STABILIZATION OF ENZYMATICALLY POLYMERIZED 2,4 DICHLOROPHENOL IN MODEL SUBSURFACE GEOMATERIALS

by

MONICA PALOMO

B.S., Universidad de Guanajuato, Guanajuato, México, 1999
M.S., Kansas State University, Kansas, 2003

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Civil Engineering
College of Engineering

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2008

Abstract

Human activities generate large amounts of chlorinated phenolic chemicals that are often introduced into the soil environment during pesticide and insecticide application, industrial releases, and accidental spills. For example, 2,4-dichlorophenol (DCP), a derivative of the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) can been found in soil within 24 hours of 2,4-D application. Horseradish peroxidase (HRP)-mediated polymerization has been proposed as an approach to remediate soils and groundwater contaminated by phenolic pollutants. Treatment with HRP results in the transformation of phenols into polyphenolic oligomers that sorb strongly or precipitate on soils surfaces. Although HRP-mediated chlorophenol stabilization has been studied extensively in surface soils, very limited scientific data is available that supports the application of this technology in subsurface materials. Hence, the focus of this study was to evaluate sorption and binding of DCP and products of HRP-mediated polymerization of DCP to model geosorbents representing subsurface geomaterials. These sorbents included two humin-mineral geomaterials and one mineral geosorbent derived from surface soils. Soil-water phase distribution of total solute in the HRP-amended systems was observed to reach equilibrium within 7 days in woodland humin-mineral soil (WHM), and within 1 day in agricultural humin-mineral (AHM) and model mineral geomaterials. For all the geomaterials used, water extraction data indicated the development of contact time-dependent resistance to extraction/dissolution of soil-associated DCP and DPP. Solute associated with WHM geomaterial was higher at the end of the study than that associated with AHM. Contact time increased DCP stabilization at all initial aqueous DCP concentrations studied. Results of this study suggest that DCP stabilization in organic geosorbents results from a combination of sorption and cross-coupling of DCP and precipitation of DPP; in inorganic soils, precipitation of DPP macromolecules is the dominant process. HRP-mediated stabilization of DCP in soils was effective and independent of the solution ionic concentration. The amount of DCP stabilized in the mineral soil was comparable to that stabilized in humin-mineral geomaterials. The research reported in this dissertation demosntrates the potential of HRP enzyme to stabilize DCP in subsurface geomaterials under variable contaminant and salt concentrations, thereby restricting its transport in the environment.

STABILIZATION OF ENZYMATICALLY POLYMERIZED 2,4 DICHLOROPHENOL IN MODEL SUBSURFACE GEOMATERIALS

by

MONICA PALOMO

B.S., Universidad de Guanajuato, Guanajuato, México, 1999
M.S., Kansas State University, Kansas, 2003

A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Civil Engineering
College of Engineering

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2008

Approved by:

Major Professor
Dr. Alok Bhandari

Copyright

Abstract

Human activities generate large amounts of chlorinated phenolic chemicals that are often introduced into the soil environment during pesticide and insecticide application, industrial releases, and accidental spills. For example, 2,4-dichlorophenol (DCP), a derivative of the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) can been found in soil within 24 hours of 2,4-D application. Horseradish peroxidase (HRP)-mediated polymerization has been proposed as an approach to remediate soils and groundwater contaminated by phenolic pollutants. Treatment with HRP results in the transformation of phenols into polyphenolic oligomers that sorb strongly or precipitate on soils surfaces. Although HRP-mediated chlorophenol stabilization has been studied extensively in surface soils, very limited scientific data is available that supports the application of this technology in subsurface materials. Hence, the focus of this study was to evaluate sorption and binding of DCP and products of HRP-mediated polymerization of DCP to model geosorbents representing subsurface geomaterials. These sorbents included two humin-mineral geomaterials and one mineral geosorbent derived from surface soils. Soil-water phase distribution of total solute in the HRP-amended systems was observed to reach equilibrium within 7 days in woodland humin-mineral soil (WHM), and within 1 day in agricultural humin-mineral (AHM) and model mineral geomaterials. For all the geomaterials used, water extraction data indicated the development of contact time-dependent resistance to extraction/dissolution of soil-associated DCP and DPP. Solute associated with WHM geomaterial was higher at the end of the study than that associated with AHM. Contact time increased DCP stabilization at all initial aqueous DCP concentrations studied. