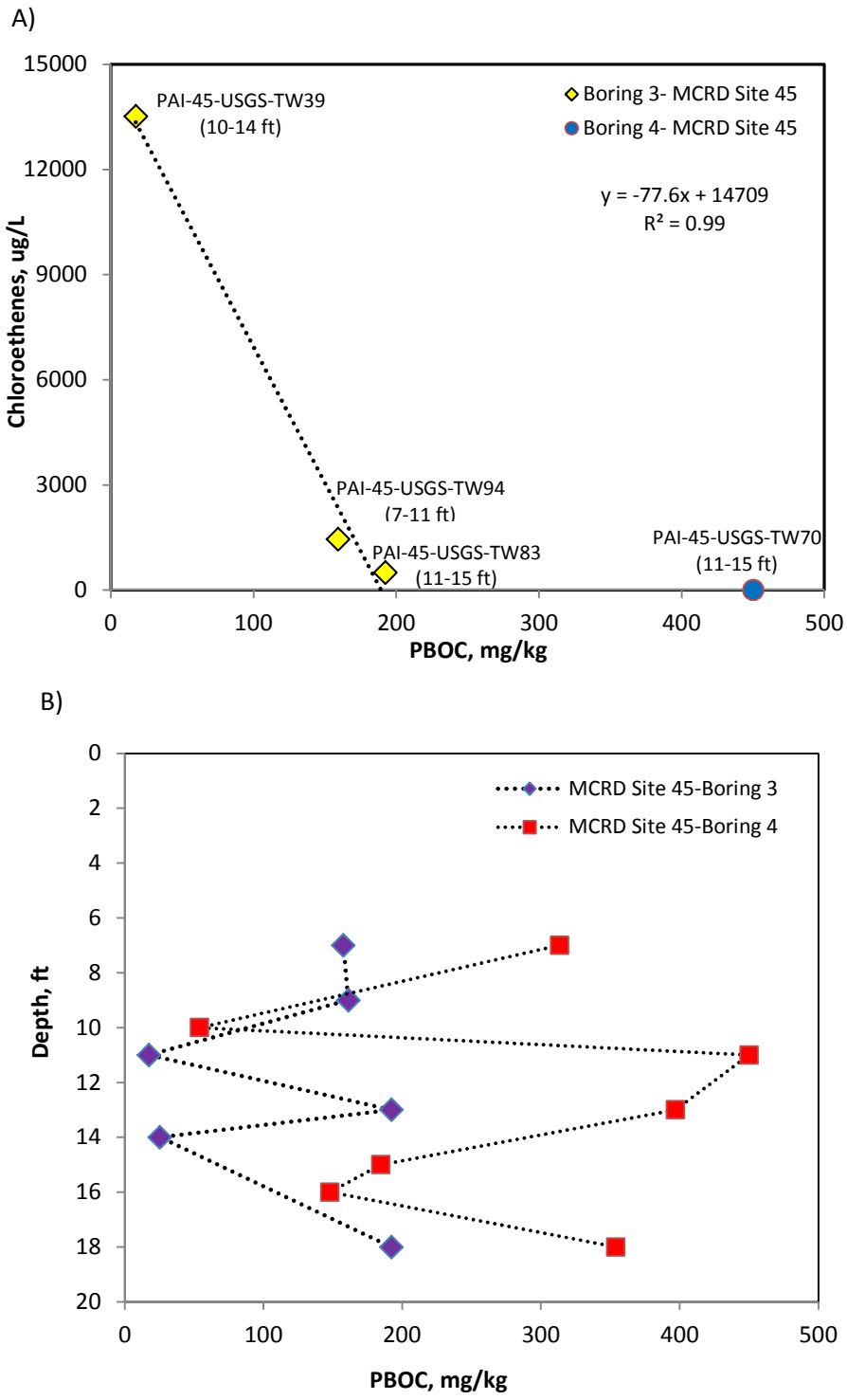

Figure 5-5. A) Chlororethenes (ug/L) in selected temporary wells versus PBOC (mg/kg) in aquifer sediments collected from Boring 3 at MCRD Site 45. B) Depth (ft) versus PBOC (mg/kg) in aquifer sediments collected from Boring 3 (inside chloroethene plume) and Boring 4 (outside chloroethene plume) at MCRD Sites 45.

Consistent with these findings, results from this study also demonstrated a similar trend of increased PBOC in aquifer sediments for other sampling locations outside the chloroethene plume relative to locations inside the plume. As shown in Figure 5-6, the quantity of PBOC in aquifer sediments outside the chloroethene plume was greater than inside of the plume by a factor of 2.0 and 1.4, respectively, for surficial upper and lower samples. PBOC was statistically significantly greater ($p<0.05$) for sediment samples collected outside the chloroethene plume in the surficial upper aquifer, when compared to sediments collected inside the plume. For the surficial lower aquifer, there was not a statistically significant difference ($p>0.05$) in PBOC levels for sediment samples collected inside and outside of the chloroethene plume.

Overall, results from this study provided an evaluation of PBOC levels in sediment samples at MCRD Site 45 by comparing differences in extractable organic carbon from aquifer sediments with minimal chloroethene exposure relative to samples collected in the PCE-contaminated source zone. Results show that chloroethene concentrations in the groundwater system inversely correlate with levels of PBOC in aquifer sediments and suggest that the long-term chloroethene exposure can contribute to the depletion of potentially bioavailable organic carbon in the aquifer groundwater system.

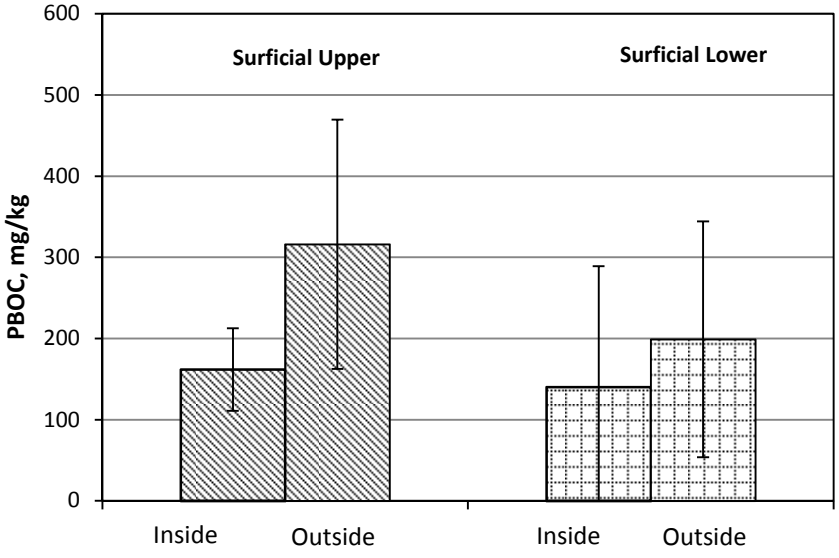

Figure 5-6. Summary of PBOC (mg/kg) for surficial aquifer sediment samples collected inside and outside of the chloroethene plume at MCRD Site 45. Standard deviations for PBOC concentrations are shown with error bars.

PCE Exposure Assay

In Figure 5-7, time-variable PBOC levels determined using laboratory PCE exposure assays are shown for aquifer sediment samples collected in the surficial upper (8-9 ft) and lower (14-15 ft) at MCRD Site 45- Boring 1. The aquifer sediments obtained from Boring 1 were known to be on the outside of the contaminant boundary based on historical records and the most recent data collected by Vroblesky et al. (2009), and thus are assumed to have no or only minimal exposure to chloroethenes. Background PBOC levels for surficial upper sediment samples collected at Boring 1 were 426.7 mg/kg with concentrations of extracted carbon from pyrophosphate and alkali extractions of 283.6 and 143.1 mg/kg, respectively. For sediments collected in the surficial lower aquifer, PBOC levels were 250.6 mg/kg with concentrations of pyrophosphate and alkali extracted carbon of 31.6 and 219.0 mg/kg, respectively.

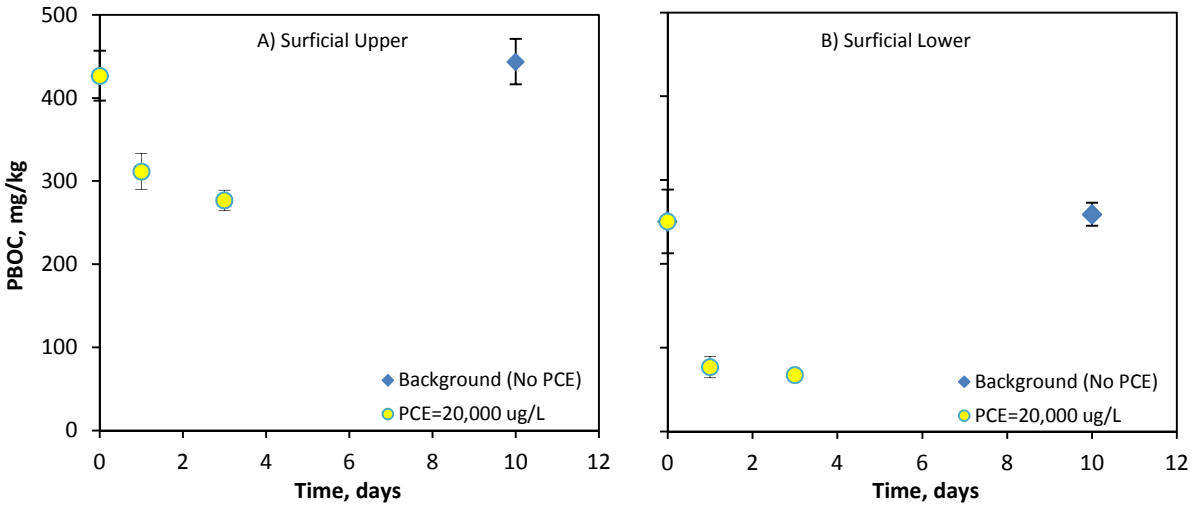

Figure 5-7. PBOC (mg/kg) versus Time (days) for selected samples collected within the surficial aquifer at MCRD Site 45-Boring 1. For (A) Surficial Upper (8-9 ft) and (B) Surficial Lower (14-15 ft) each graph illustrates the PBOC levels with respect to PCE exposure over time. Standard deviations for PBOC are shown with error bars.

For both surficial units, aquifer sediment samples exhibited significantly lower ($p<0.05$) PBOC levels after a 72-hour PCE (20,000 µg/L) exposure period. Results showed that exposed sediment samples collected in the surficial upper and lower aquifer exhibited a reduction in extractable carbon of 35% and 73%, respectively, relative to background PBOC levels, while negligible amounts of PBOC were removed from control samples containing only aquifer

sediments and Nanopure water. This finding suggests that chloroethene exposure in sediments collected within the surficial lower aquifer could more greatly impact PBOC when compared to surficial upper aquifer sediments.

Differences in the extracted carbon from pyrophosphate and alkali extractions were also observed within the surficial upper and lower aquifer sediments (Figure 5-8). For surficial upper aquifer sediments, concentrations of extracted carbon from pyrophosphate extractions contained 50% more organic carbon, when compared to alkali extractions; while extracted carbon from alkali extractions contained the dominant fraction of carbon for sediments collected in the surficial lower aquifer (Figure 5-8). Previous results from Chapter 3 suggested that pyrophosphate extractions represented the mildly-extractable organic carbon, and alkali extractions accounted for the more strongly-adsorbed and humic fractions of organic carbon associated with the sediment. Since readily biodegradable organic carbon is needed to support reductive dechlorination, differences in extracted carbon levels at the site for surficial upper and lower sediment samples in the PCE-contaminated source zone could impact the effectiveness of contaminant biotransformation within the hydrographic unit.

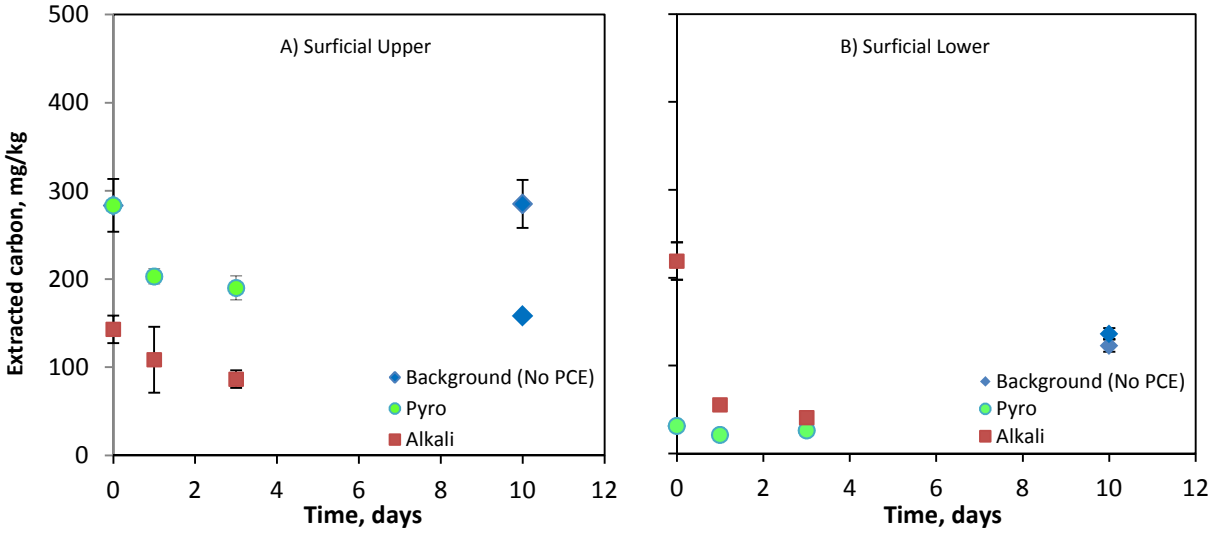

Figure 5-8. Extracted carbon (mg/kg) versus Time (days) for selected samples collected within the surficial aquifer at MCRD Site 45-Boring 1. For (A) Surficial Upper (8-9 ft) and (B) Surficial Lower (14-15 ft) each graph illustrates the extracted PBOC levels with respect to PCE exposure over time. Standard deviations for extracted carbon are shown with error bars.

Anaerobic Bioassay

Results from anaerobic bioassays are summarized in Figure 5-9 using aquifer sediment samples collected in the surficial upper (7-8 ft) and lower (11-12 ft) at MCRD Site 45-Boring 4, which are identical to the locations used in the PCE exposure assays. Bioassays were constructed under anaerobic conditions to determine the extent of reductive dechlorination supported by PBOC in aquifer sediments. Aquifer sediments obtained from Boring 4 were located on the periphery of the contaminant boundary based on historical records and data collected by Vroblesky et al. (2009), and thus have minimal chloroethene exposure.

As noted in the previous section, background PBOC levels for the surficial upper and lower sediment samples collected at Boring 4 were 313.5 and 450.0 mg/kg, respectively. Figures 5-9A and 5-9B depict the reductive dechlorination activity in terms of PCE concentration decreases and *cis*-1,2-DCE concentration increases, respectively, in the aqueous phase over time for the aquifer sediments and positive control (1.0% yeast extract) amended with an enrichment culture and PCE (9.3 µM). At each depth, sediments supported dechlorination of PCE through *cis*-1,2-DCE with trace levels of TCE (<0.1µM). Although average concentrations for cis-1,2-DCE were greater for sediment samples obtained in the surficial upper aquifer after 30 days, results indicated there was not a statistically significant difference when compared to surficial lower aquifer sediments ($p=0.36$).

Dechlorination of PCE was also observed through *cis*-1,2-DCE in the positive control with yeast extract (Figure 5-9C). Based on a 1:1 stoichiometric conversion in reductive dechlorination, chloroethene mass balance calculations indicated a loss between 40-60% of amended PCE after 30 days for the live bioassays. Loss of PCE in liquid, autoclaved controls ranged between 50-60%, but with negligible daughter products formation (results not shown). It is assumed contaminant loss was due to sorption onto the aquifer material and leakage through the septa.

Overall, the results of the anaerobic bioassay experiment were inconclusive; however, the differences in the two depths suggest further study is warranted.

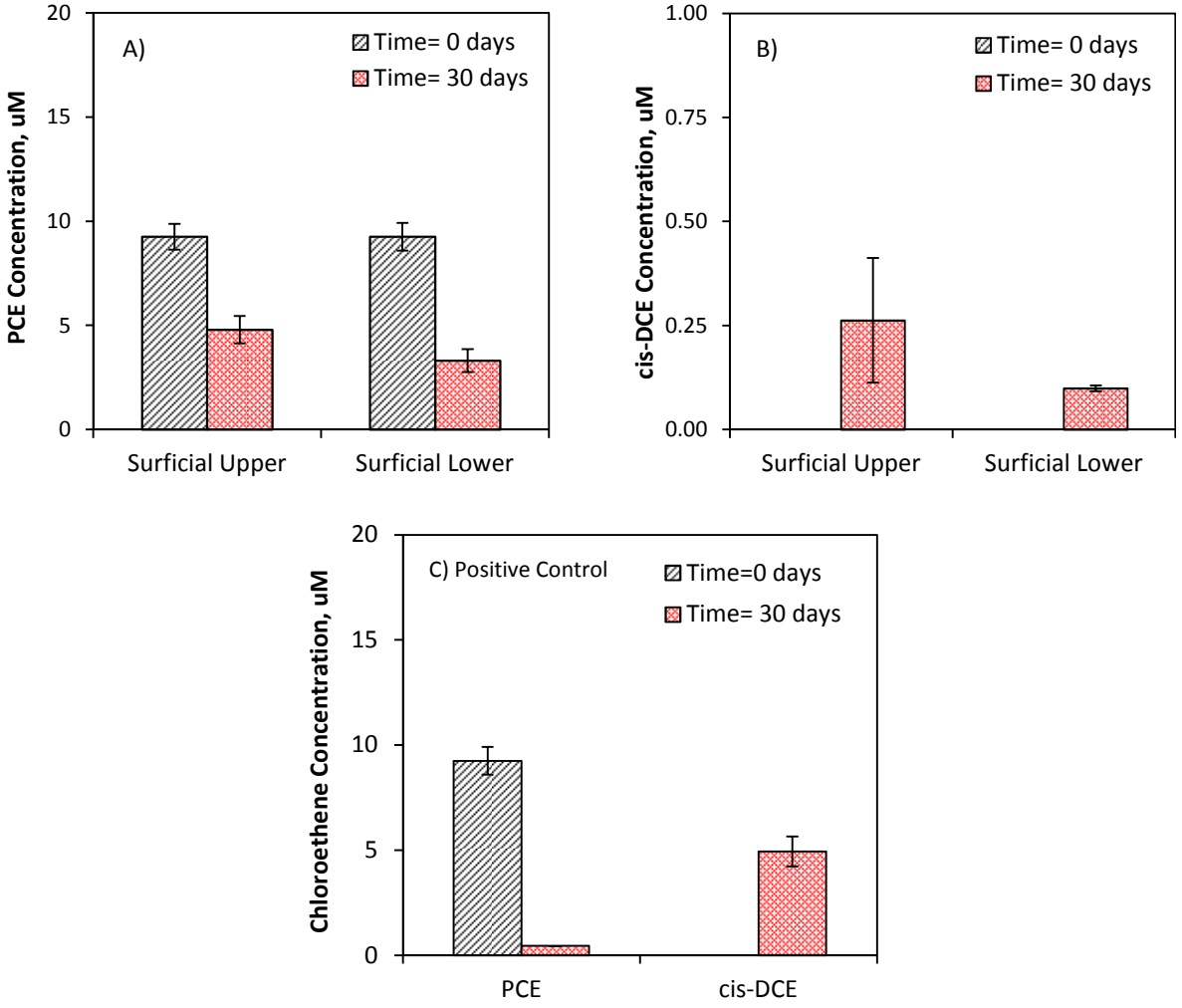

Figure 5-9. Summary of anaerobic bioassay results for surficial aquifer sediments collected at MCRD Parris Island Site 45- Boring 4 and positive yeast control. For (A) PCE (µM) with respect to time (days) and (B) *cis*-DCE with respect to time (days) each graph illustrates the chloroethene levels over time in sediment samples collected in the surficial upper (7-8 ft) and lower (11-12 ft) aquifer. Figure C shows chloroethenes (µM) with respect to time (days) for positive control (1.0% yeast extract). Standard deviations for chloroethene concentrations are shown with error bars.

Conclusions

In this study, the distribution of PBOC was investigated at a chloroethene-contaminated site. PBOC was measured using aquifer sediment samples collected at varying depths both inside and outside of a chloroethene plume. Results from this study examined the effect of long-term chloroethene exposure on PBOC depletion in aquifer sediments by evaluating the variability in PBOC levels in aquifer sediments with minimal chloroethene exposure relative to samples collected in the contaminated source zone. PBOC levels in aquifer sediments were inversely correlated with chloroethene concentrations for selected temporary wells in the contaminated source zone at the study site. Results also demonstrated a similar trend of increased PBOC in aquifer sediments outside the chloroethene plume when compared to aquifer sediments inside the plume.

Results from laboratory exposure assays also demonstrated that surficial aquifer sediments exhibited a significant depletion in PBOC after a 72-hour PCE (20,000 µg/L) exposure period. Results showed that exposed sediment samples collected in the surficial lower aquifer exhibited a greater decrease in PBOC relative to surficial upper aquifer sediments. For surficial upper aquifer sediments, concentrations of extracted carbon from pyrophosphate extractions contained 50% more organic carbon, when compared to alkali extractions; while extracted carbon from alkali extractions contained the dominant fraction of carbon for sediments collected in the surficial lower aquifer. Results further suggested that differences in extracted carbon from pyrophosphate and alkali extractions for surficial aquifer sediments could impact the level of reductive dechlorination.

The anaerobic bioassay was intended to demonstrate that under anaerobic conditions more loosely bound extractable carbon from pyrophosphate extractions would be more readily bioavailable to support reductive dechlorination, when compared to alkali extractions. Since aquifer sediments collected from the surficial upper aquifer contained 66% more pyrophosphate extractable carbon, when compared to surficial lower aquifer sediments, it was expected that surficial upper aquifer sediments would more adequately support reductive dehalogenation. Consistent with this hypothesis, greater levels of *cis*-1,2-DCE were produced in the surficial upper aquifer sediments. However, based on stoichiometry, chloroethene mass

balance calculations indicated a significant loss after 30 days for bioassays amended with PCE. In addition, liquid, killed controls showed loss of the contaminants between 50-60% with negligible daughter products formation (results not shown). This outcome did not enable accurate assessment of biotransformation in bioassays amended with PCE. Thus, it is recommended that the anaerobic bioassay experiment be repeated and longer incubation time be used to observe greater daughter product formation.

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Chapter 6. Engineering Significance

Remediation of contaminants in soil and groundwater is necessary to ensure a cleaner, safer, environment for years to come. Monitored natural attenuation (MNA) offers a remediation strategy aimed toward restoring contaminated systems to their beneficial uses. The scientific understanding of the natural attenuation processes that impact contaminant reduction allows for the implementation of effective remedial technologies. A ‘‘lines of evidence’’ approach has been adopted by the U.S. EPA (1999) as a method of documenting natural attenuation. This approach involves providing three tiers of site-specific information to demonstrate that natural attenuation is occurring. These steps include:

1. Demonstration of a reduction in contaminant mass and/or concentration levels observed over time in historical groundwater and /or soil chemistry performance data

2. Assessment and characterization of geochemical and hydrogeological data that supports natural attenuation mechanisms are active at the site and significantly influence contaminant reduction

3. Evaluation of laboratory or field studies that assess the potential of a site to undergo specific natural processes and its ability to degrade contaminants

For most sites, the effectiveness of natural attenuation is typically evaluated over short periods of time; however, when MNA becomes part of a long-term remediation strategy, processes must be sustained over the operational life of the system (U.S. National Research Council, 2000). The results of this research provide engineers and scientists with metrics for evaluating a key factor that may influence the efficacy of MNA at chlorinated solvent sites.

In this study, potentially bioavailable organic carbon (PBOC) was evaluated as a quantitative measure of the labile organic carbon fraction of aquifer sediments in relation to microbial reductive dechlorination of chlorinated solvents. The results obtained in this study have demonstrated that PBOC in aquifer sediments is directly related to reductive dechlorination at chloroethene-contaminated sites and the ability of the groundwater to attenuate a chloroethene plume at a site. Results have further shown that the level of PBOC in

aquifer sediments may be a key factor in sustaining conditions favorable for microbial reductive dechlorination.

The sustainability of reductive dechlorination at chloroethene-contaminated sites is controlled by the availability of readily-biodegradable natural organic carbon along with favorable geochemical conditions for supporting microbial dehalogenation (Chapelle et al. 2007). In this study, PBOC in aquifer sediments demonstrated direct correlations with independent field metrics related to reductive dechlorination at chloroethene-contaminated sites. The evaluation of PBOC in aquifer sediments also provided further insight on the nature of the relationships with known biodegradable organic carbon compounds in the aquifer system. Results have suggested that PBOC may an important measure of metabolizable natural organic carbon in aquifer sediments. Future research studies should further investigate the ability of PBOC to directly assess the long-term sustainability of microbially-mediated reductive dechlorination.

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Appendix A. Chapter 3

Figure 3-1. PBOC (mg/kg) versus TOC_s (mg/kg) for 15 selected study sites (NWIRP omitted from the regression –diamonds) including data from Rectanus (2006).

Figure 3-2. Mean site values for PBOC (mg/kg) versus TOC_s (mg/kg) for 15 selected study sites (NWIRP omitted from the regression) including data from Rectanus (2006).

Figure 3-3. Extracted carbon (mg/kg) using pyrophosphate and alkali solutions versus TOC_s (mg/kg) for selected study sites (NWIRP omitted).

Data for Figure 3-1, Figure 3-2, and Figure 3-3 come from the following tables.

NAS Jacksonville, FL (OU3)

Sample	Depth (ft)	Pyro (mg/kg)	Standard Deviation	Alkali (mg/kg)	Standard Deviation	TOC_s (mg/kg)
NAS JAX	50-54	27.8	2.9	40.6	16.3	146.6

NAS North Island, CA (Site 5-Unit 2)

Sample	Depth (ft)	Pyro (mg/kg)	Standard Deviation	Alkali (mg/kg)	Standard Deviation	TOC_s (mg/kg)
158	Aquifer	393.7	25.3	451.2	144.1	9134.4
159	Aquifer	52.6	7.3	166.8	45.5	239.5
160	Aquifer	50.3	3.4	205.5	105.4	412.3

NAS Pensacola, FL (WWTP)

Sample	Depth (ft)	Pyro (mg/kg)	Standard Deviation	Alkali (mg/kg)	Standard Deviation	TOC_s (mg/kg)
ORC1	Aquifer	84.9	6.4	221.9	101.1	631.8

NTC Orlando, FL (OU2)

Sample	Depth (ft)	Pyro (mg/kg)	Standard Deviation	Alkali (mg/kg)	Standard Deviation	TOC_s (mg/kg)
SB1	32-35	565.7	48.0	304.4	32.3	1819.1
SB2	32-35	570.2	51.3	503.2	199.2	1865.0

NTC Orlando, FL (OU4)

Sample	Depth (ft)	Pyro (mg/kg)	Standard Deviation	Alkali (mg/kg)	Standard Deviation	TOC_s (mg/kg)
SB1	9-14	321.1	11.5	405.9	164.6	1673.2
SB1	25-30	1145.4	109.8	123.7	29.5	2751.3

Hill AFB, UT (OU2)

Sample	Depth (ft)	Pyro (mg/kg)	Standard Deviation	Alkali (mg/kg)	Standard Deviation	TOC_s (mg/kg)
U2 1000	40-42	21.2	7.0	7.4	2.2	415.6
U2 1001	40-42	12.3	1.8	16.6	5.1	397.5
U2 1002	30-32	14.1	1.2	20.3	8.1	541.2
U2 1003	40-42	15.2	2.2	14.6	1.3	867.4

NAES Lakehurst, NJ (Sites I & J)

Sample	Depth (ft)	Pyro (mg/kg)	Standard Deviation	Alkali (mg/kg)	Standard Deviation	TOC_s (mg/kg)
MW6	Aquifer	49.0	4.0	22.0	1.8	235.9

NUWC Keyport, WA (OU1)

Sample	Depth (ft)	Pyro (mg/kg)	Standard Deviation	Alkali (mg/kg)	Standard Deviation	TOC_s (mg/kg)
NUWC	Aquifer	190.7	9.4	444.3	40.8	6299.3

Fort Lewis, WA (Qv Unit)

Sample	Depth (ft)	Pyro (mg/kg)	Standard Deviation	Alkali (mg/kg)	Standard Deviation	TOC_s (mg/kg)
BIOINJ4	7	35.5	2.6	34.6	5.5	855.0
BIOINJ4	12	39.8	2.1	19.6	2.0	660.0
BIOINJ4	15	38.3	0.8	59.4	34.5	735.0
BIOINJ4	20	31.8	0.6	15.6	6.3	625.0
BIOINJ3	8	69.4	2.0	33.0	6.9	655.0
BIOINJ3	10	46.9	6.8	19.1	3.3	615.0
BIOINJ3	12	70.6	6.3	63.9	22.3	1060.0
BIOINJ3	14	54.5	8.6	28.7	2.6	995.0

Beale AFB, CA (Site 10)

Sample	Depth (ft)	Pyro (mg/kg)	Standard Deviation	Alkali (mg/kg)	Standard Deviation	TOC_s (mg/kg)
10CO39	37-38	16.0	2.7	2.1	0.1	23.6
10CO39	46-47	13.1	1.0	2.6	0.1	22.9
10CO39	58-59	1.7	1.0	2.5	0.2	23.0
10CO39	43-44	23.9	1.3	2.5	0.3	34.9
10CO41	45-46	28.8	1.8	2.8	0.3	0.1
10CO41	59-60	2.0	0.4	1.3	0.1	0.1
10CO49	41.5-42	7.5	0.4	2.9	0.1	0.1
10CO49	45-46	5.9	0.5	2.2	0.5	0.1
10CO49	49.5-50	3.8	0.3	1.4	0.2	0.1
10CO49	54-54.5	5.5	0.5	1.8	0.3	0.1
10CO49	59-60	5.2	0.4	1.8	0.2	23.0

(Rectanus et al. 2007)

NSB Kings Bay, GA (Site 11)

Sample	Depth (ft)	Pyro (mg/kg)	Standard Deviation	Alkali (mg/kg)	Standard Deviation	TOC_s (mg/kg)
KBA13A	10-13	359.5	3.4	174.6	36.9	935.9
5/11/2002	3m	958.8	155.0	1404.5	133.0	3642.1
Outcrop	3m	3166.6	127.0	11003.1	872.0	16019.9

(Rectanus et al. 2007)

NWIRP, TX (SWMU 118)

Sample	Depth (ft)	Pyro (mg/kg)	Standard Deviation	Alkali (mg/kg)	Standard Deviation	TOC_s (mg/kg)
799E152I	43-45	218.1	25.7	223.2	73.2	26379.0
799E115I	43-45	129.3	7.5	172.3	7.2	12794.0
799E151U	28-30	151.3	22.2	204.0	80.4	40724.0

(Rectanus et al. 2007)

ACRP, MN (OU1)

Sample	Depth (ft)	Pyro (mg/kg)	Standard Deviation	Alkali (mg/kg)	Standard Deviation	TOC_s (mg/kg)
PESMW13A	38-40	18.0	1.1	175.5	95.9	182.0
PESMW10B	68-70	7.7	0.7	118.9	38.0	223.0

(Rectanus et al. 2007)

NAB Little Creek, VA (Site 12)

Sample	Depth (ft)	Pyro (mg/kg)	Standard Deviation	Alkali (mg/kg)	Standard Deviation	TOC_s (mg/kg)
MLS 12	8-12	15.5	0.4	3.0	0.3	130.0
MLS 12	10-12	34.1	5.1	9.4	0.2	147.7
MLS 12	16-19	33.2	4.1	1.8	0.8	105.5
MLS 12	20-22	142.8	2.0	51.8	4.2	831.9
MW6	8-10	12.7	1.7	2.2	0.4	135.8
MW6	10-12	5.6	0.1	0.9	0.4	59.0
MW6	18-20	204.4	12.0	53.3	0.3	868.5
MLS22	8-10	62.1	4.8	26.0	0.7	462.3
MLS22	10-12	26.3	5.1	6.1	0.8	71.7
MLS22	16-19	35.9	0.9	4.8	0.7	97.3
MLS22	20-22	268.0	13.4	55.6	3.2	1176.2
MLS20	10-12	9.6	2.3	2.8	0.2	206.8
MLS20	16-19	29.6	6.0	6.5	1.2	687.2
MLS20	20-22	182.2	10.7	51.0	2.7	669.5
MLS10	8-10	39.9	2.0	13.8	1.3	187.4
MLS 10	10-12	34.4	1.5	7.5	0.6	71.0
MLS 10	16-19	149.6	8.8	26.1	4.3	405.2
MLS 10	20-22	324.2	14.5	111.6	11.3	1184.8
MIP 08	23-24	115.0	5.6	195.1	12.6	1130.5
MIP 10	23-24	125.5	2.5	219.9	28.1	980.5

(Rectanus et al. 2007)

MCRD Parris Island, SC (Site 45)

Sample	Depth (ft)	Pyro (mg/kg)	Standard Deviation	Alkali (mg/kg)	Standard Deviation	TOC_s (mg/kg)
MCRD	8-10	96.4	2.5	114.7	8.1	335.4
MCRD	13-14	208.8	42.2	349.1	41.7	1931.5
Boring 1	3-4	351.9	37.0	177.7	29.5	3315.0
Boring 1	6-7	320.8	4.4	95.8	11.3	2915.0
Boring 1	7-8	59.4	5.3	53.3	6.2	2300.0
Boring 1	8-9	283.6	4.3	143.1	30.3	4195.0
Boring 1	10-11	37.1	2.6	48.2	10.3	1130.0
Boring 1	13-14	34.4	9.7	20.3	6.0	725.0
Boring 1	14-15	31.6	6.5	219.0	39.3	2230.0
Boring 1	16-17	29.4	5.1	34.5	10.2	815.0
Boring 2	2-3	242.9	6.5	496.9	71.5	6285.0
Boring 2	4-5	284.4	8.5	554.6	75.0	7170.0
Boring 2	6-7	182.1	38.7	73.3	16.7	1565.0
Boring 2	7-8	160.2	23.7	63.5	12.6	1120.0
Boring 2	9-10	44.8	7.9	66.3	10.8	647.5

Boring 2	11-12	44.7	6.4	425.0	111.4	589.5
Boring 2	13-14	41.4	6.5	87.4	49.4	1525.0
Boring 2	15-16	29.3	4.3	204.0	43.8	1680.0
Boring 2	18-19	19.9	4.7	19.4	10.6	387.5
Boring 3	7-8	99.7	12.1	57.9	12.9	3335.0
Boring 3	9-10	73.6	15.0	87.5	10.6	2980.0
Boring 3	11-12	9.6	4.7	7.9	0.8	383.0
Boring 3	13-14	122.2	15.8	70.2	6.9	2200.0
Boring 3	14-15	15.6	2.3	9.8	1.9	412.5
Boring 3	18-19	21.1	4.4	171.1	15.9	2120.0
Boring 4	2-3	401.7	36.6	204.0	31.1	4080.0
Boring 4	7-8	243.5	38.9	70.0	9.4	1965.0
Boring 4	10-11	41.4	6.6	12.6	1.4	871.5
Boring 4	11-12	52.7	27.9	397.4	19.6	2840.0
Boring 4	13-14	284.7	9.7	112.3	17.5	3930.0
Boring 4	15-16	30.7	4.5	153.9	14.9	1400.0
Boring 4	16-17	110.7	18.2	37.3	6.6	2335.0
Boring 4	18-19	52.4	6.4	301.6	23.0	1151.4
Boring 5	3-4	138.0	7.2	29.7	5.3	1061.0
Boring 5	5.5-6	223.2	36.0	55.6	13.9	1640.0
Boring 5	7-8	163.8	36.0	24.4	3.7	1245.0
Boring 5	10-11	23.0	4.6	3.3	0.7	271.0
Boring 5	11-12	45.9	3.5	274.8	41.4	2295.0
Boring 5	12-13	216.0	9.7	288.4	33.9	5470.0
Boring 5	15-16	42.8	2.5	140.4	1.0	1240.0
Boring 5	17-18	29.6	4.5	10.8	2.0	275.0
Boring 6	1-2	234.1	24.9	417.0	39.5	7735.0
Boring 6	6-7	73.7	25.5	41.3	8.5	999.0
Boring 6	7-8	82.7	44.5	33.9	4.2	1270.0
Boring 6	9-10	54.5	28.6	14.8	1.8	1130.0
Boring 6	11-12	51.5	14.9	47.2	4.7	1420.0
Boring 6	13-14	93.6	41.3	69.1	3.3	1735.0
Boring 6	15-16	39.1	8.5	219.8	25.3	2405.0
Boring 6	17-18	36.1	4.2	51.2	3.7	1390.0
Boring 10	1	213.3	15.1	391.1	15.5	3920.0
Boring 10	4	261.4	45.2	173.0	40.5	3340.0
Boring 10	5	102.9	18.4	82.9	6.3	1380.0
Boring 10	6	134.4	32.7	171.1	5.5	1860.0
Boring 10	7-8	89.9	6.0	115.2	17.1	1975.0
Boring 10	8-9	54.8	6.1	88.2	11.3	1375.0
Boring 10	10-11	14.8	4.4	6.4	2.9	404.5
Boring 10	11-12	109.4	14.8	143.9	13.1	1745.0
Boring 10	14-15	22.4	1.0	15.6	2.3	390.5
Boring 10	19-20	29.5	1.1	130.5	24.8	1330.0

Boring 10	23-24	56.2	5.2	85.8	8.2	1300.0
Boring 10	16-17	146.2	16.4	447.2	137.1	3290.0
Boring 12	1-2	390.7	25.9	435.5	46.2	8735.0
Boring 12	3-4	287.9	7.5	77.7	34.4	2020.0
Boring 12	5-6	71.4	3.0	15.6	5.5	764.5
Boring 12	7-8	77.9	9.8	10.8	3.1	788.0
Boring 12	9-10	255.4	15.2	281.1	25.7	4635.0
Boring 12	11-12	59.0	3.5	11.7	2.1	596.5
Boring 12	13-14	48.3	3.0	189.5	26.8	1600.0
MW21-SU	3-8ft	419.7	15.0	65.3	5.2	1034.0
MW22-SU	3-8ft	370.9	5.7	147.3	0.5	1571.0
MW21-SL	10-15ft	159.3	8.1	310.9	18.6	1408.0
MW22-SL	10-15ft	217.4	2.6	355.6	48.9	1786.0

PBOC versus TOC_s for 15 selected study sites (NWIRP omitted from the regression)

Regression Statistics						
Multiple R	0.8167					
R Square	0.6670					
Adjusted R Square	0.6643					
Standard Error	0.7557					
Observations	124.0000					
ANOVA						
	df	***SS***	***MS***	***F***	***Significance F***	
Regression	1	139.5365	139.5365	244.3526	6.5189E-31	
Residual	122	69.6676	0.5710			
Total	123	209.2040				
	Coefficients	***Standard Error***	***t Stat***	***P-value***	***Lower 95%***	***Upper 95%***
Intercept	-0.5073	0.3582	-1.4160	0.1593	-1.2165	0.2019
X Variable 1	0.8099	0.0518	15.6318	6.5189E-31	0.7074	0.9125
RESIDUAL OUTPUT						
Observation	***Predicted Y***	***Residuals***	***Standard Residuals***			
1	3.5322	0.6938	0.9219			
2	6.8790	-0.1398	-0.1857			
3	3.9299	1.4611	1.9415			
4	4.3700	1.1741	1.5600			
5	4.7156	1.0106	1.3428			
6	5.5721	1.1965	1.5898			
7	5.5922	1.3863	1.8420			
8	5.5044	1.0845	1.4410			
9	5.9072	1.2389	1.6462			
10	4.9723	-1.5766	-2.0949			
11	4.5903	-1.0537	-1.4000			
12	4.3402	-0.9790	-1.3008			
13	4.3764	-1.0240	-1.3607			
14	3.9177	0.3439	0.4570			
15	6.5781	-0.1244	-0.1653			

16	4.2026	1.1494	1.5273			
17	5.6206	0.7036	0.9348			
18	6.0581	0.2139	0.2842			
19	5.9540	0.0781	0.1037			
20	5.7621	-1.0371	-1.3780			
21	6.2488	-0.1927	-0.2561			
22	5.1865	-0.7408	-0.9843			
23	4.8270	-0.8239	-1.0948			
24	5.7370	-0.2131	-0.2832			
25	4.9218	-0.7646	-1.0159			
26	6.1939	0.2103	0.2794			
27	6.0642	0.0098	0.0130			
28	5.3483	-0.1233	-0.1639			
29	5.5901	0.1320	0.1755			
30	5.6387	-0.3155	-0.4192			
31	5.3454	-0.3823	-0.5080			
32	4.3544	-1.2986	-1.7255			
33	5.5384	-0.0035	-0.0047			
34	4.3259	-0.6864	-0.9120			
35	5.3184	-0.2433	-0.3233			
36	5.3000	-0.3443	-0.4575			
37	6.0520	0.3340	0.4438			
38	6.8428	-0.1259	-0.1673			
39	5.6569	0.2445	0.3249			
40	4.8700	-0.4044	-0.5374			
41	4.8945	-0.4095	-0.5441			
42	6.3296	-0.0446	-0.0592			
43	4.6690	-0.4107	-0.5457			
44	5.4681	0.0031	0.0042			
45	6.5762	0.0302	0.0401			
46	6.6829	0.0492	0.0654			
47	5.4502	0.0924	0.1227			
48	5.1793	0.2310	0.3070			
49	4.7354	-0.0258	-0.0342			
50	4.6594	1.4927	1.9834			
51	5.4292	-0.5713	-0.7592			
52	5.5076	-0.0555	-0.0737			
53	4.3196	-0.6482	-0.8613			
54	6.0630	-1.0031	-1.3329			
55	5.9718	-0.8896	-1.1820			
56	4.3102	-1.4477	-1.9236			
57	5.7261	-0.4669	-0.6204			

58	4.3703	-1.1355	-1.5088			
59	5.6961	-0.4375	-0.5813			
60	6.2263	0.1801	0.2393			
61	5.6346	0.1131	0.1503			
62	4.9761	-0.9864	-1.3107			
63	5.9329	0.1765	0.2345			
64	6.1960	-0.2119	-0.2816			
65	5.3600	-0.1417	-0.1883			
66	5.7743	-0.7774	-1.0330			
67	5.2016	0.6677	0.8872			
68	5.1354	-0.0132	-0.0175			
69	5.4881	0.1424	0.1892			
70	5.2649	-0.0275	-0.0365			
71	4.0300	-0.7589	-1.0083			
72	5.7603	0.0101	0.0134			
73	6.4637	-0.2404	-0.3194			
74	5.2617	-0.0510	-0.0677			
75	4.0419	-0.3453	-0.4588			
76	6.7444	-0.2657	-0.3530			
77	5.2810	-0.5225	-0.6943			
78	5.1865	-0.9479	-1.2594			
79	5.3715	-0.7794	-1.0356			
80	5.5337	-0.4418	-0.5870			
81	5.7982	-0.2418	-0.3213			
82	5.3542	-0.8850	-1.1759			
83	4.9606	-0.7107	-0.9443			
84	4.7509	-0.6669	-0.8861			
85	4.8381	-0.2564	-0.3406			
86	4.7068	-0.8479	-1.1266			
87	4.7448	-0.1161	-0.1543			
88	4.6937	-0.5048	-0.6707			
89	5.1347	-0.2328	-0.3093			
90	5.0834	-0.6620	-0.8796			
91	5.1145	1.0696	1.4212			
92	5.4533	0.7970	1.0591			
93	5.3646	0.7886	1.0478			
94	5.5572	0.7937	1.0546			
95	2.0527	0.8433	1.1205			
96	2.0295	0.7218	0.9591			
97	2.0322	-0.5943	-0.7897			
98	2.3700	0.9034	1.2004			
99	2.0322	-0.0863	-0.1147			

100	5.0338	1.2468	1.6566			
101	6.1343	1.6335	2.1704			
102	7.3341	2.2248	2.9562			
103	3.4353	-0.5193	-0.6901			
104	3.5387	0.2354	0.3128			
105	3.2656	0.2917	0.3876			
106	4.9384	0.3323	0.4415			
107	3.4705	-0.7666	-1.0186			
108	2.7951	-0.9313	-1.2375			
109	4.9733	0.5785	0.7686			
110	4.4625	0.0153	0.0203			
111	2.9531	0.5242	0.6965			
112	3.2005	0.5051	0.6712			
113	5.2189	0.5606	0.7449			
114	3.8109	-1.2928	-1.7178			
115	4.7837	-1.1966	-1.5899			
116	4.7625	0.6896	0.9162			
117	3.7313	0.2509	0.3334			
118	2.9448	0.7904	1.0502			
119	4.3558	0.8130	1.0802			
120	5.2248	0.8525	1.1327			
121	5.1868	0.5504	0.7313			
122	5.0715	0.7732	1.0274			
123	3.7076	1.5574	2.0693			
124	3.8721	0.9695	1.2882			

Mean site values for PBOC versus TOC_s for 15 selected study sites (NWIRP omitted from the regression)

Regression Statistics						
Multiple R	0.8509					
R Square	0.7240					
Adjusted R Square	0.7010					
Standard Error	0.8615					
Observations	14					
ANOVA						
	df	***SS***	***MS***	***F***	***Significance F***	
Regression	1	23.3585	23.3585	31.4728	0.0001	
Residual	12	8.9061	0.7422			
Total	13	32.2646				
	Coefficients	***Standard Error***	***t Stat***	***P-value***	***Lower 95%***	***Upper 95%***
Intercept	-0.2762	1.0353	-0.2668	0.7941	-2.5321	1.9796
X Variable 1	0.8502	0.1516	5.6101	0.0001	0.5200	1.1805
RESIDUAL OUTPUT						
Observation	***Predicted Y***	***Residuals***	***Standard Residuals***			
1	3.9644	0.2617	0.3161			
2	6.6023	-0.5155	-0.6228			
3	5.2066	0.5195	0.6277			
4	6.1164	0.7627	0.9214			
5	6.2721	0.6337	0.7656			
6	5.0971	-1.6828	-2.0331			
7	4.3690	-0.1074	-0.1298			
8	7.1618	-0.7082	-0.8556			
9	6.3178	-0.5578	-0.6739			
10	5.3803	-0.9665	-1.1677			
11	2.4768	0.1817	0.2195			
12	7.2351	1.4112	1.7050			
13	4.9737	-0.0685	-0.0828			
14	4.2392	0.8363	1.0104			

Figure 3-4. Protein and humic acid concentration (mg/kg) for 8 selected study sites.

Figure 3-5. Protein concentration (mg/kg) versus extracted carbon (mg/kg) for 8 selected study sites.

Figure 3-6. Humic acid concentration (mg/kg) versus extracted carbon (mg/kg) for 8 selected study sites.

Data for Figure 3-4, Figure 3-5, and Figure 3-6 come from the following table.

Facility Name	Site	Pyro (mg/kg)	Alkali (mg/kg)	Proteins (mg/kg)	Humic Acids (mg/kg)
NAS Jacksonville, FL	OU3	27.8±2.0	40.60±16.3	0.70	13.13
NAES Lakehurst, NJ	Sites I &J	49.0±4.0	21.96±1.8	7.50	12.15
Hill AFB, UT	OU2	15.7±3.1	14.71±4.2	19.24±17.7	6.82±2.36
NAS Pensacola, FL	WWTP	84.9±6.4	221.9±101.1	2.60	15.85
NAS North Island, CA	Site 5- Unit 2	51.4±5.4	186.15±75.4	9.18±1.5	23.62±11.24
NTC Orlando, FL	OU2	567.9±49.7	403.79±115.8	16.36±12.9	54.64±26.9
NTC Orlando, FL	OU4	733.2±60.7	264.78±97.1	28.62±29.7	45.02±10.8
NUWC Keyport	OU1	190.7±9.4	444.33±40.8	ND	108.11

Proteins versus Pyro Extracted Carbon

Linear Regression

Data source: Proteins versus Pyro Extracted Carbon

Col 3 = 5.126 + (0.0251 * Col 1)

N = 8

R = 0.688 Rsqr = 0.473 Adj Rsqr = 0.386

Standard Error of Estimate = 7.940

	Coefficient	Std. Error	t	P
Constant	5.126	3.645	1.407	0.209
Col 1	0.0251	0.0108	2.322	0.059

Analysis of Variance:

	DF	SS	MS	F	P
Regression	1	340.015	340.015	5.394	0.059
Residual	6	378.226	63.038		
Total	7	718.242	102.606		

Normality Test (Shapiro-Wilk) Passed (P = 0.815)

Constant Variance Test: Passed (P = 0.619)

Power of performed test with alpha = 0.050: 0.471

The power of the performed test (0.471) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists. Negative results should be interpreted cautiously.

Proteins versus Pyro Extracted Carbon

Regression Statistics						
Multiple R	0.6880					
R Square	0.4734					
Adjusted R Square	0.3856					
Standard Error	7.9396					
Observations	8					
ANOVA						
	df	***SS***	***MS***	***F***	***Significance F***	
Regression	1	340.0151	340.0151	5.3938	0.0592	
Residual	6	378.2265	63.0377			
Total	7	718.2415				
	Coefficients	***Standard Error***	***t Stat***	***P-value***	***Lower 95%***	***Upper 95%***
Intercept	5.1262	3.6445	1.4066	0.2092	-3.7916	14.0440
X Variable 1	0.0251	0.0108	2.3225	0.0592	-0.0013	0.0515
RESIDUAL OUTPUT						
Observation	***Predicted Y***	***Residuals***	***Standard Residuals***			
1	5.8250	-5.1247	-0.6972			
2	6.4167	2.7638	0.3760			
3	7.2564	-4.6520	-0.6329			
4	19.3803	-3.0199	-0.4108			
5	23.5301	5.0861	0.6919			
6	5.5199	13.7165	1.8660			
7	6.3551	1.1422	0.1554			
8	9.9120	-9.9120	-1.3485			

Proteins versus Alkali Extracted Carbon

Regression Statistics						
Multiple R	0.0105					
R Square	0.0001					
Adjusted R Square	-0.1665					
Standard Error	10.9405					
Observations	8					
ANOVA						
	df	***SS***	***MS***	***F***	***Significance F***	
Regression	1	0.0797	0.0797	0.0007	0.9802	
Residual	6	718.1618	119.6936			
Total	7	718.2415				
	Coefficients	***Standard Error***	***t Stat***	***P-value***	***Lower 95%***	***Upper 95%***
Intercept	10.3975	6.2567	1.6618	0.1476	-4.9120	25.7070
X Variable 1	0.0006	0.0246	0.0258	0.9802	-0.0596	0.0609
RESIDUAL OUTPUT						
Observation	***Predicted Y***	***Residuals***	***Standard Residuals***			
1	10.4233	-9.7230	-0.9599			
2	10.5158	-1.3352	-0.1318			
3	10.5385	-7.9341	-0.7833			
4	10.6541	5.7063	0.5634			
5	10.5657	18.0505	1.7821			
6	10.4069	8.8295	0.8717			
7	10.4115	-2.9142	-0.2877			
8	10.6798	-10.6798	-1.0544			

Humic Acids versus Alkali Extracted Carbon

Linear Regression

Data source: Humic Acids versus Alkali Extracted Carbon

Col 4 = -0.509 + (0.177 * Col 2)

N = 8

R = 0.875 Rsqr = 0.765 Adj Rsqr = 0.726

Standard Error of Estimate = 17.843

	Coefficient	Std. Error	t	P
Constant	-0.509	10.204	-0.0499	0.962
Col 2	0.177	0.0401	4.417	0.004

Analysis of Variance:

	DF	SS	MS	F	P
Regression	1	6210.989	6210.989	19.508	0.004
Residual	6	1910.298	318.383		
Total	7	8121.287	1160.184		

Normality Test (Shapiro-Wilk) Passed (P = 0.867)

Constant Variance Test: Passed (P = 0.120)

Power of performed test with alpha = 0.050: 0.856

Humic Acids versus Pyro Extracted Carbon

Regression Statistics						
Multiple R	0.4260					
R Square	0.1815					
Adjusted R Square	0.0450					
Standard Error	33.2857					
Observations	8					
ANOVA						
	df	***SS***	***MS***	***F***	***Significance F***	
Regression	1	1473.6613	1473.6613	1.3301	0.2927	
Residual	6	6647.6258	1107.9376			
Total	7	8121.2871				
	Coefficients	***Standard Error***	***t Stat***	***P-value***	***Lower 95%***	***Upper 95%***
Intercept	23.6777	15.2790	1.5497	0.1722	-13.7087	61.0642
X Variable 1	0.0523	0.0453	1.1533	0.2927	-0.0586	0.1631
RESIDUAL OUTPUT						
Observation	***Predicted Y***	***Residuals***	***Standard Residuals***			
1	25.1326	-12.0071	-0.3896			
2	26.3645	-2.7452	-0.0891			
3	28.1126	-12.2627	-0.3979			
4	53.3528	1.2834	0.0416			
5	61.9919	-16.9718	-0.5507			
6	24.4974	-17.6755	-0.5736			
7	26.2361	-14.0859	-0.4571			
8	33.6412	74.4649	2.4164			

Humic Acids versus Alkali Extracted Carbon

Regression Statistics						
Multiple R	0.8745					
R Square	0.7648					
Adjusted R Square	0.7256					
Standard Error	17.8433					
Observations	8					
ANOVA						
	df	***SS***	***MS***	***F***	***Significance F***	
Regression	1	6210.9886	6210.9886	19.5079	0.0045	
Residual	6	1910.2985	318.3831			
Total	7	8121.2871				
	Coefficients	***Standard Error***	***t Stat***	***P-value***	***Lower 95%***	***Upper 95%***
Intercept	-0.5088	10.2043	-0.0499	0.9618	-25.4778	24.4601
X Variable 1	0.1773	0.0401	4.4168	0.0045	0.0791	0.2756
RESIDUAL OUTPUT						
Observation	***Predicted Y***	***Residuals***	***Standard Residuals***			
1	6.6909	6.4346	0.3895			
2	32.4987	-8.8795	-0.5375			
3	38.8404	-22.9905	-1.3917			
4	71.0917	-16.4555	-0.9961			
5	46.4415	-1.4214	-0.0860			
6	2.0993	4.7225	0.2859			
7	3.3858	8.7644	0.5305			
8	78.2805	29.8255	1.8055			

Figure 3-7. Total hydrolyzable amino acid concentration (mg/kg) versus PBOC (mg/kg) for selected study sites (NTC Orlando sites omitted from the regression).

Figure 3-8. Total hydrolyzable amino acid concentration (mg/kg) versus extracted carbon (mg/kg) for selected study sites (NTC Orlando sites omitted).

Data for Figure 3-7 and Figure 3-8 come from the following table.

Facility Name	Sample	Depth (ft)	Pyro (mg/kg)	Alkali (mg/kg)	Total HAA (mg/kg)
NAS North Island, CA	158	Aquifer	393.7±25.3	451.2±144.1	49.5
NAS North Island, CA	159	Aquifer	52.6±7.3	166.8±45.5	2.6
NAS North Island, CA	160	Aquifer	50.3±3.4	205.5±105.4	8.2
MCRD Parris Island, SC	MCRD	8-10	96.4±2.5	114.7±8.1	2.4
MCRD Parris Island, SC	MCRD	13-14	208.8±42.2	349.1±41.7	9.9
MCRD Parris Island, SC	Boring 4	2-3	401.7±36.6	204.0±31.1	14.7
MCRD Parris Island, SC	Boring 4	7-8	243.5±38.9	70.0±9.4	12.8
MCRD Parris Island, SC	Boring 4	10-11	41.4±6.6	12.6±1.4	4.9
MCRD Parris Island, SC	Boring 4	11-12	52.7±27.9	397.4±19.6	12.5
MCRD Parris Island, SC	Boring 4	13-14	284.7±9.7	112.3±17.5	12.8
NAS Pensacola, FL	ORC1	Aquifer	84.9±6.4	221.9±101.1	13.2
NAB Little Creek, VA	MLS22	10-12	26.3±5.1	6.1±0.8	2.7
NAB Little Creek, VA	MLS22	16-19	35.9±0.9	4.8±0.7	7.0
NAB Little Creek, VA	MLS 12	8-12	15.5±0.4	3.0±0.3	2.3
NAB Little Creek, VA	MLS 12	16-19	33.2±4.1	1.8±0.8	4.4
NAB Little Creek, VA	MLS10	8-10	39.9±2.0	13.8±1.3	6.0
NAB Little Creek, VA	MLS 10	20-22	324.2±14.5	111.6±11.3	44.8
Beale AFB, CA	10CO39	58-59	1.7±1.0	2.5±0.2	3.0
Beale AFB, CA	10CO49	49.5-50	3.8±0.3	1.4±0.2	0.9
NTC Orlando, FL	SB1	25-30	1145.4±109.8	123.7±29.5	1.3
NTC Orlando, FL	SB1	9-14	321.1±11.5	405.9±164.6	1.9
NTC Orlando, FL	SB1	32-35	565.7±48.0	304.4±32.3	0.8
NTC Orlando, FL	SB2	32-35	570.2±51.3	503.2±199.2	0.7

Total Hydrolyzable Amino Acid Concentration versus Pyro Extracted Carbon

Regression Statistics						
Multiple R	0.7830					
R Square	0.6131					
Adjusted R Square	0.5903					
Standard Error	0.9534					
Observations	19					
ANOVA						
	df	***SS***	***MS***	***F***	***Significance F***	
Regression	1	24.4879	24.4879	26.9387	7.37397E-05	
Residual	17	15.4534	0.9090			
Total	18	39.9413				
	Coefficients	***Standard Error***	***t Stat***	***P-value***	***Lower 95%***	***Upper 95%***
Intercept	1.9052	0.4724	4.0334	0.0009	0.9086	2.9018
X Variable 1	1.1330	0.2183	5.1902	0.0001	0.6724	1.5935
RESIDUAL OUTPUT						
Observation	***Predicted Y***	***Residuals***	***Standard Residuals***			
1	6.3258	-0.3502	-0.3780			
2	2.9745	0.9879	1.0662			
3	4.2880	-0.3710	-0.4004			
4	2.9055	1.6627	1.7945			
5	4.5078	0.8335	0.8995			
6	4.8288	-0.3876	-0.4184			
7	3.0275	0.2424	0.2617			
8	4.1079	-0.5277	-0.5695			
9	2.8454	-0.1071	-0.1156			
10	3.5955	-0.0918	-0.0991			
11	3.9295	-0.2443	-0.2636			
12	6.2136	-0.4321	-0.4664			
13	3.1636	-2.6200	-2.8277			
14	1.7601	-0.4135	-0.4463			
15	4.9490	1.0466	1.1296			
16	4.7909	0.7041	0.7599			

17	3.7045	0.0195	0.0210			
18	4.7691	-0.8051	-0.8689			
19	4.7977	0.8538	0.9215			

Total Hydrolyzable Amino Acid Concentration versus Alkali Extracted Carbon

Regression Statistics						
Multiple R	0.6762					
R Square	0.4573					
Adjusted R Square	0.4253					
Standard Error	1.5510					
Observations	19					
ANOVA						
	df	***SS***	***MS***	***F***	***Significance F***	
Regression	1	34.4544	34.4544	14.3230	0.0015	
Residual	17	40.8940	2.4055			
Total	18	75.3484				
	Coefficients	***Standard Error***	***t Stat***	***P-value***	***Lower 95%***	***Upper 95%***
Intercept	1.0551	0.7684	1.3731	0.1876	-0.5661	2.6762
X Variable 1	1.3439	0.3551	3.7846	0.0015	0.5947	2.0931
RESIDUAL OUTPUT						
Observation	***Predicted Y***	***Residuals***	***Standard Residuals***			
1	6.2986	-0.1866	-0.1238			
2	2.3234	2.7937	1.8534			
3	3.8815	1.4437	0.9579			
4	2.2415	2.5006	1.6590			
5	4.1422	1.7133	1.1367			
6	4.5229	0.8794	0.5834			
7	2.3863	-0.5846	-0.3878			
8	3.6678	-2.0998	-1.3931			
9	2.1703	-1.0700	-0.7099			
10	3.0600	-2.4538	-1.6280			
11	3.4562	-0.8331	-0.5527			

12	6.1655	-1.4506	-0.9624			
13	2.5478	-1.6357	-1.0852			
14	0.8829	-0.5526	-0.3666			
15	4.6656	0.6526	0.4330			
16	4.4780	-0.2298	-0.1525			
17	3.1893	-0.6550	-0.4345			
18	4.4521	1.5327	1.0169			
19	4.4860	0.2353	0.1561			

Figure 3-9. Functional groups of protein amino acids (mg/kg) for 5 of 7 study sites (NTC Orlando sites omitted). Acidic: aspartic acid and glutamic acid. Neutral: alanine, valine, glycine, iso-leucine, leucine, proline, threonine, serine, phenylalanine, tyrosine, and methionine. Basic: histidine and lysine.

Facility Name	Site	Acidic HAA (mg/kg)	Neutral HAA (mg/kg)	Basic HAA (mg/kg)
NAS North Island, CA	Site 5- Unit 2	2.25±3.56	10.33±3.34	1.45±2.13
MCRD Parris Island, SC	Site 45	0.08±0.12	5.74±5.01	0.00±0.00
NAS Pensacola, FL	WWTP	2.61	9.61	0.35
NAB Little Creek, VA	Site 12	1.18±0.50	7.51±11.17	0.30±0.13
Beale AFB, CA	Site 10	0.64±0.76	0.98±0.39	0.10±0.04
NTC Orlando, FL	OU2	0.02±0.00	0.39±0.05	0.00±0.00
NTC Orlando, FL	OU4	0.24±0.16	1.15±0.25	0.00±0.00

Figure 3-10. NAC (ft^{-1}) versus neutral hydrolyzable amino acid concentration (mg/kg) for 5 study sites.

Facility	Site	Neutral HAA (mg/kg)	NAC (ft^{-1})
Beale AFB, CA	Site 10	0.98±0.39	0.00247
MCRD Parris Island, SC	Site 45	5.74±5.01	0.00604
NAS Pensacola, FL	WWTP	9.61	0.01270
NAB Little Creek, VA	Site 12	7.51±11.17	0.01320
NAS North Island, CA	Site 5-Unit 2	4.65±0.17	0.00970

Appendix B. Chapter 4

Figure 4-1. Dissolved Oxygen (DO) versus PBOC for selected study sites. Standard deviations for dissolved oxygen concentrations are shown with error bars.

Figure 4-2. Hydrogen (H_2) versus PBOC for selected study sites. Standard deviations for hydrogen concentrations are shown with error bars.

Data for Figure 4-1 and Figure 4-2 come from the following tables.

Table 4-2. Summary of PBOC (mg/kg) in aquifer sediments for selected study sites.

NAES Lakehurst, NJ (Sites I & J)
Sample: MW6 (Aquifer)

Replicate	**Extracted Carbon Concentration (mg/kg)**				
	0.1% Pyro	0.1% Pyro	0.1% Pyro	0.5N NaOH	0.1% Pyro
1	30.5	12.0	2.0	17.9	6.0
2	37.2	12.6	2.3	16.4	4.4
3	34.6	12.2	3.3	16.8	4.4
Average	34.1	12.3	2.6	17.0	4.9
Standard Deviation	3.4	0.31	0.67	0.81	0.96

MCRD Parris Island, SC (Site 45)
Sample: MCRD (8-10 ft)

Replicate	**Extracted Carbon Concentration (mg/kg)**				
	0.1% Pyro	0.1% Pyro	0.1% Pyro	0.5N NaOH	0.1% Pyro
1	51.8	25.7	19.6	103.9	4.7
2	49.1	24.0	25.3	120.2	3.6
3	49.9	23.5	20.2	105.0	6.9
Average	50.3	24.4	21.7	109.6	5.1
Standard Deviation	1.4	1.2	3.1	9.2	1.7

MCRD Parris Island, SC (Site 45)
Sample: MCRD (12-13 ft)

Replicate	Extracted Carbon Concentration (mg/kg)				
	0.1% Pyro	0.1% Pyro	0.1% Pyro	0.5N NaOH	0.1% Pyro
1	127.9	31.7	26.0	237.8	103.7
2	124.5	34.4	24.3	275.4	118.7
3	207.7	25.3	24.4	190.1	121.6
Average	153.4	30.5	24.9	234.5	114.7
Standard Deviation	47.1	4.7	0.95	42.7	9.6

Hill AFB, UT (OU2)
Sample: U2 1000 (40-42 ft)

Replicate	Extracted Carbon Concentration (mg/kg)				
	0.1% Pyro	0.1% Pyro	0.1% Pyro	0.5N NaOH	0.1% Pyro
1	21.9	5.9	1.5	6.5	3.4
2	9.2	3.3	3.8	4.6	1.6
3	10.2	5.5	2.3	2.4	3.6
Average	13.8	4.9	2.5	4.5	2.9
Standard Deviation	7.0	1.4	1.2	2.1	1.1

Hill AFB, UT (OU2)
Sample: U2 1001(40-42 ft)

Replicate	Extracted Carbon Concentration (mg/kg)				
	0.1% Pyro	0.1% Pyro	0.1% Pyro	0.5N NaOH	0.1% Pyro
1	6.5	2.6	5.2	6.0	5.2
2	4.6	1.7	5.3	13.4	7.9
3	3.9	2.7	4.2	10.4	6.8
Average	5.0	2.3	4.9	9.9	6.6
Standard Deviation	1.3	0.5	0.6	3.7	1.4

Hill AFB, UT (OU2)
Sample: U2 1002(30-32 ft)

Replicate	Extracted Carbon Concentration (mg/kg)				
	0.1% Pyro	0.1% Pyro	0.1% Pyro	0.5N NaOH	0.1% Pyro
1	5.8	5.1	4.3	13.5	7.5
2	5.8	3.5	4.8	19.7	8.2
3	4.3	3.4	5.1	3.9	7.9
Average	5.3	4.0	4.7	12.4	7.9
Standard Deviation	0.9	0.9	0.4	8.0	0.4

Hill AFB, UT (OU2)
Sample: U2 1003 (40-42 ft)

Replicate	Extracted Carbon Concentration (mg/kg)				
	0.1% Pyro	0.1% Pyro	0.1% Pyro	0.5N NaOH	0.1% Pyro
1	5.9	4.7	4.2	9.2	4.2
2	5.2	8.0	4.4	10.8	3.7
3	5.0	4.3	3.9	12.4	3.6
Average	5.4	5.6	4.2	10.8	3.8
Standard Deviation	0.5	2.0	0.3	1.6	0.3

NAS Jacksonville, FL (OU3)
Sample: NAS JAX (50-54 ft)

Replicate	Extracted Carbon Concentration (mg/kg)				
	0.1% Pyro	0.1% Pyro	0.1% Pyro	0.5N NaOH	0.1% Pyro
1	12.6	6.6	11.6	51.2	7.8
2	9.8	11.0	6.9	30.1	4.4
3	9.5	10.5	5.0	23.8	4.4
Average	10.7	9.4	7.8	35.1	5.6
Standard Deviation	1.7	2.4	3.4	14.4	1.9

NAS Pensacola, FL (WWTP)
Sample: ORC1 (Aquifer)

Replicate	Extracted Carbon Concentration (mg/kg)				
	0.1% Pyro	0.1% Pyro	0.1% Pyro	0.5N NaOH	0.1% Pyro
1	58.7	13.1	20.4	191.7	30.4
2	52.3	18.6	11.5	91.2	29.5
3	55.0	12.5	12.6	299.1	23.9
Average	55.3	14.7	14.8	194.0	27.9
Standard Deviation	3.2	3.4	4.9	104.0	3.6

NTC Orlando, FL (OU2)
Sample: SB1 (32-35 ft)

Replicate	Extracted Carbon Concentration (mg/kg)				
	0.1% Pyro	0.1% Pyro	0.1% Pyro	0.5N NaOH	0.1% Pyro
1	441.3	126.9	44.6	207.4	60.0
2	434.8	84.5	48.3	263.2	64.2
3	352.5	87.3	77.0	259.1	59.3
Average	409.5	99.5	56.6	243.2	61.2
Standard Deviation	49.5	23.7	17.8	31.1	2.7

NTC Orlando, FL (OU2)
Sample: SB2 (32-35 ft)

Replicate	Extracted Carbon Concentration (mg/kg)				
	0.1% Pyro	0.1% Pyro	0.1% Pyro	0.5N NaOH	0.1% Pyro
1	360.5	92.2	100.5	615.3	114.6
2	375.6	159.6	92.6	270.8	85.2
3	358.0	102.4	69.0	349.6	74.1
Average	364.7	118.1	87.4	411.9	91.3
Standard Deviation	9.5	36.3	16.4	180.5	20.9

NTC Orlando, FL (OU4)
Sample: SB1 (9-14ft)

Replicate	Extracted Carbon Concentration (mg/kg)				
	0.1% Pyro	0.1% Pyro	0.1% Pyro	0.5N NaOH	0.1% Pyro
1	124.0	115.2	87.4	190.7	41.3
2	118.5	118.4	91.9	525.2	34.3
3	108.7	112.9	86.2	391.6	34.5
Average	117.1	115.5	88.5	369.1	36.7
Standard Deviation	7.7	2.7	3.0	168.4	4.0

NTC Orlando, FL (OU4)
Sample: SB1 (25-30 ft)

Replicate	Extracted Carbon Concentration (mg/kg)				
	0.1% Pyro	0.1% Pyro	0.1% Pyro	0.5N NaOH	0.1% Pyro
1	475.6	384.4	207.4	58.1	40.7
2	580.4	274.6	242.9	65.3	50.7
3	584.3	436.3	250.4	96.3	60.0
Average	546.7	365.1	233.6	73.2	50.5
Standard Deviation	61.7	82.5	23.0	20.3	9.7

NUWC Keyport, WA (OU1)
Sample: NUWC (Aquifer)

Replicate	Extracted Carbon Concentration (mg/kg)				
	0.1% Pyro	0.1% Pyro	0.1% Pyro	0.5N NaOH	0.1% Pyro
1	77.0	71.6	46.7	298.1	134.8
2	74.7	58.7	46.4	274.5	136.1
3	100.4	55.3	41.2	374.0	115.7
Average	84.1	61.9	44.8	315.5	128.8
Standard Deviation	14.2	8.6	3.1	52.0	11.4

NAS North Island, CA (Site 5-Unit 2)
Sample: NAS 159 (Aquifer)

Replicate	Extracted Carbon Concentration (mg/kg)				
	0.1% Pyro	0.1% Pyro	0.1% Pyro	0.5N NaOH	0.1% Pyro
1	30.6	11.2	18.6	130.7	7.4
2	28.6	10.3	12.5	137.3	5.9
3	22.4	16.5	6.9	215.6	3.6
Average	27.2	12.7	12.7	161.2	5.6
Standard Deviation	4.3	3.4	5.8	47.3	1.9

NAS North Island, CA (Site 5-Unit 2)
Sample: NAS 160 (Aquifer)

Replicate	Extracted Carbon Concentration (mg/kg)				
	0.1% Pyro	0.1% Pyro	0.1% Pyro	0.5N NaOH	0.1% Pyro
1	25.7	11.0	9.7	315.3	11.8
2	20.5	21.9	9.6	135.0	9.8
3	29.0	12.6	10.8	133.9	10.5
Average	25.1	15.2	10.0	194.8	10.7
Standard Deviation	4.3	5.9	0.7	104.4	1.1

NSB Kings Bay, GA (Site 11)
Sample: KBA-13A

Depth (ft)	Extracted Carbon Concentration (mg/kg)			
	0.1% Pyro Extractions (1-3)	Standard Deviation	0.5 NaOH/ 0.1% Pyro Extractions (4-5)	Standard Deviation
10-13	359.5	3.4	174.6	36.9

(Rectanus et al. 2007)

Beale AFB, CA (Site 10)
Sample: 10CO39

Depth (ft)	Extracted Carbon Concentration (mg/kg)			
	0.1% Pyro Extractions (1-3)	Standard Deviation	0.5 NaOH/ 0.1% Pyro Extractions (4-5)	Standard Deviation
37-38	16.0	2.7	2.1	0.1
46-47	13.1	1.0	2.6	0.1
58-59	1.7	1.0	2.5	0.2

Beale AFB, CA (Site 10)
Sample: 10CO41

Depth	Extracted Carbon Concentration (mg/kg)			
	0.1% Pyro Extractions (1-3)	Standard Deviation	0.5 NaOH/ 0.1% Pyro Extractions (4-5)	Standard Deviation
43-44	23.9	1.3	2.5	0.3
45-46	28.8	1.8	2.8	0.3
59-60	2.0	0.4	1.3	0.1

Beale AFB, CA (Site 10)
Sample: 10CO49

Depth	Extracted Carbon Concentration (mg/kg)			
	0.1% Pyro Extractions (1-3)	Standard Deviation	0.5 NaOH/ 0.1% Pyro Extractions (4-5)	Standard Deviation
41.5-42	7.5	0.4	2.9	0.1
45-46	5.9	0.5	2.2	0.5
49.5-50	3.8	0.3	1.4	0.2
54-54.5	5.5	0.5	1.8	0.3
59-60	5.2	0.4	1.8	0.2

(Rectanus et al. 2007)

Table 4-3. Summary of dissolved oxygen and hydrogen concentrations with corresponding standard deviations for selected sampling events.

NAS Pensacola, FL (WWTP)

Well ID	Date	Dissolved Oxygen (mg/L)	Hydrogen (nM)
33G12	7/16/1997	0.05	0.39
IMW66	7/16/1997	0.00	0.37
IMW66R	7/16/1997	0.00	
33G05	7/16/1997	0.00	0.62
USGS1I	7/16/1997	0.00	0.23
USGS1S	7/16/1997	0.05	0.58
USGS2I	7/16/1997	0.00	1.00
USGS2S	7/16/1997	0.05	2.00
33G12	11/4/1997	0.00	0.36
IMW66	11/4/1997	0.00	0.59
33G05	11/4/1997	0.00	0.42
USGS1I	11/4/1997	0.00	0.10
USGS1S	11/4/1997	0.40	0.52
USGS2I	11/4/1997	0.00	0.32
USGS2S	11/4/1997	0.05	0.80
33G12	1/23/1998	0.00	0.39
33G05	1/23/1998	0.00	0.28
IMW66	1/23/1998	0.20	0.26
IMW66R	1/23/1998	0.20	
USGS1I	1/23/1998	0.00	0.39
USGS1S	1/23/1998	0.00	1.75
USGS2I	1/23/1998	0.00	0.85
USGS2S	1/23/1998	0.00	7.80
Average		0.03	0.95
Standard Deviation		0.09	1.64

NAES Lakehurst, NJ (Sites I & J)

Well ID	Date	Dissolved Oxygen (mg/L)
LY	1/23/2006	0.45
MA	1/23/2006	1.23
MC	1/24/2006	2.66
MG	1/24/2006	1.97
MI	1/24/2006	2.66
MK	1/24/2006	2.78
LG	1/27/2006	0.22
LI	1/27/2006	3.8
LK	1/27/2006	0.09
LM	1/27/2006	0.07
LY	11/6/2006	0.37
MA	11/6/2006	1.35
MI	11/6/2006	3.63
MK	11/6/2006	3.31
MC	11/7/2006	6.56
MG	11/7/2006	2.68
LG	11/9/2006	0.39
LI	11/9/2006	4.3
LK	11/9/2006	0.11
LM	11/9/2006	0.18
LY	5/7/2007	0.23
MA	5/7/2007	1.58
MI	5/7/2007	3.76
MK	5/7/2007	3.27
MC	5/8/2007	7.03
MG	5/8/2007	2.74
LG	5/9/2007	0.05
LI	5/9/2007	5.62
LK	5/9/2007	0.4
LM	5/9/2007	0.8
LK	11/12/2007	0.28
MA	11/12/2007	0.98
MC	11/12/2007	2.57
LY	11/13/2007	0.07
MI	11/13/2007	2.36

MK	11/13/2007	2.4
MG	11/14/2007	2.41
LG	11/15/2007	0.14
LI	11/15/2007	3.57
LM	11/15/2007	0.07
Average		1.98
Standard Deviation		1.86

MCRD Parris Island, SC (Site 45)

Well ID	Date	Dissolved Oxygen (mg/L)	Hydrogen (nM)
PAI-45-MW04-SL	8/25/2005	0.025	
PAI-45-MW20-SL	8/25/2005	0.15	
PAI-45-MW04-SL	9/26/2006	0.025	
PAI-45-MW20-SL	9/26/2006	0.05	
PAI-45-MW20-SU	9/26/2006	0.1	
PAI-45-MW04-SL	7/24/2007	0.1	1.1
PAI-45-MW04-SU	7/24/2007	0.1	
PAI-45-MW10-SL	7/23/2007		2.2
PAI-45-MW20-SL	7/24/2007	0.1	2.9
PAI-45-MW20-SU	7/24/2007	0.15	
PAI-45- MW04-D	9/10/2007	0.05	
PAI-45-MW04-SL	9/10/2007	0.05	
PAI-45-MW04-SU	9/10/2007	0.02	
PAI-45-MW10-SL	9/10/2007	0.1	
PAI-45-MW10-SU	9/10/2007	0.025	
PAI-45-MW20-SL	9/10/2007	0.05	
PAI-45-MW20-SU	9/10/2007	1	
Average		0.07	2.07
Standard Deviation		0.03	0.91

Hill AFB, UT (OU2)

Well ID	Date	Dissolved Oxygen (mg/L)
U2-018	5/11/2006	5.10
U2-085	5/11/2006	4.30
U2-017R	5/12/2006	3.80
U2-045	5/12/2006	1.60
U2-083	5/16/2006	3.30
U2-082	5/16/2006	4.70
U2-043	5/16/2006	2.40
U2-086	5/17/2006	2.80
U2-023	5/18/2006	5.40
U2-021R	5/18/2006	6.10
U2-042	5/19/2006	4.20
U2-080	5/19/2006	2.70
U2-039	5/22/2006	0.50
U2-079	5/5/2006	2.90
U2-675	5/9/2006	4.00
U2-086	10/9/2006	4.00
U2-042	10/9/2006	5.30
U2-080	10/13/2006	2.90
U2-043	10/13/2006	2.30
U2-083	10/13/2006	2.80
U2-039	10/13/2006	2.50
U2-085	10/17/2006	4.30
U2-023	10/17/2006	5.20
U2-018	10/17/2006	4.10
U2-045	10/18/2006	6.90
U2-082	10/19/2006	5.90
U2-021R	10/19/2006	7.70
U2-079	10/9/2006	4.10
U2-675	10/13/2006	3.70
U2-017R	4/23/2007	5.70
U2-043	4/23/2007	3.40
U2-039	4/23/2007	3.00
U2-021R	4/26/2007	5.40
U2-023	4/26/2007	1.20
U2-086	4/30/2007	3.40
U2-042	4/30/2007	4.10
U2-675	4/25/2007	4.40
U2-079	4/30/2007	4.40

U2-082	5/4/2007	7.70
U2-083	5/4/2007	3.60
U2-085	5/4/2007	4.10
U2-018	5/4/2007	2.70
U2-080	5/7/2007	3.80
U2-045	5/8/2007	1.10
U2-017R	10/2/2007	5.50
U2-018	10/3/2007	2.10
U2-085	10/3/2007	3.60
U2-045	10/3/2007	2.20
U2-021R	10/4/2007	6.40
U2-043	10/5/2007	3.50
U2-039	10/5/2007	2.00
U2-086	10/8/2007	3.40
U2-042	10/8/2007	4.80
U2-083	10/16/2007	2.60
U2-082	10/16/2007	1.50
U2-080	10/16/2007	3.30
U2-675	10/9/2007	2.20
U2-079	10/9/2007	3.80
U2-023	10/16/2007	3.20
Average		3.79
Standard Deviation		1.55

NAS Jacksonville, FL (OU3)

Well ID	Screen Interval (ft)	Date	Dissolved Oxygen (mg/L)
U3C-MW31	35-40	11/1/2001	0.59
U3C-MW35	30-35	11/1/2001	0.09
U3C-MW35	35-40	11/1/2001	0.78
U3C-MW35	40-45	11/1/2001	0.55
U3C-MW35	45-50	11/1/2001	0.46
U3C-MW36	30-35	11/1/2001	0.8
U3C-MW36	35-40	11/1/2001	0.99
U3C-MW36	40-45	11/1/2001	1.13
U3C-MW36	45-50	11/1/2001	1.05
U3C-MW37	30-35	11/1/2001	0.33
U3C-MW37	35-40	11/1/2001	0.64
U3C-MW37	40-45	11/1/2001	1.03
U3C-MW37	45-50	11/1/2001	1.32

U3C-MW38	30-35	11/1/2001	0.47
U3C-MW38	35-40	11/1/2001	0.87
U3C-MW38	40-45	11/1/2001	0.26
U3C-MW38	45-50	11/1/2001	0.96
U3C-MW39	30-35	11/1/2001	0.33
U3C-MW39	35-40	11/1/2001	0.96
U3C-MW39	40-45	11/1/2001	0.48
U3C-MW39	45-50	11/1/2001	0.71
U3C-MW40	30-35	11/1/2001	0.36
U3C-MW40	35-40	11/1/2001	0.15
U3C-MW40	40-45	11/1/2001	0.61
U3C-MW40	45-50	11/1/2001	0.49
U3C-MW40	50-55	11/1/2001	1.25
U3C-MW40	55-60	11/1/2001	1.84
U3C-MW41	30-35	11/1/2001	0.83
U3C-MW41	35-40	11/1/2001	0.34
U3C-MW41	40-45	11/1/2001	0.61
U3C-MW41	45-50	11/1/2001	1.05
U3C-MW41	50-55	11/1/2001	0.49
U3C-MW41	55-60	11/1/2001	0.73
U3C-MW42	30-35	11/1/2001	0.81
U3C-MW42	35-40	11/1/2001	1.05
U3C-MW42	40-45	11/1/2001	0.79
U3C-MW42	45-50	11/1/2001	3.09
U3C-MW42	50-55	11/1/2001	0.03
U3D-MW30	30-35	11/1/2001	2.13
U3D-GEW002	27-52	11/1/2001	2.89
U3D-MW43	24-29	11/1/2001	0.23
U3D-MW43	29-34	11/1/2001	0.29
U3D-MW43	34-39	11/1/2001	0.14
U3D-MW43	39-44	11/1/2001	0.56
U3D-MW43	44-49	11/1/2001	0.35
U3D-MW43	49-54	11/1/2001	0.43
U3D-MW43	54-59	11/1/2001	2.34
U3D-MW44	24-29	11/1/2001	0.01
U3D-MW44	29-34	11/1/2001	0.32
U3D-MW44	34-39	11/1/2001	0.29
U3D-MW44	39-44	11/1/2001	1.06
U3D-MW44	44-49	11/1/2001	0.27
U3D-MW44	49-54	11/1/2001	5.8
U3D-MW44	54-59	11/1/2001	0.56

U3D-MW45	24-29	11/1/2001	0.14
U3D-MW45	29-34	11/1/2001	0.56
U3D-MW45	34-39	11/1/2001	0.53
U3D-MW45	39-44	11/1/2001	1.12
U3D-MW45	44-49	11/1/2001	0.51
U3D-MW45	49-54	11/1/2001	1.13
U3D-MW45	54-59	11/1/2001	0.28
U3D-MW46	24-29	11/1/2001	0.67
U3D-MW46	29-34	11/1/2001	0.53
U3D-MW46	34-39	11/1/2001	0.75
U3D-MW46	39-44	11/1/2001	0.44
U3D-MW46	44-49	11/1/2001	1.06
U3D-MW46	49-54	11/1/2001	1.52
U3D-MW46	54-59	11/1/2001	NA
U3D-MW47	24-29	11/1/2001	0.35
U3D-MW47	29-34	11/1/2001	0.42
U3D-MW47	34-39	11/1/2001	0.35
U3D-MW47	39-44	11/1/2001	0.72
U3D-MW47	44-49	11/1/2001	0.58
U3D-MW47	49-54	11/1/2001	0.43
U3D-MW47	54-59	11/1/2001	0.95
U3D-MW48	24-29	11/1/2001	0.43
U3D-MW48	29-34	11/1/2001	0.67
U3D-MW48	34-39	11/1/2001	0.3
U3D-MW48	39-44	11/1/2001	1.24
U3D-MW48	44-49	11/1/2001	0.85
U3D-MW48	49-54	11/1/2001	4.96
U3D-MW48	54-59	11/1/2001	1.31
Average			0.86
Standard Deviation			0.92

NTC Orlando, FL (OU2)

Well ID	Date	Dissolved Oxygen (mg/L)	Hydrogen (nM)
OLD-OU2-07B	3/25/2004	0.25	
OLD-OU2-08B	3/25/2004	0.58	
OLD-OU2-35B	3/25/2004	0.36	
OLD-OU2-37B	3/25/2004	0.33	
OLD-OU2-18B	3/26/2004	0.87	6.50
OLD-OU2-21B	3/26/2004	0.28	6.80
OLD-OU2-27B	3/26/2004	0.33	6.30
OLD-OU2-28B	3/26/2004	0.60	
OLD-OU2-31B	3/26/2004	0.50	6.20
OLD-OU2-32B	3/26/2004	0.50	2.10
OLD-OU2-33B	3/26/2004	0.60	6.10
OLD-OU2-DP02B	3/26/2004	0.23	
OLD-OU2-09B	3/27/2004	0.90	
OLD-OU2-12B	3/27/2004	0.32	
OLD-OU2-13B	3/27/2004	0.50	
OLD-OU2-14B	3/27/2004	0.83	
OLD-OU2-17B	3/27/2004	0.40	
OLD-OU2-20B	3/27/2004	0.80	
OLD-OU2-DP01B	3/27/2004	0.26	
Average		0.50	5.67
Standard Deviation		0.22	1.76

NTC Orlando, FL (OU4)

Well ID	Date	Dissolved Oxygen (mg/L)
OLD-13-58D	7/25/2006	0.35
OLD-13-59D	7/25/2006	0.16
OLD-13-60D	7/25/2006	0.36
OLD-13-61D	7/25/2006	0.17
OLD-13-62D	7/26/2006	
OLD-13-63D	7/25/2006	0.12
OLD-13-64D	7/25/2006	0.14
OLD-13-65D	7/31/2006	1.14
OLD-13-67D	7/31/2006	0.16
OLD-13-68D	7/31/2006	1.86
OLD-13-68D	7/31/2006	1.21

OLD-13-68D	7/31/2006	0.28
OLD-13-70D	7/31/2006	0.74
OLD-13-69D	8/1/2006	0
OLD-13-69D	8/1/2006	1.25
OLD-13-69D	8/1/2006	0.48
OLD-13-66D	8/2/2006	0.01
OLD-13-66D	8/2/2006	0
OLD-13-66D	8/2/2006	0
OLD-13-66D	8/2/2006	0.09
OLD-13-66D	10/24/2006	0.24
OLD-13-66D	10/24/2006	0.4
OLD-13-66D	10/24/2006	1.02
OLD-13-58D	1/21/2008	0.26
OLD-13-59D	1/22/2008	0.26
OLD-13-60D	1/22/2008	0.77
OLD-13-61D	1/21/2008	0.29
OLD-13-62D	1/22/2008	0.26
OLD-13-63D	1/17/2008	0.14
OLD-13-64D	1/23/2008	0.24
Average		0.43
Standard Deviation		0.46

NUWC Keyport, WA (OU1)

