THE ABIOTIC TRANSFORMATION OF NITROAROMATIC PESTICIDES BY
FE(II) AND DISSOLVED ORGANIC MATTER

DISSERTATION

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By

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ABSTRACT

Nitroaromatic pesticides are hydrophobic contaminants that can accumulate in sediments by the deposition of suspended solids from surface waters. Fe(II) and dissolved organic matter (DOM), present in suboxic and anoxic zones of freshwater sediments, can transform nitroaromatic pesticides in natural systems. In this study, the abiotic chemical transformation of pentachloronitrobenzene, trifluralin and pendimethalin was studied in controlled laboratory systems containing Fe(II) and fulvic acid DOM isolates, and in natural pore waters collected from a freshwater wetland. Geochemical parameters affecting reactivity in the natural wetland were also monitored.

Rapid reduction of pentachloronitrobenzene to pentachloroaniline was observed in controlled systems in the presence of Fe(II) and DOM fulvic acid isolates from surface waters (pseudo-first-order half-life, \( t_{1/2} \approx 30 \) min to 4 h). DOM in unfiltered systems inhibited iron colloid formation and possibly limited the formation of reactive Fe(II)-iron colloid surface complexes, causing reductive transformation in Fe(II)-DOM media to be slower in some cases relative to Fe(II)-only controls. Conversely, in 0.45-µm-filtered solutions pentachloronitrobenzene reduction in Fe(II)-DOM media was faster than the Fe(II)-only controls, suggesting that DOM enhances the reductive capacity of Fe(II) in the absence of iron colloids.
Differential pulse polarography (DPP) scans of natural wetland pore waters collected from Old Woman Creek (OWC; located in northern Ohio) revealed that a variety of redox-active metals exist naturally in OWC pore waters. Fe(III)-organic and Fe(II) species increased to a depth of ~30 cm from the sediment-water interface, and a maximum for dissolved Mn(II) was observed at ~6 cm depth. Dissolved Fe(II) was necessary for rapid pentachloronitrobenzene reduction in natural pore water (< 24 hr), and faster reduction was observed with increased pore water pH. Pentachloronitrobenzene reduction in “pH-adjusted” pore waters (acidified to pH 2.5 after pore water extraction and raised to the native pH (between 6.7 to 7.6) prior to reaction) was similar to that observed in a model system containing Fe(II) and an aquatic fulvic acid isolate. Conversely, pentachloronitrobenzene reduction in fresh, unaltered pore water was slower than that observed in “pH-adjusted” pore water. This indicated that the Fe(II) speciation and reductive capacity differs between unaltered and “pH-adjusted” samples due to a rearrangement of the naturally-occurring Fe complexes with pH-adjustment.

Trifluralin and pendimethalin reduction occurred in controlled systems containing Fe(II) and DOM surface water isolates, and in natural pore waters collected from OWC. Dissolved Fe(II) was necessary for trifluralin and pendimethalin reduction to occur, and pendimethalin reduction was faster in solutions containing Fe(II) and Suwannee River, Georgia, fulvic acid isolate relative to reactions containing Fe(II) and Pony Lake, Antarctica, fulvic acid isolate. DOM source material did not affect reactivity for trifluralin in similar systems. The reduction rate increased with increased pH for both compounds. Natural pore waters reduced both trifluralin and pendimethalin, and
trifluralin degraded to multiple byproducts while pendimethalin only degraded to one major byproduct. Comparison of pseudo-first-order rate constants between controlled systems containing Fe(II) and OWC fulvic acid isolate, and natural sediment pore waters collected from OWC, showed that trifluralin and pendimethalin reduction in controlled systems was an order of magnitude faster relative to natural pore waters.

This study is the first to investigate nitroaromatic pesticide reduction in the presence of Fe(II) and DOM surface water isolates, and in natural benthic pore waters that contain high concentrations of dissolved Fe(II) and DOM. Although pentachloronitrobenzene, trifluralin and pendimethalin were reduced both in controlled systems and natural pore waters, reduction in unaltered pore waters was roughly an order of magnitude slower than in systems containing Fe(II) and fulvic acid isolates. These data show that controlled systems over-estimate nitroaromatic pesticide reactivity in natural systems, and also suggest that Fe(II) is naturally complexed to Fe(II)-stabilizing ligands in anoxic environments. Future work is necessary to elucidate the speciation of Fe(II) complexes present in natural anoxic environments.
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CHAPTER 1

INTRODUCTION

1.1 Introduction

Nitroaromatic pesticides are hydrophobic organic compounds applied throughout the United States as pre-emergent treatments to prevent weed and fungus growth on a variety of agricultural crops (Grover et al., 1997; Sprankle, 1974). When not retained in agricultural soils, nitroaromatic pesticides can be transported throughout agricultural watersheds and deposited in various sedimentary environments. The environmental fate of nitroaromatic pesticides is of particular interest because many of these compounds are suspected carcinogens and liver toxicants (USEPA, 2007; USEPA, 1997) that may have deleterious effects on aquatic ecosystems (USEPA, 2004).

Post-application water and atmospheric transport of nitroaromatic pesticides have been studied by a number of investigators (Hojberg et al., 2005; Grover et al., 1997). Many of these studies focused upon batch experiments to assess sorption to agricultural soils, and soil microcosm studies to assess the ability for degradation by aerobic microorganisms (Grover et al., 1997). Although the physical environmental fate of
nitroaromatic pesticides is generally understood, the chemical fate of these compounds in sedimentary environments remains unclear.

Studies with model nitroaromatic compounds of simple structure have been conducted in order to elucidate specific reduction mechanisms affecting the fate of nitroaromatic explosives in anoxic environments. Chemical reduction of nitroaromatics to the corresponding aniline reduction products was observed in contaminated aquifers (Weissmahr et al., 1999; Hofstetter et al., 1999), and reduction of nitroaromatic probe compounds was observed in an anoxic aquifer contaminated with a landfill leachate plume (Rügge et al., 1998). Dissolved Fe(II) was found necessary for nitroaromatic reduction to occur in batch sediment slurries (Hoferkamp and Weber, 2006), and reduction by reactive Fe(II)-iron mineral species in an anoxic sediment column was linked to the production of dissolved Fe(II) by anaerobic bacteria (Heijman et al., 1995). Reactive reductants present naturally in anoxic sediment and groundwater systems are capable of degrading nitroaromatics, and although microbial metabolic byproducts serve as reactive reductants in these systems, the reduction of nitro moieties was found to occur abiotically (Heijman et al., 1995).

To date, mechanistic investigations into nitroaromatic reduction kinetics have focused upon a variety of model reductants believed to represent the reactive abiotic species present in natural anoxic environments. These reductants include electrochemically-reduced hydroquinones (Tratnyek and Macalady, 1989), HS\(^{-}\) and juglone (a hydroquinone) (Schwarzenbach et al., 1990), cysteine and iron porphyrin (Schwarzenbach et al., 1990), HS\(^{-}\) and DOM (Dunnivant et al., 1992), dissolved Fe(II) in the presence of iron minerals and clays (Hofstetter et al., 2006; Elsner et al., 2004;
Schultz and Grundl, 2000; Klausen et al., 1995) and Fe(II) in the presence of synthetic organic molecules such as catechols and thiols (Naka et al., 2006). Although Fe(II) and DOM are found to co-exist in high concentrations in some sediment pore water environments (O’Loughlin and Chin, 2004; Chin et al., 1998; Chin and Gschwend, 1991), the specific reductive capacity of Fe(II) in the presence of DOM has not been investigated.

Nitroaromatic pesticides that are sorbed to sediment particles and transported throughout agricultural watersheds eventually may settle in natural wetlands. Wetlands with large surface areas can slow surface water flow from incoming rivers and streams, and submerged vegetation can trap suspended particles (Wilson et al., 2002). Both processes can cause suspended solids to settle in the wetland sediments (Wilson et al., 2002). Some freshwater wetland sediment pore waters have been found to contain high concentrations of dissolved Fe(II) and DOM to depths of ~30 cm from the sediment-water interface (O’Loughlin and Chin, 2004; Chin et al., 1998; Chin and Gschwend, 1991). An understanding of nitroaromatic pesticide reactivity in natural pore waters containing high concentrations of dissolved Fe(II) and DOM is an important addition to the overall understanding of pesticide fate in natural systems, as these environments contain potentially reactive abiotic reductants that can degrade nitroaromatic pesticides in situ.

Pentachloronitrobenzene is a fungicide whose primary agricultural use is on cotton crops (72% of total agricultural use in 2002), and trifluralin and pendimethalin are herbicides primarily used on soybeans and cotton (combined 64% and 60%, respectively, of total agricultural use in 2002) (Figure 1.1) (USGS, 2002a,b,c). All compounds are used
in agricultural zones throughout the United States that drain into larger wetland zones with potentially high levels of dissolved Fe(II) and DOM in sedimentary pore waters.

This study focused upon the abiotic reduction of these three nitroaromatic pesticides in controlled systems containing dissolved Fe(II) and DOM isolates from surface waters, and in natural sediment pore waters collected from a temperate freshwater wetland in northern Ohio. *I hypothesize that abiotic reduction of pentachloronitrobenzene, trifluralin and pendimethalin is possible in controlled systems containing both Fe(II) and DOM, and that the three pesticides can be degraded in natural pore waters.* I tested this hypothesis with the following objectives:

- Modeling of pentachloronitrobenzene reduction kinetics in controlled systems containing Fe(II) and surface water fulvic acid isolates;
- Characterization of sediment pore water redox geochemistry from a temperate freshwater wetland;
- Modeling of pentachloronitrobenzene reduction kinetics in sediment pore waters collected from a temperate freshwater wetland; and,
- Modeling of trifluralin and pendimethalin reduction kinetics in controlled systems and natural pore waters.

The first objective was addressed through a series of kinetic reactions in controlled laboratory solutions prepared under anoxic conditions, where buffered solutions containing Fe(II) and DOM (as surface water fulvic acid isolates) were reacted with pentachloronitrobenzene. Reactions in both unfiltered and 0.45 µm filtered systems were compared to understand the effects of trace colloids on reduction kinetics, and reactions containing both dissolved Fe(II) and DOM were compared with reactions that
only contained Fe(II) to assess the effect of DOM on the Fe(II)-mediated reduction of pentachloronitrobenzene.

The second objective was addressed by collecting sediment cores from Old Woman Creek, a freshwater wetland located in northern Ohio adjacent to Lake Erie. Pore waters were extracted from sediment cores, and depth profiles of pore water redox geochemistry were generated using differential pulse polarography (DPP). Pore waters were subjected to a variety of treatments (Na⁺-cation exchange; reaction with sodium dithionite) to probe the nature of redox-active species observed in the DPP scans. The pH, dissolved Fe(II), dissolved organic carbon (DOC; used as a proxy for DOM in natural systems), total Fe, total Mn, and specific conductivity also were measured to understand OWC pore water geochemistry. Results from these analyses were used to interpret pore water reduction kinetics studies performed with pentachloronitrobenzene.

The third objective was addressed by reacting pentachloronitrobenzene with both altered and unaltered OWC pore waters. For all experiments, pore waters were combined according to Fe(II) concentrations. In some cases, the combined sample was pH-adjusted prior to reaction with pentachloronitrobenzene in order to assess the influence of Fe speciation on reduction kinetics. Reactions also were performed in unaltered combined pore waters that were not treated beyond size filtration. Experiments also were performed with Na⁺-cation exchanged pore waters to assess the role of naturally occurring DOM on reduction kinetics in the absence of Fe(II).

The final objective was addressed by reacting trifluralin and pendimethalin in both controlled reaction systems containing Fe(II) and fulvic acid isolates, and in unaltered natural pore waters collected from OWC. In the controlled systems, reactions
were performed at various pH and concentrations of Fe(II) and DOM, in order to assess kinetics under different reaction conditions. Reactions with trifluralin and pendimethalin were conducted in unaltered natural pore waters, and kinetics were compared with those observed in the controlled systems.

This work combines applied environmental chemistry and sediment redox geochemical characterization techniques in order to understand how Fe(II) and DOM interact to chemically reduce nitroaromatic pesticides. Combining methods from these diverse areas allowed me to determine both the potential fate of nitroaromatic pesticides in Fe-reducing environments, and the reactivity of naturally-occurring Fe complexes in anoxic sediment pore waters. Results from this are important for future evaluations of the biogeochemical fate of nitroaromatic pesticides and for understanding the natural reactivity of dissolved Fe in anoxic sedimentary pore waters.
1.2 Figures

Figure 1.1 Structures of nitroaromatic pesticides.

Pentachloronitrobenzene (PCNB)  Trifluralin (TR-1)  Pendimethalin (PM)
1.3. References


Wilson, C.; Matisoff, G.; Whiting, P. *The Movement of Sediment in the Old Woman Creek Watershed*. Ohio Department of Natural Resources, 2002.
CHAPTER 2

INFLUENCE OF DISSOLVED ORGANIC MATTER AND FE(II) ON THE ABIOTIC REDUCTION OF PENTACHLORONITROBENZENE

2.1 Introduction

The pollution of surface waters and sediments by synthetic organic substances present in point and non-point sources is of significant environmental concern. A 2004 study by the U.S. Environmental Protection Agency identified 43% of waterways included in the National Sediment Inventory as having probable adverse effects on human health and aquatic life due to contaminant loadings in sediments and fish (USEPA, 2004). Nitroaromatic pesticides, used on agricultural lands to control weed and fungus growth, may be transported to wetland sediments by the deposition of suspended solids (Reigart and Roberts, 1999) where they can re-equilibrate with surrounding porefluids and undergo both abiotic and biotic transformations.

Nitroaromatic and other organic contaminants have been shown to readily undergo abiotic reductive transformation in a number of controlled studies. Reduction occurred by reaction with electron donors such as bisulfide, polysulfides, and Fe(II) either in the presence of a mineral surface or dissolved organic matter (DOM) surrogates.
such as quinones and catechols (Klausen et al., 1995; Strathmann and Stone, 2001; Strathmann and Stone, 2002; Naka et al., 2006; Carlson et al., 2006; Klupinski et al., 2004; Elsner et al., 2004). For example, various Fe(II)-iron mineral systems promoted the reduction of 4-chloronitrobenzene, and Fe(II) sorbed to goethite reduced pentachloronitrobenzene (PCNB) (Klausen et al., 1995; Klupinski et al., 2004). Ascorbic acid (a DOM surrogate) was found to promote the reductive dissolution of goethite, which then facilitated the chemical reduction of 4-cyanonitrobenzene (Smolen et al., 2003). Dinitroaniline herbicides have been studied by several investigators who observed reduction by iron-oxide-bound Fe(II) (Klupinski and Chin, 2003; Wang and Arnold, 2003).

DOM present in natural systems may affect nitroaromatic reduction in anoxic environments, but to date this has been shown only in a limited number of studies (Hoferkamp and Weber, 2006; Simon et al., 2000). Others have shown that a series of nitroaromatic compounds can undergo reduction in the presence of bisulfide and DOM (Dunnivant et al., 1992). DOM also may affect the ligand environment, and thus the reductive capacity, of Fe(II) in sedimentary porefluids. For example, different Fe(II)-ligand combinations have been shown to affect the reductive capacity of Fe(II) toward Cr reduction, and a soil humic acid-Fe(II) complex was shown to efficiently reduce chromate at rates equivalent to those observed for the Fe(II)-tartrate complex (Buerge and Hug, 1998). Nonetheless, the precise nature of DOM’s role in the reduction of nitroaromatic compounds in suboxic to anoxic iron-rich sedimentary porefluids remains unclear.
The combined role of DOM and Fe(II) is relevant to the fate of organic compounds that can undergo reduction in sedimentary environments. The co-accumulation of DOM and Fe(II) in sedimentary porefluids from different lacustrine environments has been observed, where levels of Fe(II) and DOM measured in these porefluids can exceed 1mM and 3 mM (as dissolved organic carbon, DOC), respectively (Chin et al., 1998; O'Loughlin and Chin, 2004). At these levels it is plausible that reduction of nitroaromatic compounds by Fe(II) and DOM can occur to a substantial degree. In addition, bioturbation processes and rooted plants can pump oxygenated overlying water into the sediments. This results in sediment suboxic zones where iron oxide species can form and subsequently interact with Fe(II) to create Fe(II)-iron oxide surface complexes that are reactive towards nitroaromatics (Williams and Scherer, 2004).

In this study, I investigated the reduction of PCNB by Fe(II) and DOM in controlled analogues of natural sedimentary porefluids in unfiltered and 0.45-μm-filtered solutions to simulate the effect of trace Fe colloids on PCNB reduction in natural systems. I hypothesize that in 0.45-μm-filtered solutions Fe(II)-DOM complexes are the reactive reductants, but in the presence of trace oxygen, Fe(II) associated with iron oxide colloids becomes the dominant reactive phase. The major goals of this study were to model PCNB reduction kinetics in the presence of Fe(II) and DOM in unfiltered and 0.45-μm-filtered solutions, and to determine whether DOM composition affects reduction kinetics.
2.2 Experimental Details

2.2.1 Chemicals and Dissolved Organic Matter

PCNB (Sigma-Aldrich) was recrystallized in cold methanol (<0 °C). Other compounds were used as received: pentachloroaniline (PCA; Alfa Aesar), MOPS (3-[N-morpholino]propanesulfonic acid; Sigma-Aldrich, 99%), FeCl$_2$⋅4H$_2$O (Certified, Fisher Scientific), ammonium acetate (Jenneile Enterprises), 1,10-phenanthroline monohydrate (Certified ACS, Fisher Scientific), glacial acetic acid (Certified ACS Plus, Fisher Scientific), and Fe(NH$_4$)$_2$(SO$_4$)$_2$⋅6H$_2$O (Certified ACS, Fisher Scientific). Acid solutions were made from concentrated HCl (12 N, Certified ACS Plus, Fisher Scientific), and base solutions were made from solid NaOH (Mallinckrodt AR; Certified ACS, Fisher Scientific). Milli-Q water (Milli-Q UV Plus, Millipore) and methanol (HPLC Grade, Fisher Scientific) were used as solvents. XAD-8 isolates of surface water DOM from Pony Lake, Antarctica (PLFA), and Suwannee River, Georgia, USA (SRFA), were made into separate stock solutions in Milli-Q water.

2.2.2 Reaction Studies

Reaction samples and Fe measurement aliquots were prepared in a glovebox (PLAS Labs, Inc.) filled either with argon or with 95/5 nitrogen/hydrogen and equipped with an oxygen scrubber. A Beckman 240 pH/temp meter and Orion Thermo Aqua Pro pH probe were used to measure pH values for each sample. Reaction media of Milli-Q water, MOPS buffer and NaOH were prepared in glass bottles outside of the glovebox, and DOM stock solutions were added to the reaction media depending upon the reaction being studied. All reaction media were purged with argon gas for 1 min per each mL of solution, and then transferred to the glovebox. All other solutions (2 N HCl (aq)), PCNB
stock in methanol, Milli-Q water used to prepare Fe(II) spike) were purged and transferred to the glovebox in a similar fashion. An aqueous Fe(II) stock solution for the Fe(II) spike was prepared inside the glovebox with FeCl$_2$$\cdot$4H$_2$O. The Fe(II) spike was added to the reaction medium and allowed to equilibrate for 30 min to overnight, and reactivity was not affected by equilibration time, confirming the observations reported by Klupinski et al. (2004). In some experiments, acidified (pH ~3) Fe(II) stock solution was added to the premixture prior to purging the solution, the solution pH was raised to the desired level with purged NaOH inside the glovebox, allowed to equilibrate, and filtered with a 0.45-µm filter (Pall Life Sciences IC Acrodisc 25 mm syringe filter with Supor (PES) membrane) prior to use. The reaction medium then was drawn into a ground glass syringe (Popper & Sons, Inc.), and spiked with the PCNB stock solution to yield a mixture with [PCNB] = 1.0 µM and <0.1% cosolvent. Reactions were performed inside sealed glass syringes with no headspace at 20°C.

Reaction aliquots were separated at designated time points into 2-mL or 4-mL borosilicate glass autosampler vials (National Scientific) preloaded with 20 to 40 µL of 2 N HCl to quench the reaction at pH 2.0 to 3.0. The vials were sealed with Teflon-lined septa screw caps, and loaded into the autosampler for HPLC analysis using either a Waters 1525 binary HPLC pump, 2487 dual λ absorbance detector with a 717 Plus autosampler or a Shimadzu SCL-10AT pump, SIL-10A autosampler, DGU-14A degasser, SCL-10A system controller, and SPD-10A UV/Vis detector. All compounds were detected at a wavelength of 225 nm. Samples were injected in volumes of 200 to 250 µL and analytes were separated through a reverse-phase C$_{18}$ column (a Waters Nova-Pak C$_{18}$, 3.9 x 150 mm or a Restek Pinnacle II C$_{18}$, 5 µm, 150 x 4.6 mm) using either an
80/20, 77/23, or 75/25 methanol/water mobile phase acidified to pH 2.8 to 3.6 with concentrated HCl. Concentrations of PCNB and PCA in the reaction aliquots were calculated by comparison of analyte peak areas with calibration standards of known concentration. Kinetic parameters were determined by a least squares fitting of the concentration-versus-time data with the fitting program MicroMath Scientist and the regression wizard function in SigmaPlot 10.0.

Filter-sterilized control experiments were performed with a solution containing Fe(II) and PLFA, and a PLFA-only solution. Solutions were prepared and filtered with a 0.45-µm-filter in the same manner as the 0.45-µm-filtered experiments, and sterilized by further filtration through a sterile 0.2-µm filter (25 mm diameter, Pall Acrodisc) into autoclaved syringes.

2.2.3. Kinetic Models

PCNB reduction to PCA was modeled using both pseudo-zero-order and pseudo-first-order kinetic models. The pseudo-zero-order rate constant is represented by $k_{AZ}$ (Eqn 1).

$$[\text{PCNB}] = [\text{PCNB}]_0 - k_{AZ} t$$ (1)

The pseudo first-order rate constant is represented by $k_A$ (Eqn 2).

$$[\text{PCNB}] = [\text{PCNB}]_0 e^{-k_A t}$$ (2)

In Eqs 1 and 2, $t$ represents reaction time and $[\text{PCNB}]_0$ represents the initial concentration of PCNB. The value for $[\text{PCNB}]_0$ was determined during each model fit because the true initial reactant concentration could not be measured due to its immediate reduction upon addition to the reaction medium. Determination of whether a reaction proceeded via pseudo-zero- or pseudo-first-order kinetics was made by a comparison of
statistical data for the least squares fits. However, it is often difficult to identify the accuracy of a particular model when fitting the measured data points. This observation is partially due to the limits inherent in small data sets, and to the fact that both models are simplified explanations of complex reaction dynamics. All kinetic modeling performed in this study, and the rate constants obtained from these kinetic models, are only valid for situations in which [PCNB] ~ 1 μM under the conditions reported here.

I observed the appearance of a reactive intermediate in the Fe(II)-only system that was absent in the Fe(II)-DOM experiments (Figure 2.1). Thus, only the loss of PCNB was modeled in the kinetics studies. Formation of PCA was monitored to ensure that the reaction proceeded to completion ([PCA]_{final} \approx [PCNB]_{0}) and that PCNB was not lost through volatilization from or sorption to the HPLC autosampler vials.

2.2.4. Fe(II), DOC, and PCS Measurements

Aliquots (~2 mL) for Fe(II) measurements were taken from each reaction premixture (prior to reaction with PCNB) and acidified with 20 to 30 μL 2 N HCl. Each aliquot was filtered with a Milli-Q-rinsed 0.2-μm filter (Acrodisc 13 mm syringe filter with HT Tuffryn membrane) and 1 mL of the 0.2-μm-filtered aliquot was reacted with 1.0 mL of an aqueous 1,10-phenanthroline solution (~5.4 mM), 0.5 mL ammonium acetate buffer (~3.8 M) and 2.5 mL Milli-Q water. In some cases the aliquot was filtered with a 0.45-μm filter, and [Fe(II)] measured for the 0.2-μm-filtered and 0.45-μm-filtered aliquots were within 5%. The Fe(II)-1,10-phenanthroline complex absorbance was measured in plastic cuvettes at a wavelength of 508 nm (either with a Varian Cary 1 or Shimadzu UV-1201 UV/Vis spectrophotometer) zeroed to a Fe(II)-free blank containing
the colorimetric reagents. Concentration was calculated from a linear equation relating absorbance to calibration standard concentrations (range of 10 to 1000 µM Fe(II)).

DOC concentrations of fulvic acid stock solutions were measured with a Shimadzu TOC 5000 after filtering the sample with a Milli-Q-rinsed 0.45-µm filter and acidification with 2 N HCl. DOC concentrations for the reaction media were calculated from DOC concentrations of stock solutions prepared with PLFA and SRFA.

Photon correlation spectroscopy (PCS) was performed with a 90 Plus Particle Size Analyzer (Brookhaven Instruments). A ~3-mL sample was placed in a plastic 1 cm cuvette, and incident light (678 nm) was directed at the sample and detected at a 90° angle.

2.3. Results and Discussion

2.3.1. Definition of Particle-Free Reaction Media

Experiments were conducted in unfiltered and 0.45-µm-filtered solutions in order to distinguish the roles of colloidal iron and Fe(II)-DOM (PLFA and SRFA) complexes on PCNB reactivity. Unfiltered reaction media were not filtered after preparation and prior to reaction with PCNB, while particle-free reaction media were further filtered with a 0.45-µm filter (to remove colloids from solution) after preparation and prior to reaction. A 0.45-µm size was used instead of a smaller-sized filter due to practical constraints of filtration time and concerns about inadvertently removing reactive DOM fractions. The integrity of both solutions, as measured by particle formation, was monitored with PCS over the course of the experiment, and results of normalized PCS count rates represent the ratio of the reaction sample count rate to a solution blank.
In the unfiltered reaction experiments I observed a range of PCS ratios from 15 to 55 (Figure 2.2). The 0.45-µm-filtered reaction media had PCS ratios between 1 and 5, indicating that reduction occurs in a system that is operationally “colloid-free” within blank detection limits of the PCS instrument. The lower values within each range were observed in solutions containing fulvic acids, and reasons for this phenomenon are discussed elsewhere in the manuscript.

2.3.2. Reduction of PCNB in Fe(II)-only Reaction Media

Rapid reduction of PCNB to PCA (within 10 hr) was observed in both unfiltered and 0.45-µm-filtered solutions in our Fe(II)-only control reactions (Figure 2.3 and Table 2.1). PCS ratios show that colloids are present in the unfiltered systems (Figures 2.2 and 2.4) and, although expected, reduction rates did not consistently rise with [Fe(II)]. Klupinski et al. (2004) reported a positive correlation between increased reactive Fe(II)-iron oxide surface species present and PCNB reactivity. As PCS ratios for our unfiltered reactions varied by a factor of 10 to 20 amongst reaction sets, it is possible that different levels of reactive surface species present caused the observed variation in reactivity in the Fe(II)-only unfiltered reactions (Figures 2.2 and 2.4). However, the precise mechanism of reduction in the unfiltered Fe(II)-only reaction cannot be determined from the data collected, as the lack of data past 2 half-lives for some reactions obscures the definition of observed rate order for these reactions (Figures 2.3 and 2.5).

Data were collected past 2 half-lives for the 0.45-µm-filtered Fe(II)-only reactions, and I observed both pseudo-first-order and pseudo-zero-order kinetics in these systems (Table 2.1). Klupinski et al. (2004) observed mixed pseudo-first-order and pseudo-zero-order kinetics in their reactions containing Fe(II) and low concentrations of
goethite, and pseudo-zero-order kinetics in their Fe(II)-only reactions where PCS measurements detected colloids. They explained that the pseudo-zero-order mechanism for PCNB reduction in the presence of trace iron colloids is analogous to the Michaelis-Menten enzyme catalysis model. In their work, the limited reactive species involves an oxide surface that is low in concentration relative to PCNB, and the reaction proceeds by an intermediate that limits reactivity between the surface species and PCNB. In the present work, reduction may occur by a similar mechanism where an unknown intermediate limits reactivity between PCNB and the reactive Fe(II) species present. However, I am limited by my data set in providing a more detailed mechanistic interpretation of these reactions as I performed the Fe(II)-only reactions as control experiments for comparison with Fe(II)-DOM systems.

2.3.3. Effect of DOM on Reduction Kinetics for PCNB

Rapid reduction of PCNB to PCA was observed in reaction media containing both Fe(II) and DOM (Fe(II)-DOM) (Figures 2.4 and 2.6). Reduction of PCNB did not occur in reaction media containing DOM without Fe(II) (DOM-only) over the time span of the Fe(II)-DOM experiments performed (10 h) (Figure 2.7). However, the PCA byproduct was observed at or below the minimum concentration of our PCA calibration standard range (0.05 µM) in DOM-only experiments after 12 h (Figure 2.7 and 2.8). These observations confirm that [Fe(II)] in the 100s of µM are needed to facilitate rapid PCNB reduction. I suspect that either trace levels of Fe(II) are present in the fulvic acid isolates (undetectable by the colorimetric method I used) or redox active moieties were involved in the slow PCNB reduction observed in the DOM-only systems. Reactivity in both unfiltered and 0.45-µm-filtered Fe(II)-DOM systems followed a pseudo-first-order
kinetic model, and was not significantly affected by [DOC] nor the type of DOC used
within a particular pH range, except at high [Fe(II)] (Figures 2.9, 2.10 and 2.11).
Reactions performed in 0.2-µm-filter-sterilized Fe(II)-PLFA and PLFA-only solutions
showed reactivity similar to that of non-filter-sterilized reactions (Figures 2.8 and 2.10),
confirming that PCNB reduction in our reaction systems occurs abiotically.

PCNB reduction in unfiltered Fe(II)-DOM reactions was slower compared to
unfiltered Fe(II)-only systems (for reactions performed within the same set), while
reactivity in both Fe(II)-DOM systems was statistically the same within the 95%
confidence interval of the calculated rate constant (Figure 2.9). Comparisons between the
unfiltered Fe(II)-only and Fe(II)-DOM reactions can only be made within the same
reaction set as particle formation within the solutions may vary between reaction sets.
The presence of iron oxide particles for the unfiltered Fe(II)-DOM reactions presented in
Figure 2.9 was roughly a factor of 4 lower than in the Fe(II)-only system. In addition,
PCS ratios were approximately the same in the Fe(II)-DOM reaction media, regardless of
the type of DOM used (Figure 2.2).

I suspect that complexation of Fe(III) (present due to oxidation of Fe(II) by trace
O₂) with DOM interfered with the formation of iron oxide colloids. Pullin and Cabaniss
(2003) reported that the kinetics of iron colloid formation in solutions containing Fe(II)
and SRFA were slower than in solutions without SRFA. They suggested that both Fe(II)
complexation by DOM and chemical reduction of Fe(III) by DOM inhibited Fe(II)
oxidation in the presence of fulvic acids. In support of the latter mechanism Fimmen et
al. (2007) recently observed that quinone/hydroquinone redox couples in PLFA have
reduction potentials that are capable of reducing Fe(III). Additionally, competition
between Fe(II) and DOM for sorption sites on iron colloids may affect PCNB reduction by limiting the amount of surface-complexed Fe(II) available to react. Tao et al. (2000) found that the presence of fulvic acids generally inhibited metal cation sorption at high pH. Slower reduction rates for PCNB in the unfiltered Fe(II)-DOM reactions may result from inhibition of iron colloid formation by DOM, limited availability of reactive surface sites, re-reduction of Fe(III) to Fe(II) or a combination of these effects.

In the 0.45-µm-filtered reaction media, both Fe(II)-DOM (PLFA and SRFA) systems reduced PCNB at a faster rate than the Fe(II)-only systems (Figures 2.9 and 2.10; Table 2.1). At high [Fe(II)], PCNB reduction in the Fe(II)-DOM systems are statistically different from each other and the Fe(II)-only system (based upon the 95% confidence interval of the calculated rate constants) and reduction in both Fe(II)-DOM systems is faster than in the Fe(II)-only system. However, this distinction changes in reactions performed at lower [Fe(II)], where reduction in Fe(II)-DOM systems is statistically the same. Back-reduction of Fe(III) to Fe(II) by DOM might affect reduction pathways at lower [Fe(II)], and a greater abundance of reactive Fe(II)-DOM complexes at higher [Fe(II)] may contribute to greater reactivity at high pH.

The data suggest that reduction in 0.45-µm-filtered systems is regulated by solution-phase pathways in which Fe(II)-DOM complexes are possible reductants, whereby the reductive capacity of Fe(II) is enhanced by complexation with functional groups present within the DOM structure. Buerge and Hug (1998) observed a correlation between increased Cr(VI) reduction rates and the greater reductive capacity of various Fe(II) complexes with organic and inorganic ligands. Nitroaromatic reduction by Fe(II) complexed with O or reduced S functional groups has been described in controlled
studies with DOM analogues by Naka et al. (2006) who reported significant reactivity for Fe(II) complexed to specific catechol and thiol-containing multidentate ligands. Such reactive ligands are suspected to be ubiquitous within DOM, however, relative amounts of these ligands are expected to differ between DOMs from different surface water environments. In the reactions presented here, the elemental composition of the two DOMs used differs: PLFA has roughly 2, 5 and 10 times the amount of O, S, and N, respectively, compared to SRFA (Reddy et al., 1989; Brown et al., 2004). XPS data has shown that 58.9% of organic S ligands in PLFA are in the reduced form (Fimmen et al., 2007). Faster PCNB reduction kinetics in Fe(II)-PLFA media at high [Fe(II)] may result from a greater influence of Fe(II)-thiol interactions on PCNB reduction kinetics. Such variation in elemental composition and reduced S content may explain the differences in and variations of PCNB reduction kinetics observed for the 0.45-µm-filtered Fe(II)-PLFA and Fe(II)-SRFA systems.

Comparison of the unfiltered Fe(II)-DOM reactions with 0.45-µm-filtered Fe(II)-DOM reactions shows that the pseudo-first-order rate constants for unfiltered reactions can be up to an order of magnitude larger than those for the 0.45-µm-filtered Fe(II)-DOM reactions (Figures 2.10 and 2.11). The major difference between the unfiltered and 0.45-µm-filtered reactions is that colloids are generally absent (or present in significantly smaller quantities) in the 0.45-µm-filtered media (Figures 2.2 and 2.4). Thus, the faster PCNB reduction in the unfiltered reactions relative to 0.45-µm-filtered reactions reported in Figure 2.9 shows that colloidal phases enhance PCNB reduction relative to systems with limited or no colloidal phases.
I also observed formation of a reaction intermediate in the Fe(II)-only system that was absent in the Fe(II)-DOM system (Figures 2.1 and 2.12). Reduction of nitroaromatic compounds to their aniline derivatives occurs through nitroso and hydroxylamine intermediates (Larson and Weber, 1994). Klupinski et al. (2004) observed the hydroxylamine intermediate produced during abiotic reduction of PCNB mediated by Fe(II) and goethite. I suspect that the intermediate formed during reduction in our Fe(II)-only systems (both unfiltered and 0.45-µm-filtered) is the hydroxylamine intermediate. Because this intermediate is absent in the Fe(II)-DOM reactions, I propose that a different mechanism is responsible for the rate-limiting step in the Fe(II)-DOM reactions (both unfiltered and 0.45-µm-filtered), though at this time I am unable to identify the precise reaction pathway.

Kappler and Haderlein (2003) found that reduction of chlorinated aliphatic compounds by either electrochemically-reduced humic acids (HA) or anthrahydroquinone-2,6-disulfonic acid (AHQDS) proceeded over the course of ~150 h in the absence of Fe(II). To date, no studies of nitroaromatic reduction by electrochemically-reduced DOM have been reported. However, Tratnyek and Macalady (1989) reported methyl parathion reduction by electrochemically-reduced hydroquinones in model systems. Though hydroquinone moieties in DOM are potential nitroaromatic reductants in natural freshwater sediment systems, the data presented here show much faster PCNB reduction in systems containing both Fe(II) and DOM relative to DOM-only systems, and reactivity is related to the [Fe(II)] in solution. I propose that specific Fe(II)-DOM complexes are the reactive reductants in the 0.45-µm-filtered Fe(II)-DOM systems.
Though I discuss a number of potential effects occurring in both filtered and unfiltered Fe(II)-DOM reaction systems, it is important to note the limitations of these interpretations. Previous research has shown that DOM is capable of complexing Fe(II) (Rose and Waite, 2003), however, I need to know the exact functional character of DOM in order to provide an experimentally-derived mechanistic interpretation of our kinetic results. Current research on DOM functional character and Fe(II)-complexation capacity lacks this level of detail, and prevents me from precisely defining the reactive Fe(II) complexes present in the Fe(II)-DOM systems.

Tas and Pavlostathis (2005) investigated the reduction of PCNB to PCA and the dechlorination of PCA by bacteria under methanogenic conditions. They found that autoclaved culture controls at pH ~ 7.8 with [Fe(II)] ~ 500 µM, sulfides and low levels of vitamin B$_{12}$ reduced PCNB to PCA at similar rates relative to reactions performed with live bacteria in culture media of the same composition, and at faster rates than culture media control blanks. Dechlorination of PCA only occurred in solutions with live bacteria, likely the result of halorespiring bacterial activity. Though the culture media contained sulfides and vitamin B$_{12}$ (other potentially reactive PCNB reductants), the important point is that microbial exudates, which are DOM precursors, are suspected of promoting reduction of PCNB in their autoclaved control system. Although bacteria may play a direct role in the further degradation of nitroaromatic reduction byproducts, they also play an important yet indirect role during reduction of the parent nitroaromatic compound by generating the reactive reductants in natural systems (Heijman et al., 1995).
2.4. Environmental Significance

These results indicate that iron colloid-mediated reduction may be the dominant pathway in natural systems containing high concentrations of iron colloids in solution, such as in the suboxic zones of freshwater sediments. Fe(II)-DOM mediated reduction is expected to be significant in anoxic sediment porefluids as well as in lakes that undergo either permanent or seasonal anoxia in the hypolimnion. If PCNB is readily reduced by chemical reductants, it is also possible for other NAPs (that are recalcitrant in oxic environments) to be reductively transformed to other substances in environments that contain Fe(II) and DOM. The precise role of DOM during reduction is an important area for future investigations on specific reactive complexes that form between Fe(II) and DOM ligands.
2.5. Tables

<table>
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<th>Reaction</th>
<th>Order</th>
<th>$k_s$ (min$^{-1}$) or $k_{AZ}$ (µM min$^{-1}$) (x $10^{-2}$)</th>
<th>Error$^a$ (min$^{-1}$ or µM min$^{-1}$)</th>
<th>pH</th>
<th>[Fe(II)]$^{b}$ (µM)</th>
<th>[DOC] (mM-C)</th>
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$^a$Rate constant error reported as one half of the 95% confidence interval for the kinetic fit.

$^b$Concentrations represent values for the 0.45-µM fraction of the reaction media.

$^c$Reactions performed with 6-month-old PLFA stock solution. $^d$Reactions performed with 3-day-old PLFA stock solution. $^e$Reactions only performed to one half-life; pseudo-first-order kinetics are assumed. $^f$Reactions performed with week-old PLFA stock under filter-sterilized conditions.

**Table 2.1.** Table of rate constants for reactions performed in 0.45-µm-filtered Fe(II)-only, Fe(II)-PLFA and Fe(II)-SRFA reactions. Reaction conditions are listed for each reaction.
2.6. Figures

Figure 2.1. HPLC chromatograms at \([\text{PCNB}] \approx 0.4[\text{PCNB}]_0\) for PCNB reduction in Fe(II)-only (solid line) and Fe(II)-DOM (dashed line) unfiltered solutions at pH 7.80 ± 0.01, \([\text{Fe(II)}]_{\text{aq}} = 480 \pm 30 \mu\text{M}\) and \([\text{DOC}] = 1.3 \text{ mM-C (as SRFA)}\) for the reaction containing DOM. The intermediate peak occurs at retention time (RT) = 4.6 min, the PCA peak occurs at RT = 8.1 min, and the PCNB peak occurs at RT = 11.7 min.
Figure 2.2. Normalized PCS count rates (count rate reaction sample/count rate reaction blank) for reactions containing Fe(II), Fe(II) and SRFA, or Fe(II) and PLFA. Reactions were performed in unfiltered and 0.45-µm filtered solutions, and were normalized to the Fe(II)-free solution phase or to a Milli-Q blank. Where not shown, error bars are smaller than symbols.
Figure 2.3. Reduction of PCNB (solid symbols) and pseudo-first-order rate equation fitted lines in Fe(II)-only unfiltered system at [Fe(II)] and pH as indicated in the figure legend. Rate constant errors, shown in the figure legend, are reported as half of the 95% confidence interval for the integrated rate fit.
Figure 2.4. PCNB reduction in unfiltered Fe(II)-only and Fe(II)-DOM systems. A) Fe(II)-only at pH 7.62, [Fe(II)]$_{aq}$ = 460 µM and observed PCS ratios (ratio to estimated blank values), with pseudo-zero-order kinetics, $k_{AZ} = 2.73 \pm 1.33 \times 10^{-2}$ µM min$^{-1}$. B) Fe(II)-SRFA system at pH 7.82, [Fe(II)]$_{aq}$ = 800 µM, [SRFA] = 1.8 mM-C, and observed PCS ratios (ratio to SRFA-MOPS solution without Fe(II)), with pseudo-first-order kinetics, $k_{A} = 2.23 \pm 0.15 \times 10^{-2}$ min$^{-1}$. C) Fe(II)-PLFA system at pH 7.79, [Fe(II)]$_{aq}$ = 430 µM, [PLFA] = 1.7 mM-C and observed PCS ratios (ratio to PLFA-MOPS solution without Fe(II)), with pseudo-first-order kinetics, $k_{A} = 2.22 \pm 0.26 \times 10^{-2}$ min$^{-1}$. Rate constant error for the integrated rate fit is reported at the 95% confidence interval. Data for the Fe(II)-SRFA reaction presented (Figure 2.3.B) are from a different reaction set than the Fe(II)-only and Fe(II)-PLFA reactions (Figures 2.3.A and 2.3.C, respectively).
Figure 2.5. Pseudo-zero-order kinetic fits for PCNB reduction in unfiltered Fe(II)-only systems. Rate constant error in the legend is reported as one half of the 95% confidence interval for the rate constant. Error of the pseudo-zero-order fit results in a negative value for these kinetic points.
Figure 2.6. PCNB reduction in A) unfiltered Fe(II)-DOM systems, and B) 0.45-µm-filtered Fe(II)-DOM systems. For unfiltered systems (A), Fe(II)-PLFA reactions were performed at pH 7.81, [Fe(II)] = 270 µM, [DOC] = 1.80 mM-C with pseudo-first-order $k_A = 3.08 \pm 0.57 \times 10^{-2}$ min$^{-1}$; Fe(II)-SRFA reactions were performed at pH 7.72, [Fe(II)] = 250 µM, [DOC] = 1.78 mM-C, with pseudo-first-order $k_A = 0.97 \pm 0.08 \times 10^{-2}$ min$^{-1}$. For 0.45-µm-filtered systems (B), Fe(II)-PLFA reactions were performed at pH 7.82, [Fe(II)] = 220 µM, [DOC] = 1.28 mM-C with pseudo-first-order $k_A = 0.38 \pm 0.04 \times 10^{-2}$ min$^{-1}$; Fe(II)-SRFA reactions were performed at pH 7.82, [Fe(II)] = 230 µM, [DOC] = 1.27 mM-C, with pseudo-first-order $k_A = 0.32 \pm 0.04 \times 10^{-2}$ min$^{-1}$. Rate constant errors are reported as half of the 95% confidence interval for the integrated rate fit.
Figure 2.7. PCNB (filled circles) reduction in A) PLFA-only and B) SRFA-only reaction media at pH 7.73 ± 0.04 and [DOC] 1.28 ± 0.01 mM-C in 0.45-µm-filtered media. The PCA byproduct (empty circles) appeared after ≈ 12 h, and did not increase past a [PCA] = 0.07 µM over a period of 40 h (PLFA-only reaction) or [PCA] = 0.04 µM over a period of 45 h (SRFA-only reaction). (Note that the calibration limit of the HPLC for this run was 0.05 µM.)
Figure 2.8. PCNB reduction in A) non-sterile 0.45-µm-filtered systems and B) 0.2-µm-filter-sterilized systems. [DOC] = 1.28 mM-C and pH = 7.80 ± 0.04 for the reactions. At 1642 ± 51 min (27.4 ± 0.9 h), [PCA] = 0.05 µM for the non-sterile reaction (A) and [PCA] = 0.04 µM for the sterile reaction (B).
Figure 2.9. Plots of reaction half-lives calculated from pseudo-first-order rate constants for PCNB reduction in unfiltered and 0.45-µm-filtered solutions containing either Fe(II) only, or Fe(II) and DOM (as PLFA or SRFA). For the unfiltered Fe(II)-only reaction, [Fe(II)] = 530 µM; for all other reactions, [Fe(II)] = 850 ± 30 µM. Fe(II) concentrations reported are for 0.2-µm-filtered aliquots ([Fe(II)]<sub>aq</sub>), except for the 0.45-µm-filtered Fe(II)-SRFA reactions in which [Fe(II)] is from the 0.45-µm fraction. [DOC] = 1.6 ± 0.5 mM-C for reactions containing DOM, except for the 0.45-µm-filtered Fe(II)-PLFA reaction with a [DOC] = 0.84 mM-C. For all reactions, MOPS-buffered pH = 7.72 ± 0.08. Half-life errors are reported at the 95% confidence interval, and values for 0.45-µm-filtered Fe(II)-DOM reactions represent the means of triplicate experiments.
Figure 2.10. Pseudo-first-order rate constants versus [Fe(II)] (0.45-\(\mu\)m fraction) for reactions performed in 0.45-\(\mu\)m-filtered Fe(II)-only, Fe(II)-PLFA and Fe(II)-SRFA systems at pH 7.80 \(\pm\) 0.05. Rate constants and specific reaction conditions are listed in Table 2.1.
Figure 2.11. Pseudo-first-order rate constants versus $[\text{Fe(II)}]$ (0.2-$\mu$m fraction) for reactions performed in unfiltered Fe(II)-PLFA and Fe(II)-SRFA systems at pH 7.73 $\pm$ 0.11.
Figure 2.12. Plot of PCNB, PCA and intermediate chromatogram peak areas during reduction in a 0.45-μm-filtered Fe(II)-only system at pH 7.77 and [Fe(II)] = 480 μM. Peak areas are plotted here in order to be able to show the formation and disappearance of the intermediate. Concentrations of the intermediate cannot be calculated, as I did not possess calibration standards for use with the HPLC analyses.
2.7. References


3.1 Introduction

Wetlands act as critical biogeochemical buffer zones between overland runoff and receiving waters. In particular, oxidation-reduction (redox) chemistry in the sediment column plays an important role in early sediment diagenesis (Gavrill and Angelidis, 2006; Audry et al., 2007; Anschutz et al., 2007), the cycling of dissolved species between surface waters and sediments (Caetano et al., 1997; Gavrill and Angelidis, 2006; Audry et al., 2007), and the environmental fate of contaminants (Lyngkilde and Christensen, 1992; Myneni et al., 1997). Fe and dissolved organic matter (DOM) are important biotic and abiotic electron transfer mediators in anoxic environments (Scott et al., 1998; Nevin and Lovley, 2000) whose geochemistry is affected by surface water hydrology (Caetano et al., 1997; Bally et al., 2004).