Well ID	Date	Dissolved Oxygen (mg/L)	Hydrogen (nM)
MW 1-41	6/11/2001	0.3	2
MW 1-5	6/13/2001	0.3	0.8
P1-10	6/13/2001	0.2	2
MW 1-16	6/14/2001	0.2	1.7
MW 1-4	6/14/2001	0.5	2.05
P1-6	6/14/2001	0.2	1.8
P1-7	6/14/2001	0.2	0.2
P1-8	6/14/2001	0.1	0.7
P1-9	6/14/2001	0.1	6.7
MW 1-41	6/10/2002	0.8	2.2
P1-5	6/10/2002	0.1	1.7
P1-10	6/12/2002	0.1	0.3
MW 1-16	6/13/2002	0.9	6.1
MW 1-4	6/13/2002	0.1	2.4
MW 1-5	6/13/2002	0.5	3.4

P1-6	6/13/2002	0.1	1.6
P1-8	6/13/2002	0.3	0.6
P1-9	6/13/2002	0.6	
P1-7	6/14/2002	1.3	0.2
Average		0.36	2.03
Standard Deviation		0.33	1.81

NAS North Island, CA (Site 5-Unit 2)

Well ID	Date	Dissolved Oxygen (mg/L)
S5-MW-12	1/6/1998	0.12
S5-MW-14	1/6/1998	0.81
S5-MW-15	1/6/1998	0.30
S5-MW-10	1/7/1998	0.01
S5-MW-17	1/7/1998	0.39
S5-MW-20	1/7/1998	0.01
S5-MW-21	1/8/1998	0.59
S5-MW-12	4/14/1998	0.34
S5-MW-14	4/14/1998	0.81
S5-MW-15	4/15/1998	0.44
S5-MW-10	4/16/1998	0.01
S5-MW-17	4/16/1998	0.88
S5-MW-20	4/16/1998	0.06
S5-MW-21	4/16/1998	0.20
S5-MW-14	7/7/1998	0.57
S5-MW-15	7/8/1998	0.35
S5-MW-10	7/9/1998	0.15
S5-MW-12	7/9/1998	0.07
S5-MW-17	7/9/1998	0.48
S5-MW-20	7/9/1998	0.08
S5-MW-21	7/9/1998	0.28
Average		0.33
Standard Deviation		0.28

NSB Kings Bay, GA (Site 11)

Well ID	Date	Dissolved Oxygen (mg/L)	Hydrogen (nM)
KBA-11-13A	11/10/97	0.00	1.20
KBA-PS-3	11/10/97	0.00	1.38
KBA-11-13A	11/3/98	0.00	0.51
USGS-1	11/03/98	0.00	0.82
USGS-15	11/3/98	0.00	
USGS-2	11/3/98	0.00	
USGS-3	11/3/98	0.00	0.66
USGS-4	11/3/98	0.00	0.68
USGS-5	11/3/98	0.00	0.88
USGS-6	11/3/98	0.00	1.15
USGS-7	11/3/98	0.00	1.55
Average		0.00	0.98
Standard Deviation		0.00	0.36

Beale AFB, CA (Site 10)

Well ID	Date	Dissolved Oxygen (mg/L)
10M005MW	8/25/2008	7.57
10C028MW	8/25/2008	4.57
10C003MW	8/25/2008	8.17
10M007MW	8/25/2008	8.23
10C035RW	8/26/2008	5.36
10C055RW	8/27/2008	4.11
10C051RW	8/27/2008	0.15
10C054RW	8/27/2008	0
10M004MW	8/28/2008	0.92
10C029MW	8/25/2008	8.91
10C027MW	8/25/2008	8.59
10R003MW	8/25/2008	8.57
10R001MW	8/26/2008	0.82
10C037RW	8/26/2008	15.5
10C049MW	8/26/2008	0
10C036RW	8/27/2008	6.68
10C025MW	9/2/2008	1.98
10C026MW	9/2/2008	0.78
10C033RW	9/2/2008	8

10C040RW	9/2/2008	6.99
10C045RW	9/2/2008	5.75
10C047MW	9/2/2008	0.7
10C048MW	9/2/2008	0.79
10C050RW	9/2/2008	0.82
10L001MW	9/2/2008	6.08
10C025MW	11/17/2008	0
10C028MW	11/17/2008	0
10C003MW	11/17/2008	7.66
10C027MW	11/17/2008	8.17
10C035RW	11/18/2008	5.89
10M004MW	11/18/2008	0
10M005MW	11/18/2008	7.78
10M007MW	11/18/2008	7.39
10R003MW	11/18/2008	9.91
10C051RW	11/19/2008	0
10C026MW	11/17/2008	0
10C049MW	11/18/2008	0
10C047MW	11/19/2008	0
10C048MW	11/19/2008	0
10C050RW	11/19/2008	0
10C055RW	11/19/2008	0
10C054RW	11/19/2008	0
Average		6.42
Standard Deviation		0.83

Table 4-4. Hyperbolic and linear regression fit data for PBOC in aquifer sediments and groundwater parameters for selected study sites.

Dissolved Oxygen (mg/L) versus PBOC (mg/kg)

Nonlinear Regression

Data Source: DO versus PBOC

Equation: Hyperbola, Hyperbolic Decay, 2 Parameter
f=(a*b)/(b+x)

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9808	0.9621	0.9583	0.3976

	Coefficient	Std. Error	t	P
a	19.7790	8.0807	2.4477	0.0344
b	6.1245	3.4127	1.7946	0.1030

Analysis of Variance:

	DF	SS	MS
Regression	2	59.5386	29.7693
Residual	10	1.5808	0.1581
Total	12	61.1194	5.0933

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	1	40.0882	40.0882	253.5973	<0.0001
Residual	10	1.5808	0.1581		
Total	11	41.6689	3.7881		

Statistical Tests:

Normality Test (Shapiro-Wilk) Passed (P = 0.5360)

W Statistic= 0.9429 Significance Level = 0.0500

Constant Variance Test Passed (P = 0.7160)

Fit Equation Description:
[Variables]
x = col(1)
y = col(2)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
[Parameters]
a = max(y) ''Auto {{previous: 19.779}}
b = x50(x,y,0.1) ''Auto {{previous: 6.12446}}
[Equation]
f=(a*b)/(b+x)
fit f to y
''fit f to y with weight reciprocal_y
''fit f to y with weight reciprocal_ysquare
[Constraints]
[Options]
tolerance=1e-10
stepsize=1
iterations=200

Number of Iterations Performed = 10

Hydrogen (nM) versus PBOC (mg/kg)

Linear Regression
Data source: Hydrogen versus PBOC

Col 2 = -0.379 + (0.00511 * Col 1)

N = 5

R = 0.789 Rsqr = 0.622 Adj Rsqr = 0.496

Standard Error of Estimate = 1.375

	Coefficient	Std. Error	t	P
Constant	-0.379	1.369	-0.277	0.800
Col 1	0.00511	0.00230	2.222	0.113

Analysis of Variance:

	DF	SS	MS	F	P
Regression	1	9.342	9.342	4.939	0.113

Residual	3	5.675	1.892
Total	4	15.016	3.754

Normality Test (Shapiro-Wilk) Passed (P = 0.518)

Constant Variance Test: Passed (P = 0.050)

Power of performed test with alpha = 0.050: 0.327

The power of the performed test (0.327) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.

Figure 4-3. Boxplots of the natural attenuation capacity (NAC) for selected study sites displaying minimal, moderate, and high reductive dechlorination activity. Boxes represent the 25-75% quartile range for NAC values. Center lines and whiskers indicate median values and data ranges for the NAC, respectively.

Natural Attenuation Capacity (NAC) and Reductive Dechlorination Activity

Facility	Site	NAC (ft^{-1})	Reductive Dechlorination
Hill AFB, UT	OU2	0.00135	Minimal
NAES Lakehurst, NJ	Sites I & J	0.000949	Minimal
Beale AFB, CA	Site 10	0.002470	Minimal
MCRD Parris Island, SC	Site 45	0.006040	Moderate
NAS Pensacola, FL	WWTP	0.01270	Moderate
NAB Little Creek, VA	Site 12	0.01320	Moderate
NAS North Island, CA	Site 5-Unit 2	0.00970	Moderate/High
NTC Orlando, FL	OU4	0.02270	Moderate/High
NUWC Keyport, WA	OU1	0.00861	High
NSB Kings Bay, GA	Site 11	0.0120	High

NAC versus Reductive Dechlorination Activity

One Way Analysis of Variance

Data source: NAC versus Reductive Dechlorination Activity

Normality Test (Shapiro-Wilk) Passed (P = 0.208)

Equal Variance Test: Passed (P = 0.700)

Group Name	N	Missing	Mean	Std Dev	SEM
Col 1	3	0	0.00159	0.000788	0.000455
Col 2	3	0	0.0106	0.00400	0.00231
Col 3	4	0	0.0133	0.00645	0.00323

Source of Variation	DF	SS	MS	F	P
Between Groups	2	0.000245	0.000123	5.425	0.038
Residual	7	0.000158	0.0000226		
Total	9	0.000403			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.038).

Power of performed test with alpha = 0.050: 0.558

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor:

Comparison	Diff of Means	t	P	P<0.050
Col 3 vs. Col 1	0.0117	3.212	0.044	Yes
Col 2 vs. Col 1	0.00906	2.333	0.102	No
Col 3 vs. Col 2	0.00261	0.718	0.496	No

Figure 4-4. NAC for total chloroethenes (ft^{-1}) versus PBOC (mg/kg) for selected study sites.

Facility	Site	PBOC (mg/kg)	NAC (ft^{-1})
Hill AFB, UT	OU2	30.4±2.69	0.00135
NAES Lakehurst, NJ	Sites I & J	70.9	0.000949
Beale AFB, CA	Site 10	12.5±9.4	0.002470
MCRD Parris Island, SC	Site 45	211.0±9.6	0.006040
NAS Pensacola, FL	WWTP	306.8	0.01270
NAB Little Creek, VA	Site 12	266.0	0.01320
NAS North Island, CA	Site 5-Unit 2	237.6±25.7	0.00970
NTC Orlando, FL	OU4	1269.1±139.0	0.02270
NUWC Keyport, WA	OU1	635.0	0.00861
NSB Kings Bay, GA	Site 11	534.1	0.0120

Appendix C. Chapter 5

Figure 5-3. Frequency (f) vs ln PBOC for selected sampling locations at MCRD Site 45.

Figure 5-4. A) PBOC (mg/kg) versus solid-phase TOC_s (mg/kg) for selected samples collected within the surficial aquifer and confining unit at MCRD Site 45. B) PBOC (mg/kg) versus solid-phase TOC_s (mg/kg) for surficial upper and lower aquifer sediments at MCRD Site 45.

Data for Figure 5-3 and Figure 5-4 come from the following tables.

Depth Interval (ft)	Boring Location	PBOC (mg/kg)	Standard Deviation	Solid-phase, TOC_s (mg/kg)	Standard Deviation	ln PBOC (mg/kg)
7.0-8.0	MCRD Site 45-Boring 1	112.7	9.0	2300.0	254.6	4.7
8.0-9.0	MCRD Site 45-Boring 1	426.7	30.0	4195.0	304.1	6.1
10.0-11.0	MCRD Site 45-Boring 1	85.3	12.8	1130.0	56.6	4.4
13.0-14.0	MCRD Site 45-Boring 1	54.8	12.4	725.0	77.8	4.0
14.0-15.0	MCRD Site 45-Boring 1	250.6	38.2	2230.0	84.9	5.5
16.0-17.0	MCRD Site 45-Boring 1	63.9	8.5	815.0	49.5	4.2
18.0-19.0	MCRD Site 45-Boring 1	958.4	49.9	12800.0	848.5	6.9
7.0-8.0	MCRD Site 45-Boring 2	223.7	20.7	1120.0	70.7	5.4
9.0-10.0	MCRD Site 45-Boring 2	111.0	17.6	647.5	20.5	4.7
11.0-12.0	MCRD Site 45-Boring 2	469.7	105.4	589.5	31.8	6.2
13.0-14.0	MCRD Site 45-Boring 2	128.8	53.5	1525.0	77.8	4.9
15.0-16.0	MCRD Site 45-Boring 2	233.3	41.5	1680.0	42.4	5.5
18.0-19.0	MCRD Site 45-Boring 2	39.3	13.4	387.5	378.3	3.7
21.0-22.0	MCRD Site 45-Boring 2	582.1	83.8	12295.0	1067.7	6.4
7.0-8.0	MCRD Site 45-Boring 3	157.6	19.7	3335.0	1110.2	5.1
9.0-10.0	MCRD Site 45-Boring 3	161.1	19.7	2980.0	212.1	5.1
11.0-12.0	MCRD Site 45-Boring 3	17.5	4.0	383.0	18.4	2.9
13.0-14.0	MCRD Site 45-Boring 3	192.3	20.4	2200.0	198.0	5.3
14.0-15.0	MCRD Site 45-Boring 3	25.4	4.1	412.5	33.2	3.2
18.0-19.0	MCRD Site 45-Boring 3	192.2	14.3	2120.0	396.0	5.3
19.0-20.0	MCRD Site 45-Boring 3	1349.3	112.4	66375.0	9015.6	7.2
7.0-8.0	MCRD Site 45-Boring 4	313.5	33.6	1965.0	134.4	5.7

10.0-11.0	MCRD Site 45-Boring 4	54.0	8.0	871.5	6.4	4.0
11.0-12.0	MCRD Site 45-Boring 4	450.0	32.0	2840.0	99.0	6.1
13.0-14.0	MCRD Site 45-Boring 4	397.0	8.6	3930.0	353.6	6.0
15.0-16.0	MCRD Site 45-Boring 4	184.6	19.3	1400.0	127.3	5.2
16.0-17.0	MCRD Site 45-Boring 4	147.9	22.3	2335.0	233.3	5.0
18.0-19.0	MCRD Site 45-Boring 4	354.0	27.0	1151.4	521.3	5.9
19.0-20.0	MCRD Site 45-Boring 4	2531.6	304.8	79830.0	36981.7	7.8
7.0-8.0	MCRD Site 45-Boring 5	188.2	39.7	1245.0	21.2	5.2
10.0-11.0	MCRD Site 45-Boring 5	26.3	5.3	271.0	43.8	3.3
11.0-12.0	MCRD Site 45-Boring 5	320.7	37.9	2295.0	120.2	5.8
12.0-13.0	MCRD Site 45-Boring 5	504.4	39.8	5470.0	297.0	6.2
15.0-16.0	MCRD Site 45-Boring 5	183.2	1.7	1240.0	70.7	5.2
17.0-18.0	MCRD Site 45-Boring 5	40.3	6.5	275.0	21.2	3.7
19.0-20.0	MCRD Site 45-Boring 5	2580.5	233.9	72415.0	18886.8	7.9
7.0-8.0	MCRD Site 45-Boring 6	116.6	40.4	1270.0	113.1	4.8
9.0-10.0	MCRD Site 45-Boring 6	69.3	29.3	1130.0	70.7	4.2
11.0-12.0	MCRD Site 45-Boring 6	98.7	10.7	1420.0	56.6	4.6
13.0-14.0	MCRD Site 45-Boring 6	162.7	43.9	1735.0	49.5	5.1
15.0-16.0	MCRD Site 45-Boring 6	258.9	27.4	2405.0	431.3	5.6
17.0-18.0	MCRD Site 45-Boring 6	87.3	7.9	1390.0	70.7	4.5
19.0-20.0	MCRD Site 45-Boring 6	603.3	79.1	11045.0	1492.0	6.4
20.0	MCRD Site 45-Boring 7	754.9	163.2	76245.0	8025.7	6.6
7.0-8.0	MCRD Site 45-Boring 8	174.2	16.6			5.2
9.0-10.0	MCRD Site 45-Boring 8	251.5	6.4			5.5
11.0-12.0	MCRD Site 45-Boring 8	46.0	6.7			3.8
13.0-14.0	MCRD Site 45-Boring 8	283.8	36.3			5.6
15-16.0	MCRD Site 45-Boring 8	1911.7	28.1			7.6
7.0-8.0	MCRD Site 45-Boring 9	140.8	17.6			4.9
10.0-11.0	MCRD Site 45-Boring 9	20.3	2.4			3.0
11.0-12.0	MCRD Site 45-Boring 9	27.6	2.4			3.3
14.0-15.0	MCRD Site 45-Boring 9	194.2	17.3			5.3
19.0-20.0	MCRD Site 45-Boring 9	943.0	123.4			6.8
7.0-8.0	MCRD Site 45-Boring 10	205.0	11.1	1975.0	247.5	5.3
8.0-9.0	MCRD Site 45-Boring 10	143.0	12.6	1375.0	63.6	5.0
10.0-11.0	MCRD Site 45-Boring 10	21.2	6.2	404.5	70.0	3.1