Saturated wetland sediments quickly establish redox zones where oxygen is depleted in the first few millimeters depth from the sediment-water interface (Brendel and Luther, 1995; Taillefert et al., 2000; Trouwborst et al., 2006), which requires microorganisms to rely on mineral and other terminal electron acceptors to complete metabolic processes. Reduction of Fe(III) to Fe(II) occurs during dissimilatory reduction,
where ferric iron minerals serve as terminal electron acceptors during microbial metabolism (Lovley et al., 1987). Some biotic processes involve the complexation of Fe(III) by siderophore-type compounds and solubilization of iron oxide minerals (Kraemer, 2004; Taillefert et al., 2007a). Humic materials and dissolved organic matter (DOM) also act as terminal electron acceptors, or as mediators for electron transfer to iron oxide minerals (Lovley et al., 1996; Scott et al., 1998; Nevin and Lovley, 2000). The abiotic reductive dissolution of iron minerals by iron-chelating organic molecules has been observed (Smolen et al., 2003; Maurice et al., 1995), and organically-complexed Fe(III) also was found to act as an oxidant in anoxic zones of sediments (Luther et al., 1992; Luther et al., 1996). Both biotic and abiotic processes involving Fe and DOM affect the reductive capacity of sediment pore waters, and the distribution of reactive species available to participate in redox chemistry.

Fe and DOM have been found in micromolar to millimolar concentrations in benthic pore waters. A number of studies with salt marsh pore waters showed an increase in Fe concentration with depth from the sediment-water interface in the absence of sulfides (Luther et al., 1992; Brendel and Luther, 1995; Luther et al., 1996). Freshwater pore waters also are known to contain high concentrations of dissolved Fe(II) and DOM (Chin and Gschwend, 1991; Chin et al., 1998; O’Loughlin and Chin, 2004; van Griethuysen et al., 2005). The combined presence of Fe(II) and DOM at high concentration in pore waters from a wide range of sedimentary environments warrants attention towards their combined reactivity in sediment pore water systems. Reduction of metals and organic contaminants by Fe(II) and DOM has been studied in controlled
laboratory systems by several investigators using Fe(II) and synthetic DOM chemical analogs such as simple organic acids, catechols and thiols (Buerge and Hug, 1998; Naka et al., 2006; Kim and Strathmann, 2007), and in systems containing Fe(II) and fulvic acid isolates (Hakala et al., 2007/Chapter 2). The reductive synergy of Fe(II) and DOM observed in controlled laboratory systems may extend to natural pore waters that contain high levels of dissolved Fe(II) and DOM.

A number of investigators have used nitroaromatic probe compounds to study Fe electron transfer kinetics in soil columns, contaminated aquifer material, and batch sediment slurries. Heijman et al. (1995) explored the combined role of microbial metabolism and electron transfer properties of reduced Fe in sediment columns using a series of nitroaromatic probe compounds. They found that, though reduction of the nitroaromatic probe compounds was an abiotic process related to concentrations of Fe(II) in the sediment column reactor, reaction kinetics depended upon the regeneration of the reactive Fe(II) reductant by microbial activity. Rügge et al. (1998) studied the reduction of nitroaromatic compounds in an anaerobic aquifer contaminated with a landfill leachate plume. They observed reduction of nitrobenzene in the presence of aqueous landfill leachate material devoid of aquifer sediments, which contained high concentrations of dissolved Fe(II) and organic matter. Hoferkamp and Weber (2006) studied the reduction of p-cyanonitrobenzene in batch sediment slurries with different terminal electron acceptors. They found that p-cyanonitrobenzene reduction increased as the solution-phase levels of dissolved Fe(II) increased. However, Fe(II) concentrations alone did not explain their observations and suggest that other factors such as Fe(II) speciation affects p-
cyanonitrobenzene reduction in the sediment slurries (Hoferkamp and Weber, 2006). Finally, Hakala et al. (2007) reported that homogeneous solutions of Fe(II) and fulvic acids in controlled laboratory systems were able to reduce nitroaromatic compounds albeit at slower rates than those observed in the presence of iron oxides (Klupinski et al., 2004).

To date I am unaware of any study that has examined the reductive capacity of Fe(II) and DOM present naturally in benthic pore waters. The lack of such data is in part due to the difficulties involved in collecting pore waters anoxically and preventing the alteration of the chemical constituents during transport from the field site to the lab. In this study, I used differential pulse polarography (DPP) to characterize pore water redox geochemistry and nitroaromatic reduction kinetic studies to probe the redox capacity of naturally-occurring Fe in freshwater sediment pore waters. I also examined how handling and preservation approaches of the pore water samples can alter their chemistry and reactivity. I hypothesize that the pore water pH and Fe(II) concentration play a major role during nitroaromatic reduction in the pore waters, and that Fe(II) speciation is the predominant factor affecting electron transfer reactions in natural systems containing Fe(II) and DOM.
3.2  Fieldwork, Materials and Methods

3.2.1.  Field Site Description and Sampling

Old Woman Creek National Estuarine Research Reserve (OWC) is an eutrophic freshwater wetland that drains into Lake Erie. Major sources of wetland sediments include the Berea sandstone, Ohio shale, and lacustrine, till and soil deposits (Frizado et al., 1986). The sampling site (Figure 3.1) sediment mineralogy was characterized as predominantly quartz, illite and plagioclase with smaller percentages of chlorite, K-feldspar, calcite, dolomite, siderite and pyrite (Frizado et al., 1986). Siderite (FeCO$_3$) and pyrite (FeS$_2$) combined were less than 5% of the bulk mineralogy (Frizado et al., 1986). Hydrous metal oxide content of the sediments ranged from 1.2 to 6.6 g/kg-sediment, and hydrous oxide-related Fe content ranged from 5.2 to 18.4 mM Fe/kg-sediment (Frizado et al., 1986).

Sediment cores were collected from OWC in the lower part of the wetland, close to the boundary between the wetland and Lake Erie (Figure 3.1). The surface water chemistry in this location is representative of the mixing zone between the chemically distinct upper wetland and Lake Erie surface waters. Sediments were collected in plastic tubes (cellulose acetate butyrate; Benthos, Inc.) with a punch-core technique and sealed with rubber stoppers immediately after collection. Although the punch-core technique may compress sediment cores, agreement of the geochemical profile results with those reported by others (Brendel and Luther, 1995; Taillefert et al., 2007b) confirms that this method is acceptable for this study. The cores were transported to the field site wet laboratory, and transferred to a Jahnke-type core squeezer fitted with 16 staggered ports.
spaced 2 cm apart to a total length of 30 cm from the sediment-water interface. (Figure 3.2) (Jahnke, 1988).

Pore waters were extracted by pressurizing the core squeezer from the bottom, and were collected in 95/5 nitrogen/hydrogen-purged ground glass syringes after passage through 5 cm ports fitted with acid-cleaned 70 µM Porex filters (Interstate Specialty Products) and 3-way luer stopcocks (Medex Technologies). The first ~2 mL of pore water was discarded through the open stopcock port in order to preserve anoxic conditions. Pore waters used for geochemical analyses either were analyzed by syringe port, or, in cases where pore water volumes were limited, various syringes from a particular depth range were combined. Pore waters used for kinetic experiments were combined according to Fe(II) concentrations, as PCNB reduction kinetics were dependent upon levels of dissolved Fe(II) in the pore waters.

Natural levels of $H^+$, dissolved Fe(II), and DOC were measured to obtain depth profiles of pore water chemistry. Pore water pH was measured at the OWC field site (Radiometer pH M84 pH probe/meter combination or Beckman 240 pH/temp meter and Orion Thermo Aqua Pro pH probe) inside a glovebox under a 95/5 nitrogen/hydrogen v/v atmosphere with a Pd-catalyzed $O_2$ scrubber (Coy Labs). Aliquots for Fe(II), total Fe and total Mn measurements were prepared by acidifying freshly-collected porewaters with 2 N HCl (~10 µL HCl for every mL porefluid) and filtering the acidified sample with a Milli-Q rinsed 0.45 µm membrane filter (Pall Life Sciences IC Acrodisc 25 mm syringe filter with Supor polyethylene sulfone membrane). For the Fe(II) measurement, a 1 mL aliquot was reacted with 2.5 mL Milli-Q water, 1 mL of 5.0 mM 1,10-phenanthroline and
0.5 mL of a 3.82 mM ammonium acetate dissolved in an 18% v/v glacial acetic acid aqueous solution, and the absorbance of the complex at 508 nm was measured spectrophotometrically (either with a Varian Cary 1 UV-Vis Spectrophotometer, Shimadzu UV-1201 UV/Vis Spectrophotometer, or Beckman-Coulter DV520). Total Fe and total Mn were measured with a GBC Avanta Sigma atomic absorption spectrometer (GBC Scientific Equipment Company) in air/acetylene mode at OWC. Pore waters used for DOC measurements were acidified and 0.45 µm-filtered, and concentrations measured with a Shimadzu TOC 5000. I also examined how acidification of the samples could alter the pore water chemistry, because some samples were acidified in order to slow Fe(II) oxidation, which simplified the transport of the samples from the field to the lab in Columbus, Ohio (OSU). Pore waters to be used for experiments either were unaltered and stored in glass serum vials with rubber stopper septa (“unaltered” pore waters), or acidified with 2 N HCl (~10 µL HCl for every mL of pore water) and re-adjusted to the initial pore water pH prior to use in DPP and kinetic experiments (“pH-adjusted” pore waters).

3.2.2. Chemicals and Fulvic Acid Isolate

Pentachloroaniline (PCA; Alfa Aesar), MOPS (3-[N-morpholino]propanesulfonic acid]; Sigma-Aldrich, 99%), FeCl₂·4H₂O (Certified, Fisher Scientific; Alfa Aesar), ammonium acetate (Jenneile Enterprises; Fisher Certified ACS), 1,10-phenanthroline monohydrate (Certified ACS, Fisher Scientific), glacial acetic acid (Certified ACS Plus, Fisher Scientific), Fe(NO₃)₂·(SO₄)₂·6H₂O (Certified ACS, Fisher Scientific), MnCl₂·4H₂O (Baker Analyzed Reagent), H₂SO₄ (Certified ACS, Fisher Scientific), and Na₂S₂O₄
(EMD Chemicals) were used as received. Pentachloronitrobenzene (PCNB) (Sigma-Aldrich) was recrystallized in cold methanol (<0°C) prior to use. Concentrated HCl was used to prepare acid solutions, and solid NaOH was used to make base solutions (Mallinckrodt AR; Fisher Certified ACS). Methanol (HPLC Grade, Fisher Scientific) and Milli-Q water (Milli-Q UV Plus, Millipore) were used to prepare solutions and HPLC mobile phase. Old Woman Creek surface water fulvic acid (OWCFA) was isolated by the XAD-8 method (Leenheer, 1981) and a stock solution made of OWCFA and Milli-Q was used for the controlled system experiment.

3.2.3. Differential Pulse Polarography

All differential pulse polarography (DPP) scans were performed with a Metrohm 797 VA Computrace analyzer with a hanging drop mercury electrode. Scans were performed in differential pulse mode, which measures current during intervals of a stepwise change in applied voltage. DPP is similar to the square wave voltammetry employed successfully by others in the measurement of redox-active species present in natural sediments (Luther et al., 1992; Brendel and Luther, 1995; Luther et al., 1996; Taillefert et al., 2000), although the step function differs slightly. Mercury was pressurized to 15 psi with Ar gas. Potentials were applied from –0.1 V to –1.8 V with a 10 s equilibration time, 0.099 V/s sweep rate (voltage step = 0.01 V, voltage step time = 0.100 s), pulse amplitude of 0.05 V and pulse time of 0.04 s. All solutions were measured in a cell fitted with an inert air supply that created an anoxic chamber.

Calibration standards were prepared by filling the cell with 20 mL of either a 28 mM or 50 mM MOPS solution at pH 7.77 to 7.90. Aliquots of acidified FeCl₂·4H₂O or
MnCl₂·4H₂O aqueous solutions were added directly to the Ar-purged MOPS solution in the measurement cell, and calibration curves were plotted as current versus concentration. However, Fe(II) and Mn(II) concentrations measured with DPP did not agree with metal concentrations measured by the 1,10-phenanthroline colorimetric method or by atomic absorption (Table 3.1). The Fe(II) and Mn(II) calibration standards were in a salt form, while Fe(II) and Mn(II) measured in the pore waters likely is complexed with DOM due to the excess [DOC] relative to the metal species in the pore waters. Discrepancies between the calibration and natural sample solution matrix can affect the current measured, and metal-organic complexes can affect DPP measurements, as observed by Taillefert et al. (2007a).

Pore water samples were analyzed by DPP either immediately after collection at OWC, or after a specific modification to probe chemical properties of the pore waters as described below. All samples were purged with Ar gas for 1 to 5 min, and analyzed at pH ~8.0. The pH of the DPP-measured samples was much higher than the original pH due to carbon dioxide outgassing during the Ar purging.

### 3.2.4. Kinetic Studies with PCNB

I selected PCNB as the probe compound because it is a mononitroaromatic compound, and therefore is expected to undergo six-electron stepwise reduction to pentachloroaniline (PCA). PCNB also was selected because I am able to compare its reduction in natural pore waters with previous work conducted in controlled systems containing Fe(II) and goethite, and Fe(II) and surface water fulvic acid isolates (Klupinski et al., 2004; Hakala et al., 2007), and because it is an agricultural fungicide.
used in the United States whose presence in natural waters could compromise water quality.

Kinetic studies were performed with both unaltered and pH-adjusted pore waters at the OWC and the OSU laboratories. Kinetic studies at OWC were performed with both unaltered and pH-adjusted pore waters that were kept anoxic after extraction from the sediment cores. The “pH-adjusted” pore waters reacted at OSU were stored at 4°C until use, filtered with a glass fiber syringe tip filter (Gelman Sciences A/E) and purged with Ar prior to transfer to the glovebox. “Unaltered” pore waters reacted at OSU were transported in sealed serum vials on ice and immediately stored in the glovebox upon arrival.

All kinetic experiments were performed in an anoxic glovebox (Coy Labs at OWC; Plas Labs at OSU) under a 95/5 nitrogen/hydrogen v/v atmosphere and equipped with a Pd-catalyst O₂ scrubber (Coy Labs). For experiments where pore water pH was adjusted, Ar-purged 1 N or 2 N HCl or NaOH were used. Prior to reaction, all pore waters were re-filtered with a 1.0 µm glass fiber syringe tip filter or 0.45 µm membrane filter through a 3-way stopcock into the reaction syringe, or drawn into the reaction syringe from a serum vial. For the majority of reactions, I selected the 0.45 µm filter as the cutoff for aqueous species rather than a smaller-sized filter because of concern with altering the natural DOM fractions available to react. In the case of the microbial control experiments, pore waters were filter-sterilized with 0.2 µm membrane filters (25 mm diameter, Pall Acrodisc) into an autoclaved syringe.
Controlled system reaction media were prepared by combining Milli-Q water, MOPS buffer, OWCFA stock solution, and FeCl$_2$·4H$_2$O in a glass bottle outside of the glovebox at pH ~3. The reaction premixture was purged with Ar gas for 1 minute per each mL of solution, and then transferred to the glovebox. The solution pH was raised to the desired level with Ar-purged 2N NaOH inside the glovebox, filtered with a 0.45 µm filter, and allowed to equilibrate overnight. Prior to starting the experiment, the reaction mixture was filtered once again with a 0.45 µm filter, and drawn into a ground glass syringe for the experiment.

For all experiments, PCNB in methanol stock solution was spiked into the reaction media with a target 0.1% v/v methanol/water. PCNB reaction aliquots were quenched at designated time points in 2 mL amber HPLC vials using 20 µL of 2 N HCl or 40 µL of 1 N HCl, and sealed with Teflon-lined septa screw caps. The PCNB parent compound and PCA reduction product were assayed by HPLC (OWC: Waters 501 HPLC pump with Rheodyne injector and Waters 486 tunable absorbance detector; Columbus: Shimadzu SCL-10AT pump, SIL-10A Autosampler, DGU-14A degasser, SCL-10A system controller, and SPD-10A UV/Vis detector). All compounds were detected at a wavelength of 225 nm. Analytes were separated through a reverse-phase C$_{18}$ column (Waters Nova-Pak C$_{18}$, 4 µm, 3.9 x 150 mm; Restek Pinnacle II C$_{18}$, 5 µm, 150 x 4.6 mm) using either an 80/20, 77/23 or 75/25 v/v methanol/water mobile phase acidified to pH 2.8 – 3.5 with concentrated HCl. Concentrations of PCNB and PCA were calculated by comparison with calibration standards of known concentration. Reaction kinetic parameters were determined by least-squares fitting of the natural log of concentration
versus time data with Sigma Plot. The \([\text{PCNB}]_0\) for all reactions was calculated from the linear regression fit, as PCNB reacts immediately with the pore water or controlled system media thus affecting my ability to assess \([\text{PCNB}]_0\) accurately.

Fe(II) concentrations for pore waters and controlled system reactions were measured spectrophotometrically (Cary UV/Vis Spectrophotometer) using the 1,10-phenanthroline method described above with 0.45 \(\mu\)m filtered or 0.2 \(\mu\)m filtered (Milli-Q rinsed; Acrodisc 13 mm syringe filter with HT Tuffryn membrane) acidified samples. DOC concentrations were measured for 0.45 \(\mu\)m filtered pore water reaction media using a Shimadzu TOC 5000, and were calculated for controlled system reaction media based upon DOC concentrations of the OWCFA stock solution.

3.3. Results and Discussion

3.3.1. Establishment of Pore Water Redox Chemistry

An increase in \([\text{Fe(II)}]\) and \([\text{DOC}]\), and decrease in pH, were observed with depth in pore waters from OWC, although \([\text{Fe(II)}]\) and \([\text{DOC}]\) varied temporally (Figure 3.3). Concentrations of Fe(II) ranged from \(< 100\) to \(\sim 1200\) mM, pH ranged from 6.7 to 7.6, and \([\text{DOC}]\) ranged from 1.2 to 2.9 mM C (Figure 3.3). These profile trends are consistent with previous work on OWC sediment pore fluids (Sacco, 1996; Chin et al., 1998). One exception occurs with the May 2006 data, where \([\text{Fe(II)}]\) showed no overall increase with depth.

DPP measurements of Ar-purged pore waters revealed 3 main redox-active species, and one minor peak that is not present in all samples (Figure 3.4). Fe(II) and Mn(II) peaks, located at \(-1.27\) V and \(-1.43\) V, respectively, were verified with calibration
standards. Although the initial pore water pH is between 6.7 and 7.6, all pore waters were purged with Ar gas prior to DPP measurement in order to maintain a common pH (~8.0) during the measurements. Comparison of pre- and post- Ar-purge DPP scans show that the Fe(II) and Mn(II) peaks remain unchanged in both measurements, however, peak potential and current changes occur between –0.1 and –0.7 V (Figure 3.4). This likely is the result of changes in the speciation of the metal-organic complexes due to pH changes resulting from the Ar purge. Although iron sulfide complexes and other dissolved sulfur species have been measured by others within this half-cell potential range (–0.1 to –0.7 V) in natural pore waters (Rozan et al., 2000), the neutral pH of unpurged pore waters (range 6.7 to 7.6), high dissolved Fe(II) content, lack of sulfidic smell and lack of black precipitate (which would indicate an iron sulfide mineral, per Hoferkamp and Weber (2006)) indicated that the contribution of inorganic sulfur species towards OWC pore water chemistry is minimal.

I identified the peak at a half-cell potential around –0.43 V as an Fe(III)-organic peak by treating a fraction of pore water with a chemical reductant, after Brendel and Luther (1995) (Figure 3.5). They tentatively identified a peak at –0.7 V in their measurements of Boston Harbor pore waters as Fe(III) colloids and/or Fe(III)-organic complexes, and Taillefert et al. (2000) found that the different Fe(III)-organic size fractions identified in controlled laboratory experiments also were found in pore waters in a sediment core from the New Jersey shelf. Taillefert et al. (2002) also found soluble Fe(III)-organic complexes in creek sediment pore waters where low concentrations of sulfides were present, and Taillefert et al. (2007b) observed an increase in Fe(III)-organic
aqueous complexes with depth in sediments from a creek bank adjacent to a salt marsh. These observations confirm that Fe(III)-organic peaks are present in sediment pore waters, however, this is the first study to document a steady increase in the Fe(III)-organic peak to a depth of ~30 cm in freshwater wetland sediment pore waters (Figures 3.6–3.8). Furthermore, comparison of colorimetric Fe(II) and atomic absorption Fe measurements reveals that, while the majority of Fe in the pore waters is Fe(II), dissolved Fe(III) species exist and increase in concentration with depth in wetland sediment pore waters (Table 3.1; Figure 3.9), corroborating the observations of Luther et al. (1996) and Taillefert et al. (2000).

In order to verify that the disappearance of the peak at −0.43 V was a result of chemical reduction of Fe(III)-organic species to Fe(II) by sodium dithionite, I monitored the [Fe(II)] for unaltered and dithionite-treated pore waters. A total Fe measurement also was performed by reacting a fraction of pore water with hydroxylamine HCl for 48 h to reduce all of the Fe in the sample to Fe(II), and Fe(II) was measured by the 1,10-phenanthroline method. The [Fe(II)] was 190 µM in the hydroxylamine HCl-treated sample, and 160 ± 10 µM for untreated and dithionite-treated pore waters. Although the Fe(II) peak at −1.27 V in the dithionite-treated pore water has a lower current than the untreated pore water, the [Fe(II)] measurements performed with the 1,10-phenanthroline method verify that the total dissolved Fe remains unchanged during a short reaction time with dithionite. No change in the Fe(II) peak at −1.27 V occurs likely due to the formation of Fe(II)-thiosulfate complexes that are not visible within the DPP scan
window. Pore waters reacted for more than 12 h with dithionite formed a black precipitate and no dissolved Fe(II) was detectable in these solutions.

Pore waters were treated with a Na

\(^{+}\)

-saturated cation-exchange cartridge in order to remove dissolved metal cations from solution. This treatment removed the three major redox-active peaks (Figure 3.5). The peak at –1.0 V was not completely removed from fresh pore waters after the cation exchange treatment, and Fe(II) was detected at low levels (~10 µM) with the 1,10-phenanthroline method (Figure 3.5). No Fe(II) was detectable by the 1,10-phenanthroline colorimetric method in pore waters that were acidified to pH ~2.5, then treated with a cation-exchange cartridge and pH adjusted to ~7.8. This indicates that the neutral species most likely contains Fe(II), though the precise nature of the complex remains unclear from this information. I therefore identify the major redox-active components of OWC porewaters as Fe(II), Mn(II) and Fe(III)-organic species.

Changes in the main redox-active species are observed with depth in OWC sediment pore waters, where levels of Fe(III)-organic and Fe(II) species increase with depth, and Mn(II) reaches a maximum in shallow pore waters (Figures 3.6–3.8). These observations are consistent with previous accounts of pore water redox speciation changes with depth (Brendel and Luther, 1995; Taillefert et al., 2000, 2002, 2007b). In addition to DPP measurements, pore water [DOC] and [Fe(II)] (colorimetric) were measured with depth for all cores, and in most cases show an increase in concentration with depth (Figures 3.6–3.8). For the August 2007 core, specific conductivity, Fe\(_T\), and Mn\(_T\) also were measured, and all measurements showed an increase with depth in the
sediments except for Mn, which remained at a constant level after reaching a maximum in the shallow pore waters (Figure 3.9).

DPP profiles for the June and July 2007 pore waters both show an steady increase with Fe(II) and Fe(III)-organic peak currents with depth from the sediment-water interface (Figures 3.6, 3.7). The Mn(II) peak current increases until ~ 6 cm depth in both cores, and either remains constant or lowers slightly with depth in the cores (Figures 3.6, 3.7). For both cores, the [Fe(II)] measured colorimetrically increases with depth. The [DOC] reaches a maximum around 6 cm depth in the June 2007 core, and remains at a constant level in the July 2007 core (Figures 3.6, 3.7).

During the period in between the June and July 2007 sampling trips, the mouth of the wetland remained isolated from Lake Erie due to the formation of a sand barrier beach between the wetland and Lake Erie. Wetland sediments remained waterlogged during this time, and were generally undisturbed. Under these static hydrologic conditions, benthic activity by larger organisms and microbial metabolic reactions are the main factors expected to affect pore water redox chemistry. The DPP profiles were similar for both the June and July 2007 cores, although the July 2007 cores showed higher peak currents relative to the June 2007 cores (Figures 3.6, 3.7). This observation reflects either a slight difference in sediment (and hence, dissolved pore water) metal concentrations between cores, enhanced microbial metabolic activity in between the two sampling trips, or a combination of both effects.

The August 2007 pore water profiles differed relative to the June and July 2007 profiles (Figure 3.8). The DPP measurements from August 2007 show that pore waters
from 1.4 and 5.9 cm average depth are similar to surface waters, while results from 3.0, 13.2 and 25.6 cm average depth are more similar to DPP scans of the pore waters collected during June and July 2007. A major storm event occurred one week prior to the August 2007 sampling trip, and the influx of precipitation and surface waters from the surrounding watershed broke the barrier beach in between the wetland and Lake Erie. Changes in surface hydrologic conditions likely affected the redox chemistry of the sediment pore waters, however, this was not investigated more fully with this work.

Another interesting feature of the August 2007 pore water profiles is that Fe(II) in some cases is detected with the colorimetric method while the corresponding peak is absent in the DPP measurements (Table 3.1; Figure 3.8). One explanation for this phenomenon is the presence of dissolved Fe(II) species that are undetectable by reaction with mercury at the working electrode tip. On the other hand, Fe(II) in OWC pore waters may be complexed with certain organic ligands that reduce the peak current of Fe(II) measured by DPP, the converse of an enhancement of Fe(II) peak current with certain organic ligands observed by others (Taillefert et al., 2007a). However, the exact nature of this discrepancy was not investigated further with this study.

OWC pore water redox geochemistry is dominated by Fe(III)-organic, Fe(II) and Mn(II) species, and levels of Fe(II) and Fe(III)-organic complexes increase with depth in the pore waters. Mn(II) reaches a maximum around 6 cm depth, and either remains constant or decreases slightly at depths > 6 cm. Under static hydrologic conditions and a well-developed redox profile, I expect dissolved Fe(II) to act as an important redox-
active metal capable of participating in electron-transfer reactions in OWC sediment pore waters.

3.3.2. Kinetic studies with Pentachloronitrobenzene

PCNB is reduced in natural pore waters and obeys pseudo-first-order kinetics in both unaltered and “pH-adjusted” pore waters (Figure 3.10). In some cases, however, reduction observed in unaltered pore waters only weakly fit a pseudo-first-order model (Figure 3.11). These results corroborated the observations of Klausen et al. (1995) who reported deviation from first order behavior for contaminants reacting in natural samples. However, in order to remain consistent while comparing kinetics results, all PCNB reduction kinetics reported here are modeled with a pseudo-first-order mechanism. Microbial reduction of PCNB was not an influence during the kinetic studies reported here, as reduction in 0.45 µm filtered and 0.2 µm filter-sterilized experiments was the same within the 95% confidence interval of the calculated rate constants (Figure 3.11).

Fe(II) is necessary for reduction to occur, as pore waters treated with a Na+-saturated cation exchange cartridge showed no reduction, or very limited reduction, over the same time frame during which reduction occurred in pore waters with Fe(II) in the hundreds of micromolar concentration (Figure 3.12). In some cases, not all of the Fe(II) was removed from the cation-exchanged pore water, indicating that some Fe(II) is strongly bound to DOM within these systems. In Figure 3.12.A, the [PCNB] inexplicably drops off after ~ 12 h in the reaction with Fe(II) and I currently have no explanation for this phenomenon. Reduction also is dependent upon pH alteration of the sample, as PCNB transformation in the “pH-adjusted” pore waters occurred much more quickly as
compared to reactions performed in unaltered pore waters (Figure 3.13). Surprisingly, PCNB kinetics in the “pH-adjusted” pore waters show similar reaction rates to artificial solutions containing Fe(II), OWCFA, and MOPS buffer (Figure 3.13).

An experiment was performed to assess pore water reactivity towards PCNB in “pH-adjusted” and “unaltered” pore waters to elucidate possible explanations for these highly divergent differences in reactivity. A fresh combined pore water (pore water combined from various syringes with high [Fe(II)]) was divided into two vials within 24 h after collection inside the glovebox at the OWC laboratory. Pore water in one vial was acidified to pH 2.5 and allowed to equilibrate for ~1 h, and subsequently treated with 2 N NaOH to raise the pH to the original pH of the fresh pore water. The other portion of pore water was left unaltered. Each solution was drawn into a separate syringe and spiked with the PCNB stock solution. Reduction was monitored at 5 min and 48 h, as pore water volumes were severely limited. At 48 h, roughly 60% of PCNB reacted to PCA in the “pH-adjusted” pore waters, while only ~11% of PCNB reacted in “unaltered” pore waters (Figure 3.14). The pH adjustment of pore water significantly enhanced its ability to reduce PCNB.

DPP measurements were performed on the “pH-adjusted” and “unaltered” pore waters used for the reactions presented in Figure 3.14. The current of the Fe(III)-organic peak in the “pH-adjusted” pore waters is smaller relative to the current measured for the Fe(III)-organic peak in the “unaltered” sample, while the Fe(II) and Mn(II) peaks remain unchanged (Figure 3.15). In order to test the effect of pH adjustment over longer periods of time, fresh pore water was acidified to pH ~ 2.5, exposed to ambient atmosphere, and
allowed to equilibrate at pH ~2.5 for 24 h (most “pH-adjusted” pore waters were acidified for a minimum of 24 h before use in PCNB kinetic studies). After equilibration, the pore water was purged with Ar gas and the pH was adjusted to ~7.8. DPP scans showed that the Fe(III)-organic peak completely disappeared when the pore water was allowed to equilibrate under acidic conditions for 24 h (Figure 3.16).