11.0-12.0	MCRD Site 45-Boring 10	253.4	11.5	1745.0	91.9	5.5
14.0-15.0	MCRD Site 45-Boring 10	38.1	2.5	390.5	0.7	3.6
19.0-20.0	MCRD Site 45-Boring 10	160.0	23.9	1330.0	226.3	5.1
23.0-24.0	MCRD Site 45-Boring 10	142.0	9.7	1300.0	396.0	5.0
16.0-17.0	MCRD Site 45-Boring 10	593.5	153.0	3290.0	28.3	6.4
21.0-22.0	MCRD Site 45-Boring 10	996.0	72.2	9900.0	1145.5	6.9
7.0-8.0	MCRD Site 45-Boring 11	190.9	6.8			5.3
9.0-10.0	MCRD Site 45-Boring 11	315.4	40.9			5.8
15.0-16.0	MCRD Site 45-Boring 11	206.8	25.1			5.3
17.0-18.0	MCRD Site 45-Boring 11	42.4	3.9			3.7
19.0-20.0	MCRD Site 45-Boring 11	1916.8	150.4			7.6
9.0-10.0	MCRD Site 45-Boring 12	536.5	33.1	4635.0	982.9	6.3
11.0-12.0	MCRD Site 45-Boring 12	70.7	1.6	596.5	65.8	4.3
13.0-14.0	MCRD Site 45-Boring 12	237.8	28.6	1600.0	212.1	5.5
15.0-16.0	MCRD Site 45-Boring 12	3321.1	433.8	152525.0	6583.2	8.1

Table 5-1. Summary of groundwater chloroethene concentrations for selected temporary monitoring wells at MCRD Site 45 (provided by Vroblesky et al. 2009).

Temporary Monitoring Well (TW)	Sampling Date	Screen Interval (ft)	PCE (µg/L)	TCE (µg/L)	cis-1,2-DCE (µg/L)	VC (µg/L)	Total Chloroethenes (µg/L)
PAI-45-USGS-TW94	3/5/2008	7-11	331	863	254	0.3	1448.3
PAI-45-USGS-TW39	6/27/2007	10-14	5240	7600	628	50	13518
PAI-45-USGS-TW83	3/5/2008	11-15	29.2	415	55	0.3	499.5
PAI-45-USGS-TW70	6/28/2007	11-15	0.25	0.25	4.21	0.5	5.21

Figure 5-5. A) Chlororethenes (ug/L) in selected temporary wells versus PBOC (mg/kg) in aquifer sediments collected from Boring 3 at MCRD Site 45. B) Depth (ft) versus PBOC (mg/kg) in aquifer sediments collected from Boring 3 (inside chloroethene plume) and Boring 4 (outside chloroethene plume) at MCRD Sites 45.

Chlororethenes (ug/L) versus PBOC (mg/kg)

Linear Regression

Data source: Chloroethenes versus PBOC

Col 1 = 14643.247 - (75.985 * Col 2)

N = 4

R = 0.993 Rsqr = 0.987 Adj Rsqr = 0.980

Standard Error of Estimate = 898.019

	Coefficient	Std. Error	t	P
Constant	14643.247	981.648	14.917	0.004
Col 2	-75.985	6.219	-12.219	0.007

Analysis of Variance:

	DF	SS	MS	F	P
Regression	1	120399943.959	120399943.959	149.298	0.007
Residual	2	1612878.021	806439.011		
Total	3	122012821.980	40670940.660		

Normality Test (Shapiro-Wilk) Failed (P = 0.043)

Constant Variance Test: Failed (P = <0.001)

Power of performed test with alpha = 0.050: 0.814

Depth (ft) versus PBOC (mg/kg) in aquifer sediments collected from Boring 3 (inside chloroethene plume) and Boring 4 (outside chloroethene plume) at MCRD Sites 45.

Depth Interval	Boring Location	PBOC	Standard Deviation	Solid-phase, TOC_s	Standard Deviation
		(mg/kg)		(mg/kg)	
7.0-8.0	MCRD Site 45 Boring 3	157.6	83.8	3335.0	1067.7
9.0-10.0	MCRD Site 45 Boring 3	161.1	19.7	2980.0	1110.2
11.0-12.0	MCRD Site 45 Boring 3	17.5	19.7	383.0	212.1
13.0-14.0	MCRD Site 45 Boring 3	192.3	4.0	2200.0	18.4
14.0-15.0	MCRD Site 45 Boring 3	25.4	20.4	412.5	198.0
18.0-19.0	MCRD Site 45 Boring 3	192.2	4.1	2120.0	33.2
7.0-8.0	MCRD Site 45 Boring 4	313.5	14.3	1965.0	396.0
11.0-12.0	MCRD Site 45 Boring 4	450.0	8.0	2840.0	6.4
10.0-11.0	MCRD Site 45 Boring 4	54.0	32.0	871.5	99.0
13.0-14.0	MCRD Site 45 Boring 4	397.0	8.6	3930.0	353.6
15.0-16.0	MCRD Site 45 Boring 4	184.6	19.3	1400.0	127.3
16.0-17.0	MCRD Site 45 Boring 4	147.9	22.3	2335.0	233.3
18.0-19.0	MCRD Site 45 Boring 4	354.0	27.0	1151.4	521.3

Depth (ft) versus PBOC (mg/kg) in aquifer sediments collected from Boring 3 and Boring 4 at MCRD Sites 45.

t-test

Data source: Depth versus PBOC

Normality Test (Shapiro-Wilk) Passed (P = 0.548)

Equal Variance Test: Passed (P = 0.119)

Group Name	N	Missing	Mean	Std Dev	SEM
Col 1	6	0	124.356	81.102	33.110
Col 2	8	0	320.578	192.980	68.229

Difference -196.222

t = -2.323 with 12 degrees of freedom. (P = 0.039)

95 percent confidence interval for difference of means: -380.271 to -12.173

The difference in the mean values of the two groups is greater than would be expected by chance; there is a statistically significant difference between the input groups (P = 0.039).

Power of performed test with alpha = 0.050: 0.480

Figure 5-6. Summary of PBOC (mg/kg) for surficial aquifer sediment samples collected inside and outside of the chloroethene plume at MCRD Site 45. Standard deviations for PBOC concentrations are shown with error bars.

Sampling Zone	PBOC (mg/kg)	PBOC (mg/kg)
	Inside Plume	Outside Plume
Surficial Upper (SU)	161.8±51.0	315.9±153.5
Surficial Lower (SL)	140.2±148.9	199.0±145.2

PBOC (mg/kg) for surficial upper aquifer sediment samples collected inside and outside of the chloroethene plume at MCRD Site 45

Data source: Inside versus Outside for Surficial Upper

Normality Test (Shapiro-Wilk) Passed (P = 0.669)

Equal Variance Test: Failed (P < 0.050)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test

Data source: Inside vs Outside for Surficial Upper

Group	N	Missing	Median	25%	75%
Col 1	12	0	159.352	122.627	200.823
Col 2	6	0	314.410	171.328	454.142

Mann-Whitney U Statistic= 13.000

T = 80.000 n(small)= 6 n(big)= 12 (P = 0.035)

The difference in the median values between the two groups is greater than would be expected by chance; there is a statistically significant difference (P = 0.035)

PBOC (mg/kg) for surficial lower aquifer sediment samples collected inside and outside of the chloroethene plume at MCRD Site 45

Data source: Inside versus Outside for Surficial Lower

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test

Data source: Inside vs Outside for Surficial Lower

Group	N	Missing	Median	25%	75%
Col 4	21	0	87.285	26.953	223.796
Col 5	8	0	195.692	90.006	357.216

Mann-Whitney U Statistic= 51.500

T = 152.500 n(small)= 8 n(big)= 21 (P = 0.118)

The difference in the median values between the two groups is not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.118)

Figure 5-7. PBOC (mg/kg) versus Time (days) for selected samples collected within the surficial aquifer at MCRD Site 45-Boring 1. For (A) Surficial Upper (8-9 ft) and (B) Surficial Lower (14-15 ft) each graph illustrates the PBOC levels with respect to PCE exposure over time. Standard deviations for PBOC are shown with error bars.

Figure 5-8. Extracted PBOC (mg/kg) versus Time (days) for selected samples collected within the surficial aquifer at MCRD Site 45-Boring 1. For (A) Surficial Upper (8-9 ft) and (B) Surficial Lower (14-15 ft) each graph illustrates the extracted PBOC levels with respect to PCE exposure over time. Standard deviations for extracted carbon are shown with error bars.

Data for Figure 5-7 and Figure 5-8 come from the following tables.

Surficial Upper (8-9 ft)- Boring 1 at MCRD Site 45

Time, days	Pyro Extractions (mg/kg)	Standard Deviation	Alkali Extractions (mg/kg)	Standard Deviation	PBOC (mg/kg)	Standard Deviation
(Background) 0	283.6	6.8	143.1	15.7	426.7	30.0
1	203.0	8.6	108.6	37.3	311.6	87.4
3	190.1	13.7	86.6	10.1	276.7	28.2
(Background) 10	285.4	31.4	158.3	4.2	443.7	27.3

Surficial Lower (14-15 ft)- Boring 1 at MCRD Site 45

Time, days	Pyro Extractions (mg/kg)	Standard Deviation	Alkali Extractions (mg/kg)	Standard Deviation	PBOC (mg/kg)	Standard Deviation
(Background) 0	31.6	3.3	219.0	21.2	250.6	38.2
1	21.3	2.7	55.5	4.1	76.8	13.0
3	26.3	2.2	40.8	1.6	67.2	1.6
(Background) 10	123.0	7.2	136.2	6.5	259.2	13.6

Surficial Upper (8-9 ft)- Boring 1 at MCRD Site 45

Paired t-test:

Data source: Surficial Upper-Boring 1

Normality Test (Shapiro-Wilk) Passed (P = 0.935)

Treatment Name	N	Missing	Mean	Std Dev	SEM
Col 1	3	0	426.698	29.987	17.313
Col 2	3	0	276.728	28.156	16.256
Difference	3	0	149.970	57.743	33.338

t = 4.498 with 2 degrees of freedom. (P = 0.046)

95 percent confidence interval for difference of means: 6.528 to 293.411
The change that occurred with the treatment is greater than would be expected by chance; there is a statistically significant change (P = 0.046)

Power of performed test with alpha = 0.050: 0.628

Surficial Lower (14-15 ft)- Boring 1 at MCRD Site 45

Paired t-test:

Data source: Surficial Lower-Boring 1

Normality Test (Shapiro-Wilk) Passed (P = 0.463)

Treatment Name	N	Missing	Mean	Std Dev	SEM
Col 1	3	0	250.604	38.183	22.045
Col 2	3	0	67.157	1.568	0.905
Difference	3	0	183.447	37.156	21.452

t = 8.551 with 2 degrees of freedom. (P = 0.013)

95 percent confidence interval for difference of means: 91.146 to 275.749
The change that occurred with the treatment is greater than would be expected by chance; there is a statistically significant change (P = 0.013)

Power of performed test with alpha = 0.050: 0.973

Figure 5-9. Summary of anaerobic bioassay results for surficial aquifer sediments collected at MCRD Parris Island Site 45- Boring 4 and positive yeast control. For (A) PCE (µM) with respect to time (days) and (B) *cis*-DCE with respect to time (days) each graph illustrates the chloroethene levels over time in sediment samples collected in the surficial upper (7-8 ft) and lower (11-12 ft) aquifer. Figure C shows chloroethenes (µM) with respect to time (days) for positive control (1.0% yeast extract). Standard deviations for chloroethene concentrations are shown with error bars.

Surficial Upper (7-8 ft)- Boring 4 at MCRD Site 45

Time, days	PCE Concentration (µM)	Standard Deviation	cis-DCE Concentration (µM)	Standard Deviation
0	9.3	0.62	0.00	0.00
30	4.8	0.66	0.26	0.27

Surficial Lower (11-12 ft)- Boring 4 at MCRD Site 45

Time, days	PCE Concentration (µM)	Standard Deviation	cis-DCE Concentration (µM)	Standard Deviation
0	9.3	0.62	0.00	0.00
30	3.3	0.55	0.10	0.01

Positive Control (Yeast Extract 1.0%)

Time, days	PCE Concentration (µM)	Standard Deviation	cis-DCE Concentration (µM)	Standard Deviation
0	9.3	0.62	0.00	0.00
30	0.46	0.05	4.9	0.71

Cis-DCE Production in Surficial Upper (7-8 ft) and Lower (11-12 ft) Aquifer Sediments

t-test

Data source: Cis-DCE Production in Surficial Upper and Lower Aquifer Sediments

Normality Test (Shapiro-Wilk) Passed (P = 0.111)

Equal Variance Test: Passed (P = 0.420)

Group Name	N	Missing	Mean	Std Dev	SEM
Col 1	3	0	0.262	0.271	0.156
Col 2	3	0	0.0989	0.00674	0.00389

Difference 0.163

t = 1.045 with 4 degrees of freedom. (P = 0.355)

95 percent confidence interval for difference of means: -0.271 to 0.598

The difference in the mean values of the two groups is not great enough to reject the possibility that the difference is due to random sampling variability. There is not a statistically significant difference between the input groups (P = 0.355).

Power of performed test with alpha = 0.050: 0.057

The power of the performed test (0.057) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists. Negative results should be interpreted cautiously.