Differences in PCNB reduction in the “pH-adjusted” and unaltered pore waters can be explained by the speciation of redox-active metals in natural pore waters. Although the metal-DOM speciation in benthic pore waters remains undocumented, a few inferences about Fe(II) speciation in OWC pore waters can be made by comparison with metal speciation in other systems. Fe(III) in the presence of fulvic acids and absence of iron hydroxides was found to form organic complexes with the innermost coordination shell consisting of O and N ligands present in soil organic matter (Gustafsson et al., 2007) and O ligands in the case of Fe(III) complexed with natural organic matter in surface waters (Vilgé-Ritter et al., 1999). Although Fe(II) speciation in anoxic pore waters remains poorly understood, Fe(II) is expected to behave as a soft metal in natural systems. In this capacity, Fe(II) can complex with O, N and S functional groups within DOM, similar to Cd complexes identified by Karlsson et al. (2005). Rose and Waite (2005) reported a weak correlation between the stability constants for Fe(II) and Fe(III) complexes with natural organic matter in surface waters, and suggested that similar DOM functional groups are involved in complexing both Fe(II) and Fe(III). It is possible for Fe(II) and Fe(III) to complex with similar ligands within pore water DOM.
In the pH-adjusted pore waters, complex dissociation and formation kinetics are suspected of influencing the Fe(II) ligand environment. In a study using Suwannee River fulvic acid isolate (SRFA), Fe(II) and Fe(III), Rose and Waite (2003) observed that the dissociation of both strong and weak Fe(III)-SRFA complexes occurred more slowly than Fe(II)-SRFA complexes (0.032 x 10⁴ s⁻¹, 2.0 x 10⁴ and 7.87 x 10⁴ s⁻¹, respectively). Formation of Fe(II)-SRFA complexes was faster than Fe(III)-SRFA complexes (2.45 x 10⁻⁴ M⁻¹s⁻¹ and 6.0 x 10⁻⁶ M⁻¹s⁻¹, respectively) (Rose and Waite, 2003).

The data from Rose and Waite (2003) complement the observations presented in this study. Pore waters allowed to reside at low pH over short periods of time (~ 1 h) resulted in a slight decrease in the current of the Fe(III)-organic peak (Figure 3.15). Because Fe(III)-organic complexes are not kinetically favored to form relative to Fe(II)-organic complexes (Rose and Waite, 2003), and an Fe(III)-organic peak was observed in the “pH-adjusted” DPP scan shown in Figure 3.15, I conclude that some Fe(III)-organic complexes did not completely dissociate during the short-term pH adjustment period. On the other hand, the Fe(III)-organic peak completely disappeared in DPP scans of pore waters that were subject to low pH conditions for ~24 h before being raised. Aqueous Fe(III) is not detectable within the DPP scan window. This phenomenon provides evidence that all of the Fe(III)-organic complexes, including both weak and strong Fe(III) complexes, had completely dissociated.

Others have shown that synthetic catechol and other oxygen-containing ligands capable of complexing Fe(II) actually enhance the reductive capacity of Fe(II) towards Cr(VI) and nitroaromatic compounds (Buerge and Hug, 1998; Naka et al., 2006). We
suspect that pH-adjustment of the pore waters results in the formation of Fe(II)-DOM complexes with much lower reduction potentials relative to the Fe(II)-DOM complexes that exist in native OWC pore waters, and highlights how sample treatment can affect the study of electron transfer reactions in natural pore waters.

3.4. Fe(II) and DOM in Natural Pore Waters

The data presented here show that Mn(II), Fe(III)-organic, Fe(II) and DOM are present naturally in OWC pore waters. Nitroaromatic reduction reactions performed to probe the electron transfer capacity of OWC pore waters reveal that Fe(II) is an important reductant in these systems, but that such reactivity is intimately tied to the speciation of Fe(II). Although details regarding the nature of Fe(II)-DOM speciation in anoxic wetland sediment pore waters is unknown to date, I hypothesize that dissolved Fe(II) present naturally in pore waters is complexed with Fe(II)-stabilizing ligands, rendering them less reactive and that the existence of stable Fe(III)-DOM complexes ties up ligands that can potentially make Fe(II) a better reductant. When pore water is subject to pH-adjustment, Fe(II) is able to complex with newly-available Fe(III)-stabilizing ligands, which significantly enhances Fe(II) reductive capacity towards PCNB.

The formation of relatively stable Fe(II)-DOM complexes in natural pore waters depends upon various dissolution and sorption reactions that occur in wetland sediments. Chin et al. (1998) hypothesized that Fe(II) and DOM are released to sediment pore water through the reductive dissolution of organic matter-coated iron hydroxides present on the surface of wetland sediments. Gybos et al. (2007) reported the release of DOM, Mn, and Fe(II) and a rise in pH in incubated anaerobic wetland soils, and a release of DOM (but
not Fe(II)) from aerobic wetland soils at pH 7. Avena and Koopal (1998) reported fast and reversible desorption of humic acids from iron oxide-coated silicon plates with changes in pH, and very limited desorption caused by dilution effects. It is possible that a combination of the reductive dissolution of DOM-coated iron hydroxides and pH-driven desorption of DOM occurs in OWC sediments, thereby affecting Fe(II) speciation with DOM and its reductive capacity in natural systems. An understanding of the specific complexation environment of Fe(II) in aqueous anoxic systems and the formation of Fe(II) complexes in these environments are important areas for future investigation.
### 3.5. Tables

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- **a** Averaged depth from the sediment-water interface of combined pore waters
- **b** Half-cell potential of peak in electrochemical scan
- **c** Current of peak in electrochemical scan
- **d** Concentration calculated from electrochemical scan and electrochemical calibration curve
- **e** Concentration measured colorimetrically with the 1,10-phenanthroline method
- **f** Concentration measured by atomic absorption

### Table 3.1. Comparison of Fe(II) and Mn(II) concentrations measured with DPP, colorimetrically and by atomic absorption. Pore waters were collected in August 2007 and the data correspond to data presented in Figure 3.8. Where shown, n/a indicates the absence of a peak in the DPP scan.
3.6. Figures

**Figure 3.1:** Aerial photograph of Old Woman Creek wetland, Ohio, courtesy of David Klarer. The star represents the sampling location for all coring trips. The water body north of the wetland mouth is Lake Erie.
Figure 3.2. Jahnke-type core squeezer. The clamped plastic tube to the left of the squeezer supplies the 95/5 nitrogen/hydrogen gas to purge the syringes prior to squeezing.
Figure 3.3 Sediment pore water chemistry profiles of A) Fe(II), B) pH and C) DOC. Lines are shown to highlight data points with depth, and are not statistical fits of the data. Values from August 2007 data represent the average of depths of pore waters that were combined to collect the data.
Figure 3.4. Influence of Ar-sparging on DPP measurements of pore water collected from one syringe port. The same pore water was measured pre- and post-Ar sparging.
Figure 3.5. A) Unaltered pore water combined from multiple syringe ports (solid line) and a fraction of the same combined pore water treated with sodium dithionite (dotted line). B) Unaltered pore water combined from multiple syringe ports (solid line) and a fraction of the same pore water treated with an ion-exchange cartridge (dash-dot line). The peak at ~ -1.0 V appeared randomly in pore water samples and was not identified in this study.
Figure 3.6. Geochemical data for June 2007 pore waters. A) DPP scans for pore waters collected from different depths (depth vs. peak current profiles are shown in B and C). An increase in current indicates an increase in concentration of the redox-active species. B) Pore water [Fe(II)] with depth as measured by the 1,10-phenanthroline method (top x-axis), and current of the Fe(II) and Mn(II) peaks with depth at half-cell potentials indicated in the legend (bottom x-axis). C) Pore water [DOC] with depth (top x-axis), and current of the Fe(III)-organic peak with depth at half-cell potentials as indicated in the legend (bottom x-axis). In B and C, lines are point-to-point and do not represent statistical fits of the data.
Figure 3.7. Geochemical data for July 2007 pore waters. A) DPP scans for pore waters collected from different depths (depth vs. peak current profiles are shown in B and C). An increase in current indicates an increase in concentration of the redox-active species. B) Pore water [Fe(II)] with depth as measured by the 1,10-phenanthroline method (top x-axis), and current of the Fe(II) and Mn(II) peaks with depth at half-cell potentials indicated in the legend (bottom x-axis). C) Pore water [DOC] with depth (top x-axis; values for some depths are not reported due to limited sample volumes), and current of the Fe(III)-organic peak with depth at half-cell potentials as indicated in the legend (bottom x-axis). In B and C, lines are point-to-point and do not represent statistical fits of the data.
Figure 3.8. DPP scans for combined pore waters collected in August 2007. Depth vs. peak current profiles are shown in C and D. Each depth point represents the average depth for the combined pore waters. A) Entire core. B) Magnification of current axis to show detail for surface water (0 cm), 1.4 cm, 5.9 cm and 3.0 cm scans. C) Depth profiles of Fe(II) and Mn(II) peak currents. D) Depth profiles of Fe(III)-organic peak currents. For C and D, the reported depth is an average of depths of combined pore waters, and lines are shown to connect data points and do not represent statistical fits of the data.
Figure 3.9. Geochemical data for pore waters from August 2007 (same pore waters analyzed in Figure 3.8). A) Total Mn (top x-axis), total Fe, and Fe(II) measured by the 1,10-phenanthroline method (bottom x-axis). B) Specific conductivity (top x-axis) and DOC concentrations (bottom x-axis).
Figure 3.10. PCNB reduction in natural pore waters. A) pH-adjusted (see description of method in the text), pH_{t=0} = 7.66, pH_{t=end} = 7.41, [Fe(II)] = 810 µM, [DOC] = 2.15 mM C, pseudo-first-order $k_{obs} = 0.683 \pm 0.026$ h^{-1} ($R^2 = 0.9902$). B) Unaltered, pH_{t=0} = 7.77, [Fe(II)] = 380 µM, [DOC] = 3.22 mM-C, pseudo-first-order, $k_{obs} = 0.042 \pm 0.004$ h^{-1} ($R^2 = 0.9314$). Rate constant errors are reported as the standard error for the integrated rate fit of the observed data. For both A and B, the main plot shows the integrated rate fit and the inset shows the disappearance of PCNB, formation of PCA and mass balance (PCNB+PCA) during the course of each reduction reaction. For the insets, PCNB is represented by solid circles, PCA is represented by open circles, and the mass balance (PCNB + PCA) is represented by solid triangles.
Figure 3.11. Kinetic data for PCNB reduction in natural pore waters for 0.45 µm filtered unsterile and 0.2 µm filtered sterile solutions. For the unsterile reaction, pH$_{t=0}$ = 7.54, pH$_{t=end}$ = 7.69, [Fe(II)] = 410 µM, [DOC] = 1.24 mM C, and pseudo-first-order, $k_{obs} = 0.023 \pm 0.002$ h$^{-1}$ ($R^2 = 0.9392$). For the sterile reaction, pH$_{t=0}$ = 7.55, pH$_{t=end}$ = 7.65, [Fe(II)] = 420 µM, [DOC] = 1.49 mM C, and pseudo-first-order $k_{obs} = 0.026 \pm 0.003$ h$^{-1}$ ($R^2 = 0.9308$). Note that the last observed data point for the unsterile reaction was not included in the fit because it appeared that the reaction stopped after ~1 $t_{1/2}$. Higher [Fe(II)] and [DOC] in the sterile sample are due to trace compounds present in the sterilized filter.
Figure 3.12. Reduction of PCNB to PCA in “unaltered” pore waters A) with natural levels of Fe(II), and B) treated with a cation-exchange cartridge. Reactions were performed at pH 7.20 ± 0.01, [DOC] = 1.92 ± 0.07 mM C, and [Fe(II)] = 250 µM for (A) and 10 µM for (B).
Figure 3.13. Log of concentration-normalized pseudo-first-order $k_{obs}$ for PCNB reduction in “pH-adjusted” and “unaltered” pore waters, and in controlled systems containing Fe(II) and OWCFA. Where not shown error bars (representing the 95% confidence interval for the rate constant fit) are smaller than symbols. Rate constants were normalized to Fe(II) concentrations in the µM scale, and to DOC by µM C.
Figure 3.14. PCNB reduction to PCA in unaltered and “pH-adjusted” pore waters after 48 h of reaction; [Fe(II)] = 160 µM and [DOC] = 2.15 mM C for both reactions. For the “unaltered” pore waters, pH\(_0\) = 7.46 and pH\(_\text{end}\) = 7.45. For the “pH-adjusted” reaction, the pore water was acidified to pH 2.26 and re-raised to pH\(_0\) = 7.52. For the “pH-adjusted” pore waters, pH\(_\text{end}\) = 7.62.
Figure 3.15. DPP scans for pore waters used for PCNB reduction experiments reported in Figure 12. [Fe(II)] and [DOC] are the same as reported in Figure 12. Each solution was purged with Ar gas prior to measurement, thus the pH of the solutions is ~7.9.
Figure 3.16. DPP scans of pore water from July 2007. The “unaltered” pore water scan is an average of scans from 0.5 cm and 4.5 cm depths (collected at pH ~8) and is shown as a comparison for the “pH-adjusted” pore water, which is a combination of pore waters from 0.5 cm, 4.5 cm and 6.5 cm depth that was acidified immediately after collection and the pH was raised to ~7 inside the DPP sample cell.
3.7. References


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4.1. Introduction

The environmental fate of pesticides is of concern due to their potential deleterious effects on aquatic ecosystems. Dinitroaniline herbicides are a particular class of pesticide used widely throughout the United States as a pre-emergent treatment on a variety of agricultural crops (Grover et al., 1997; USGS 2002a,b). Although these compounds generally are retained in the soil column when incorporated into agricultural soils (Grover et al., 1997), erosion and surface water transport of suspended solids results in widespread contamination of sediments in United States waterways (USEPA, 2004).

Natural wetlands are capable of degrading contaminants in both surface waters and sediments through several biotic and abiotic pathways. Contaminated particles can settle in wetland sediments due to the slowing of water flow, and emergent vegetation naturally present in such environments can trap suspended particles (Wilson et al., 2002). The fate of hydrophobic compounds, such as dinitroaniline herbicides, in the sediment phase is of particular interest as such compounds either are recalcitrant or can be transformed by microbes and abiotic species present naturally in benthic sediments.
Benthic sediment pore waters have been found to contain high concentrations of dissolved Fe(II) (in the hundreds of micromolar concentration range) and dissolved organic matter (DOM; measured as dissolved organic carbon (DOC), up to ~ 3 mM C) in some environments (Chin and Gschwend, 1991; Chin et al., 1998; O’Loughlin and Chin, 2004). Dissolved Fe(II) and surface water fulvic acid isolates are known to promote the abiotic reduction of pentachloronitrobenzene (PCNB) in controlled laboratory solutions (Hakala et al., 2007) and complete reduction of PCNB to pentachloroaniline (PCA) has been observed abiotically in natural pore waters containing high concentrations of Fe(II) and DOM (Chapter 3). The abiotic reduction of dinitroaniline herbicides, which contain nitro moieties susceptible to reduction, has the potential to occur in the presence of Fe(II) and DOM in both controlled systems and natural pore waters.

Trifluralin (TR-1) and pendimethalin (PM) are dinitroaniline herbicides used extensively for pre-emergent weed control on agricultural lands. (TR-1 is used to describe trifluralin because multiple trifluralin derivatives exist, and are denoted as “TR-xx” elsewhere in this chapter). United States Geological Survey (USGS) agricultural pesticide use maps for 2002 show that a combined 60% of TR-1 use in the United States was on soybean and cotton crops (roughly 3.0 and 2.6 million lbs, respectively) (USGS, 2002a). TR-1 also was used on wheat, sugarcane, sunflower seed, dry beans, tomatoes, sorghum and green bean crops (USGS, 2002a). PM was used primarily on soybeans, cotton and corn (a total 80% of use; 5.13, 2.62 and 2.51 million lbs, respectively), and also was used to treat sugarcane, peanuts, rice, sunflower seed, potatoes, tobacco and dry onions (USGS, 2002b). Several investigators have reported reduction of TR-1 and PM in
controlled laboratory systems in the presence of Fe(II)\textsubscript{aq}, Fe(II)/goethite, and HS/juglone reductant systems (Klupinski and Chin, 2003; Wang and Arnold, 2003). However, to date the specific role of dissolved Fe(II) and DOM on the fate of TR-1 and PM in natural systems remains unclear.

In this study, reduction kinetics of TR-1 and PM were studied in controlled laboratory systems containing Fe(II) and surface water fulvic acid isolates (“controlled systems”), and in natural pore waters collected from freshwater wetland sediments. I hypothesize that dissolved Fe(II) is necessary for reduction to occur; that degradation kinetics will differ between TR-1 and PM; that the DOM source material and pH will affect reduction in controlled systems; and that reduction occurs in natural pore waters. The major goals of this study were to model TR-1 and PM reduction kinetics in the presence of Fe(II) and DOM isolates, and to compare results from controlled laboratory systems with natural benthic pore waters.

4.2. Materials and Methods

4.2.1. Chemicals and Dissolved Organic Matter

Trifluralin (TR-1; 2,6-dinitro-\textit{N},\textit{N}-dipropyl-4-(trifluoromethyl)benzenamine; Riel-de Haën, 99.1% purity) and derivative standards (all from Dow AgroSciences, ≥ 99% purity) TR-2 (2,6-dinitro-\textit{N}-propyl-4-(trifluoromethyl)benzenamine), TR-4 (3-nitro-\textit{N}^2,\textit{N}^2-dipropyl-5-(trifluoromethyl)-1,2-benzenediamine), TR-6 (3-nitro-5-(trifluoromethyl)-1,2-benzenediamine) and TR-15 (2-ethyl-4-nitro-6-(trifluoromethyl)-1\textit{H}-benzimidazole) were used as received and made into separate stock solutions in methanol (HPLC Grade, Fisher Scientific). Pendimethalin (PM; N-(1-ethylpropyl)-3,4-
dimethyl-2,6-dinitroaniline; Riel-de Haën, 98.4 % purity) was used as received and made into a stock solution in methanol. Methanol and Milli-Q water (18 MΩ water; Milli-Q UV Plus, Millipore) were used as solvents. MOPS (3-[N-morpholino]propanesulfonic acid]; Sigma-Aldrich, 99%), FeCl₂·4H₂O (Certified, Fisher Scientific; Alfa Aesar), Fe(NH₄)₂(SO₄)₂·6H₂O (Certified ACS, Fisher Scientific), ammonium acetate (Jenneile Enterprises; Certified ACS, Fisher Scientific), 1,10-phenanthroline monohydrate (Certified ACS, Fisher Scientific), glacial acetic acid (Certified ACS Plus, Fisher Scientific), concentrated HCl (Certified ACS, Fisher Scientific), concentrated H₂SO₄ (Certified ACS, Fisher Scientific) and solid NaOH (Mallinckrodt AR; Certified ACS, Fisher Scientific) were used as received. Fulvic acid isolates, used as the DOM source in controlled system experiments, were prepared by the XAD-8 method (Leenheer, 1981) using surface waters collected from Pony Lake, Antarctica; Suwannee River, Georgia, USA; and Old Woman Creek, Ohio, USA.

4.2.2. Preparation of Controlled System Reaction Media

All controlled system reactions were performed at the Columbus, Ohio, laboratory (OSU) in a glovebox (Plas Labs) equipped with a Pd-catalyzed O₂ scrubber under a 95/5 v/v nitrogen/hydrogen atmosphere. All solutions prepared outside of the glovebox were purged with Ar gas for 1 min per mL solution prior to transport into the glovebox and use in experiments. Controlled system reaction media were prepared using aqueous MOPS stock solution diluted with Milli-Q, and for reactions containing DOM, an aqueous stock solution prepared with the fulvic acid isolate was added to the reaction medium. FeCl₂·4H₂O was added to the reaction medium either as a solid or a pH ~ 3 stock solution.
for reactions containing Fe(II). The solution pH was adjusted with 2 N NaOH inside the glovebox and pH was measured using a Beckman 240 pH/temp meter and Orion Thermo Aqua Pro pH probe. All solutions were filtered with a Milli-Q rinsed 0.45 µm membrane filters (Pall Life Sciences IC Acrodisc 25 mm syringe filter with Supor polyethylene sulfone membrane) and allowed to equilibrate for a minimum of ~30 min. Previous work showed that nitroaromatic reactivity was unaffected by reaction medium equilibration time (Klupinski et al., 2004; Hakala et al., 2007). After equilibration, each solution was drawn into a ground glass syringe (Popper & Sons). Solutions used for filter-sterilized control reactions were filtered through a sterile 0.2 µm membrane filter (25 mm diameter, Pall Acrodisc) using a sterile 3-port valve (Medex Technologies) into an autoclaved ground glass syringe.

4.2.3. Sediment Coring and Pore Water Collection

Sediment cores were collected during August 2007 from Old Woman Creek National Estuarine Research Reserve (OWC), a federally-protected wetland located adjacent to Lake Erie in Huron, Ohio. Intact sediment cores were collected from the lower wetland using a punch-core sampling technique, and transported to the OWC main laboratory. At the OWC lab, sediment cores were hydraulically transferred into core squeezers (Jahnke, 1988) equipped with 16 extraction ports fitted with ¾” screw to female luer fittings and 70 µm Porex rod filters (Interstate Specialty Products). The top and bottom of the squeezer were sealed with Teflon plungers and mounted on Unistrut beam supports. Each screw to female luer fitting was sealed with a 3-port valve, and the valve was fitted with a 10 cc or 20 cc ground glass syringe. The 3-port valves and
syringes were evacuated with 95/5 nitrogen/hydrogen v/v gas to remove O₂. Cores were pressurized by forcing the bottom plunger into the core, and pore waters were forced from the sediments into the ground glass syringes.

After collection, pore waters were immediately transferred to a glovebox (Coy Labs) under 95/5 nitrogen/hydrogen v/v equipped with a Pd-catalyzed O₂ scrubber, and filtered through a 0.45 µm membrane filter directly into glass serum vials. The glass serum vials were sealed with rubber septa in the glovebox at OWC, and then were transported on ice to OSU. At OSU, the serum vials were transferred to the glovebox and stored under 95/5 nitrogen/hydrogen v/v with a Pd-catalyzed O₂ scrubber. Pore waters were drawn into ground glass syringes immediately before reaction with TR-1 and PM.

4.2.4. Measurement of pH, Fe(II) and DOM

Prior to reaction with TR-1 and PM, aliquots of the reaction medium were separated to measure pH and [Fe(II)] for all controlled and pore water reaction samples, and to measure DOM concentrations (for the pore water). The [Fe(II)] was measured by reacting acidified sample aliquots with 1,10-phenanthroline and measuring the complex absorbance at 508 nm (Cary 1 UV/Vis Spectrophotometer), as described in Chapter 3. The DOM concentrations were measured as DOC using a Shimadzu TOC 5000. For controlled systems, [DOC] for fulvic acid stock solutions were used to calculate DOC concentrations for the reaction solution. Pore water [DOC] was measured directly from acidified pore water aliquots.
4.2.5. Kinetics Studies

Ar-purged TR-1 and PM stock solutions were injected directly into the reaction medium-filled ground glass syringes using a glass-tip micropipet, with a 0.1% methanol composition in solution. Kinetic time points were collected by adding a ~2 mL aliquot of the reacted solution to a borosilicate glass autosampler vial preloaded with ~20 µL of 2 N HCl. Reaction vials were sealed with screw caps equipped with Teflon-lined septa. In the case for one reaction with TR-1 in pore waters, aliquots were added directly to the HPLC vials and analyzed without acid quenching in order to detect acid-sensitive reaction byproducts. This method was used successfully by Klupinski and Chin (2003) to detect TR-1 degradation products in systems containing Fe(II) and goethite.

Reaction aliquots were analyzed by HPLC using a Shimadzu SCL-10AT pump, SIL-10A Autosampler, DGU-14A degasser, SCL-10A system controller, and SPD-10A UV/Vis detector equipped with a Restek Pinnacle II C$_{18}$, 5 µm, 150 x 4.6 mm column with an integrated guard column. Both TR-1 and PM were monitored at $\lambda = 225$ nm with a 1.0 mL/min flow rate and 250 µL injection volume. In most cases, the mobile phase consisted of an isocratic 77/23 methanol/water v/v mobile phase acidified to pH ~ 3 with 12 N HCl. For some TR-1 kinetics studies, a gradient method was used for the mobile phase (55/45 methanol/water v/v linearly increased to 82/18 methanol/water v/v at 27 min), as developed by Klupinski and Chin (2003). Concentrations of TR-1 and PM were determined by preparing dilute calibration standards in Milli-Q water from concentrated methanol stock solutions, and assaying the standards by HPLC. TR-2, TR-4, TR-6 and
TR-15 were diluted from methanol stock solutions and analyzed by HPLC to verify the byproduct peak retention times.

Pseudo-first-order rate constants ($k_{\text{obs}}$) were calculated from ln concentration-versus-time data. Initial concentrations of the two herbicides were calculated during the rate fit, as the true initial concentration could not be determined due to immediate reaction of TR-1 and PM upon addition to the reaction medium. All reaction kinetics for the Fe(II)-DOM controlled systems and natural pore waters were modeled using a pseudo-first-order model, as reactions performed to three half-lives in controlled systems showed kinetic rate fits with $R^2 \geq 0.99$.

4.3. Results and Discussion

4.3.1. Controlled Systems

TR-1 and PM degradation follows pseudo-first-order kinetics in controlled systems containing Fe(II) and DOM fulvic acid isolates (Fe(II)-DOM) (Figure 4.1). Both compounds degrade in control reactions containing buffer and Fe(II) (Fe(II)-only), however, neither react in solutions containing only MOPS and DOM (DOM-only) nor buffer-only solutions (MOPS-only) (Figure 4.2). Although others have shown that nitroaromatic reduction is possible by electrochemically-reduced DOM analogs in solutions without Fe(II) (Tratnyek and Macalady, 1989), the data presented here show that dissolved Fe(II) is necessary for rapid reduction of both TR-1 and PM to occur. High concentrations of aqueous Fe(II) previously was found necessary for nitrobenzene and PCNB reduction to occur in systems containing Fe(II) and an iron oxide surface (Williams and Scherer, 2004; Klupinski et al., 2004). Wang and Arnold (2003) studied
Fe(II)-only reduction of TR-1 and PM, however, rate constants for Fe(II)-only reduction were larger in their study relative to those found in this study. A direct comparison between their rate constants and the experiments performed here is not possible because of differences in the Fe(II)-only control reactions. In addition, the inconsistent kinetic rate orders observed in the Fe(II)-only reactions for both TR-1 and PM (both pseudo-zero-order and pseudo-first-order kinetics were observed) precludes the ability to quantitatively compare reduction rates in Fe(II)-only and Fe(II)-DOM controlled systems.

The $k_{obs}$ for TR-1 reduction in a filter-sterilized solution containing both Fe(II) and DOM is slightly slower than the $k_{obs}$ for a comparable reaction in 0.45 µm filtered reaction media (Figure 4.3). The discrepancy observed for TR-1 reduction in these two reactions likely is due to a difference in the reductants available to react with TR-1 in these systems. DOM is operationally defined as being ≤ 0.45 µm in size; a reactive DOM fraction between 0.45 µm and 0.2 µm may have been removed from the system that is critical during TR-1 reduction. Previous work showed that colloidal material in between 0.2 µm and 0.45 µm does not affect reduction of mono-nitroaromatic compounds (Hakala et al., 2007). Therefore, I assume that colloidal material is not a major reductant towards TR-1 in these systems. Furthermore, TR-1 reduction mediated by anaerobic microorganisms in moist soils amended with organic substrate was found to have half-lives on the order of 4 days (Parr and Smith, 1973). In all of the experiments presented here, TR-1 degraded by at least 70% within 2.5 days. Faster reduction in the controlled Fe(II)-DOM systems presented here indicates that the abiotic pathway is dominant in controlled system reactions.
The \( k_{\text{obs}} \) for PM reduction in filter-sterilized solutions is within the 95% confidence interval of the \( k_{\text{obs}} \) for non-sterile solutions (Figure 4.3). These results are similar to those reported for PCNB reduction in non-sterile and filter-sterilized Fe(II)-DOM reaction media (Hakala et al., 2007/Chapter 2). Wang and Arnold (2003) found that only one nitro group on PM is reduced during reduction in the presence of Fe(II), and proposed that the less sterically-hindered nitro group was the reactive ring substituent. I suspect that the less sterically-hindered nitro group is the reactive oxidant in our PM reactions, and that PM thus behaves as a “mono-nitroaromatic-like” compound. Therefore, it is subject to reduction by Fe(II)-DOM by a mechanism similar to that of PCNB. Microorganisms such as soil fungi were found are capable of reducing PM, however, reduction rate constants were not reported for these reactions (Singh and Kulshrestha, 1991).

TR-1 reduction was faster than PM reduction under similar reaction conditions (Figures 4.1 and 4.3). In a study on quinone and iron porphyrin-mediated reduction of a suite of substituted mononitroaromatic compounds (NACs), Schwarzenbach et al. (1990) found that second-order reduction rate constants for NACs of a particular base structure differed based upon the location of ring substituents relative to the nitro group being reduced. They also found that electron-withdrawing substituents increased the NAC reduction rate (Schwarzenbach et al., 1990). TR-1 contains a trifluoromethyl substituent attached to the main benzene ring, an electron-withdrawing moiety that enhances the ability for the nitro substituents to accept electrons from a reductant. On the contrary, PM contains two methyl substituents on the main ring, electron-donating moieties that
decrease the ability for the non-sterically-hindered nitro group to become reduced. Wang and Arnold (2003) also noted that reduction of the reactive nitro group in PM is affected by the electron-donating substituents on the PM ring. The data presented here complements previous work that showed an effect of compound structure on nitroaromatic reduction.

The \( k_{\text{obs}} \) for both TR-1 and PM increase with pH at constant [Fe(II)] and [DOC] (as PLFA) (Figure 4.4). An increase in reduction rate with an increase in pH has been observed by others for nitroaromatic reduction in reaction media containing dissolved Fe(II) (Klupinski and Chin, 2003; Wang and Arnold, 2003; Klupinski and Chin, 2004). The formation of reactive Fe(II) complexes with organic ligands is a pH-dependent process, and certain Fe(II)-catechol complexes that are highly reactive towards NACs were found to increase in concentration with increases in solution pH (Naka et al., 2006). DOM from various environments contains a wide variety of functional groups with a wide range of pK\(_a\)s (da Silva and Tauler, 2006). When deprotonated, particular ligands become available to complex metals, and potentially can enhance the reductive capacity of Fe(II). Naka et al. (2006) found that a specific Fe(II)-catechol complex was responsible for 4-chloronitrobenzene reduction in controlled systems. Buerge and Hug (1998) found a positive correlation between Cr(VI) reduction rates and the one-electron reduction potential of Fe(II) complexes with synthetic organic ligands. The identity of specific reactive Fe(II)-DOM complexes responsible for reduction in these systems is unknown due to the heterogeneous nature of DOM.
Triplicate reactions in systems containing Fe(II) and SRFA or PLFA show no difference in rate constants for TR-1 reduction (Figure 4.5). TR-1 reduction occurs through multiple pathways, during which a variety of intermediates and byproducts are formed (Klupinski and Chin, 2003; Wang and Arnold, 2003). Particular Fe(II)-DOM species that are reactive towards TR-1 either promote different reduction pathways that occur at the same rate in the Fe(II)-PLFA and Fe(II)-SRFA systems, or that promote similar reaction pathways at the same rate in both systems.

A difference is observed for PM reduction in systems containing Fe(II) and SRFA or PLFA, where reduction in Fe(II)-SRFA systems is faster than in Fe(II)-PLFA systems (Figure 4.5). This is in contrast to previous work, in which PCNB was reduced faster in the presence of Fe(II)-PLFA relative to Fe(II)-SRFA (Hakala et al., 2007/Chapter 2). Although PM reacts as a mono-nitroaromatic-like compound in systems containing Fe(II), reduction trends actually differ between PM and PCNB. This likely is due to the effect of other structural features on reduction.

4.3.2. Natural Pore Waters

Both TR-1 and PM are reduced in natural pore waters that contain dissolved Fe(II) in the hundreds of micromolar and high concentrations of DOC (Figure 4.6). As observed in the controlled systems, TR-1 is reduced faster than PM due to the effect of pesticide structure on degradation kinetics, as discussed above. Previous work with natural pore waters has shown that rapid nitroaromatic reduction is abiotic in these systems (Chapter 3).
Multiple byproducts are observed during TR-1 reduction in natural pore water (Figure 4.7). HPLC peak position calibration with TR-1, TR-4, TR-2, TR-15, and TR-6 standard solutions shows that TR-1 disappears over time as TR-4 is formed. It appears that TR-2 also forms during the reaction, however, the retention time of the TR-2 standard peak (23.45 min) differs slightly from the retention time for the peak in the pore waters (23.38 min) closest to the TR-2 standard (Figure 4.7). It appears that TR-2 and TR-4 are final products of reduction within the time frame of the experiments performed in this study. These results are contrary to previous work conducted by Klupinski and Chin (2003) in systems containing Fe(II) and goethite, in which TR-2 was unidentified in their reactions.

The difference in the TR-1 reaction pathway in natural pore water and controlled systems containing Fe(II) and goethite may result from the interaction between TR-1 reduction products and DOM. Aniline byproducts of nitroaromatic reduction have been found to covalently bond with humic substances (Weber et al., 1996; Thorn et al., 1996), and Grover et al. (1997) reported that TR-1 reduction byproducts are capable of binding with soil organic matter. I suspect that the interaction of TR-1 reduction byproducts with DOM present naturally in sediment pore waters affects TR-1 reduction in natural systems, and may explain why the TR-6 diamine byproduct is not observed during reduction in natural pore waters even though it is observed as a major byproduct in reactions containing Fe(II) and goethite (Klupinski and Chin, 2003).

Only one major byproduct is observed during PM reduction in natural pore waters, and at this time I am unable to determine whether this is an intermediate or a final
reaction product as PM reduction only was monitored to ~ 60 h (< 1 t_{1/2}) (Figure 4.8). Wang and Arnold (2003) observed a single reduction product in controlled systems containing Fe(II) and identified this compound as having one nitro and one amine moiety, which they assumed was the product of reduction of the less-sterically-hindered nitro group. In their study with soil fungi, Singh and Kulshrestha (1991) observed both a nitro reduction and dealkylation byproduct when PM was reduced by *Fusarium oxysporum* and *Paecliomyces varioti*, and only observed the dealkylation byproduct when PM was reduced by *Rhizoctonia bataticola*. Because the reaction systems in the current study represent abiotic reduction pathways, the byproduct formed during PM reduction likely represents reduction of the sterically unhindered nitro group.

The [Fe(II)]- and [DOC]-normalized k_{obs} for reduction in natural pore waters is roughly an order of magnitude smaller than the normalized k_{obs} for controlled systems containing Fe(II) and OWCFA (Figure 4.9). In Chapter 3, PCNB reduction in unaltered pore waters was roughly an order of magnitude slower than reduction observed in controlled systems containing Fe(II) and DOM at the same pH, [Fe(II)] and [DOC]. The same results are observed in this study. Fe(II) speciation in benthic pore waters likely differs from the controlled systems containing Fe(II) and fulvic acid isolates, and an effect of this difference is observed in the reactivity of both TR-1 and PM. An understanding of the behavior of TR-1 and PM in natural pore waters thus requires use of unaltered natural samples as controlled analogues of natural systems can over-estimate reduction rates.
4.4. **Environmental Significance**

These results indicate that Fe(II) and DOM are capable of reducing dinitroaniline herbicides, and that reduction of TR-1 and PM is possible in natural benthic pore waters containing high levels of dissolved Fe(II) and DOM. These findings are valuable for predicting the fate of TR-1 and PM in freshwater wetlands located in agricultural watersheds, and in riparian buffer strips that contain high levels of dissolved Fe(II) and DOM in anoxic sediment zones. The role of specific Fe(II)-DOM complexes during dinitroaniline herbicide degradation, and the identification of byproducts formed during reduction, are important areas for future research.
4.5. Figures

**Figure 4.1.** Reduction of TR-1 and PM in controlled systems containing Fe(II) and DOM fulvic acid isolates in a ~28 mM MOPS buffer solution. Reaction conditions, rate constants and errors (reported at the 95% confidence interval for the integrated rate fit) are reported in the legend.
Figure 4.2. Control reactions for TR-1 and PM reduction in DOM-only, MOPS-only, and MOPS + Fe(II) reaction media at pH 7.79 ± 0.04. A) TR-1 degradation only occurs in reaction media containing Fe(II). For the kinetic data shown: [OWCFA] = 1.28 mM C, [PLFA] = 0.85 mM C, [SRFA] = 1.21 mM C. For the MOPS + Fe(II) reaction, [Fe(II)] = 720 µM. B) PM degradation only occurs in reaction media containing Fe(II). For the kinetic data shown: [OWCFA] = 1.28 mM C, [PLFA]_{low} = 0.60 mM C, [PLFA]_{high} = 2.1 mM C, [SRFA] = 4.7 mM C. For the MOPS + Fe(II) reaction, [Fe(II)] = 790 µM.
Figure 4.3. [DOC]- and [Fe(II)]-normalized $k_{obs}$ for TR-1 and PM reduction in non-sterile and 0.2 µm filter-sterilized solutions. For the TR-1 reactions, pH = 7.80, [DOC] = 1.20 mM C (as PLFA) and [Fe(II)] = 665 ± 15 µM. For the PM reactions, pH = 7.39, [DOC] = 1.25 mM C (as PLFA) and [Fe(II)] = 790 ± 10 µM.
Figure 4.4. The log of [Fe(II)]- and [DOC]-normalized rate constants versus pH for TR-1 and PM reduction in controlled systems containing Fe(II) and PLFA. For the TR-1 reactions, [DOC] = 1.2 mM C and [Fe(II)] = 730 ± 50 µM. For the PM reactions, [DOC] = 1.55 ± 0.3 mM C and [Fe(II)] = 840 ± 60 µM. Error bars represent the 95% confidence interval for the integrated rate fit, and where not shown are smaller than the symbols.
Figure 4.5. [Fe(II)]- and [DOC]-normalized rate constants for triplicate experiments in Fe(II)-PLFA and Fe(II)-SRFA reaction media. Solid bars represent the mean of the [Fe(II)]- and [DOC]-normalized pseudo-first-order rate constant, $k_{obs}$, for the triplicate experiments, and error bars represent 2 standard deviations of the means. For the TR-1 reactions, pH = 7.81 ± 0.02, [DOC] = 0.87 ± 0.04 mM C, [Fe(II)] = 650 ± 20 µM. For the PM reactions, pH = 7.79 ± 0.01, [DOC] = 1.35 ± 0.35 mM C, [Fe(II)] = 680 ± 30 µM.
Figure 4.6. Reduction of TR-1 and PM in natural pore waters. For both reactions, pH = 7.77, [DOC] = 3.22 mM C, and [Fe(II)] = 380 µM. For TR-1, pseudo-first-order, $k_{obs} = 0.0262 \pm 0.0033$ h$^{-1}$. For PM, pseudo-first-order, $k_{obs} = 0.0035 \pm 0.0008$ h$^{-1}$. Rate constant error represents the 95% confidence interval for the integrated rate fit.
Figure 4.7. HPLC chromatograms for TR-1 reduction in natural pore waters and structures for TR-1 and derivatives measured by HPLC using the gradient method described in the methods section. For the chromatograms, $t_0$ indicates the first reaction aliquot collected, and $t_{\text{end}}$ represents the last reaction aliquot collected. The retention times for TR-1 and TR derivative calibration standards are listed underneath the chemical structures above. Peaks were observed at the following retention times during TR-1 reduction in natural pore waters, as highlighted by the vertical dotted lines in the chromatogram (retention time error represents two standard deviations of the average retention time for the peak): $5.34 \pm 0.03$ min; $6.08 \pm 0.06$ min; $7.64 \pm 0.12$ min; $9.36 \pm 0.09$ min; $16.58 \pm 0.19$ min; $19.99 \pm 0.24$ min; $23.11 \pm 0.27$ min; $28.06 \pm 0.28$ min; $29.49 \pm 0.28$ min; and $32.00 \pm 0.26$ min.
Figure 4.8. Chromatograms of reaction aliquots from PM reduction in natural pore waters collected using the isocratic method described in the methods section. For the chromatograms, $t_0$ indicates the first reaction aliquot collected, and $t_{end}$ represents the last reaction aliquot collected. The last kinetic aliquot (top line) represents $\sim l t_{1/2}$ for the reaction. The PM peak in reaction aliquot chromatograms was adjusted to the PM calibration standard to account for retention time drift during the HPLC run.
Figure 4.9. Comparison of [Fe(II)]- and [DOC]-normalized $k_{\text{obs}}$ for TR-1 and PM reduction in natural OWC pore waters and controlled systems containing Fe(II) and OWCFA. For all reactions, pH = 7.79 ± 0.02 and [Fe(II)] = 360 ± 20 µM. For reactions in pore waters, [DOC] = 3.22 mM C and for controlled systems, [DOC] = 2.01 mM C. Error bars represent [Fe(II)]- and [DOC]-normalized 95% confidence intervals for the integrated rate fit for each reaction presented.
4.6. References


Wilson, C.; Matisoff, G.; Whiting, P. *The Movement of Sediment in the Old Woman Creek Watershed*. Ohio Department of Natural Resources. June 2002.
5.1. Conclusions

Pentachloronitrobenzene (PCNB), trifluralin (TR-1) and pendimethalin (PM) were degraded both in controlled systems containing Fe(II) and fulvic acid DOM isolates, and in natural sediment pore waters. Reactivity in both systems was affected by nitroaromatic structure, and proceeded in the following order for reactions at the same pH, [Fe(II)] and [DOC]: PCNB > TR-1 > PM. Reduction in controlled systems containing Fe(II) and DOM was slower than in unaltered pore waters for all three compounds; for PCNB, reduction in pH-adjusted pore waters also was faster than in unaltered systems.

PCNB, TR-1 and PM reduction rates were affected by Fe(II) speciation in the reaction solutions. In controlled system reactions, Fe(II) is the only dissolved metal available for interaction with the DOM isolates. Therefore, all ligands are available to interact with Fe(II), including thiols, thiol-like ligands, catechols and catechol-type ligands. It is likely that specific reactive Fe(II)-DOM complexes exist in the controlled systems containing Fe(II) and DOM fulvic acid isolates.

Unaltered natural pore waters contain Fe(III)-organic species in addition to dissolved Fe(II). The pH-adjustment of pore waters (acidified to pH ~ 2.5 for ~1 h to
overnight, then raised to the original pore water pH) causes Fe(III)-organic complexes to
dissociate, as observed with the differential pulse polarography (DPP) data in the present
work. I hypothesize that the reductive capacity of Fe(II) towards PCNB in “pH-adjusted”
pore waters is increased relative to “unaltered” pore waters because of a ligand
rearrangement induced by pH adjustment. Although a very small concentration of
reactive DOM ligands are naturally complexed with Fe(III) in the pore waters (up 13% of
Fe in the sample is Fe(III)), when Fe(II) occupies Fe(III)-stabilizing ligands in “pH-
adjusted” pore waters, the Fe(II) reduction potential is decreased.

The data presented here indicate that Fe(II) present naturally in OWC pore waters
is complexed with Fe(II)-stabilizing ligands that slightly increase the Fe(II) reduction
potential in unaltered pore waters relative to the “pH-adjusted” systems. However, the
actual speciation of these natural Fe(II)-DOM complexes is unknown. Attempts to study
these specific coordination environment of Fe(II)-DOM complexes in natural systems are
inhibited by the inherent difficulties in working with Fe(II) and DOM in these sensitive
natural samples. Computational chemistry methods and x-ray absorption spectroscopy
(XAS) were used in an attempt to identify naturally-occurring Fe(II)-DOM complexes.

Computational chemistry was employed to predict the coordination environment
and thermodynamic properties of Fe(II)-organic complexes that are known NAP
reductants. Density functional calculations using B3LYP with a Stuttgart RSC basis set
were performed on simple Fe(II) and Fe(III) complexes with water and hydroxide during
this study. More extensive computational modeling was outside the scope of this study,
due to computational convergence issues and the difficulty in predicting the Fe(II)
coordination environment in natural systems. XAS was employed to identify the coordination environment of Fe(II) in natural pore waters. I collected Fe K-edge spectra for OWC pore waters as the National Synchrotron Light Source (NSLS) in Upton, New York. Pore water Fe(II) was detected at high enough concentration for spectral acquisition using Fe K-edge EXAFS (extended x-ray absorption fine structure spectroscopy). However, due to the complexity in handling oxygen-sensitive samples, oxidation is likely to have occurred at some point during pore water transport to the NSLS, or during sample preparation in the XAS cell. Future work towards identifying the Fe(II) speciation environment in natural systems is crucial for an understanding of Fe(II) reactivity in anoxic environments.

5.2. Future Work

The actual speciation of Fe(II) in anoxic pore waters remains unclear. A variety of methods can be used in the future to probe the speciation and reductive capacity of anoxic Fe(II)-DOM complexes present naturally in anoxic pore waters. Some methods have been successfully used to probe the nature of metal-organic complexes in oxic systems (e.g., x-ray absorption spectroscopy and liquid chromatography/mass spectrometry) and the reactivity of various reductants (e.g., kinetic studies). However, problems inherent in handling oxygen-sensitive samples can make these analyses extremely difficult.

One could perform kinetic studies with nitroaromatic compounds of various structure to probe the specific reductive capacity of naturally-occurring Fe(II) complexes. Others have used such studies successfully in the past to probe the reductive capacity of
specific reductants in controlled systems. Performing similar work with natural pore waters can provide a more detailed picture of the redox capacity of the active pore water reductants relative to nitroaromatic compounds of varied reduction potential. Ultimately, this work could show the redox capacity of natural Fe(II)-DOM complexes relative to other reductants present in anoxic environments.

Future work with XAS can provide a highly sensitive level of detail regarding Fe speciation in natural pore waters. An air-tight sampling cell needs to be designed and constructed, and an understanding of the effects of beam damage on redox-sensitive samples during analysis needs to be well-understood, before any serious analysis of pore water spectra can be conducted. Prior to pore water analysis, the natural samples can be subjected to size fractionation in order to separate larger colloidal and nanoparticle components from the dissolved species. If the aqueous redox chemistry challenges can be handled prior to pore water analysis, XAS could be a very powerful tool for characterizing natural Fe speciation in anoxic pore waters.

Both kinetic studies with pore waters, and XAS analysis of pore waters, could be used as a basis for calculating the reduction potentials of specific Fe complexes in natural pore waters. Computational chemistry can provide more detailed information regarding the thermodynamics of Fe complexes identified with other methods, and also can provide information regarding the geometry of specific complexation environments. Kinetic studies, XAS and computational chemistry can be used individually or in combination to more fully probe the actual nature of Fe speciation in anoxic pore waters.


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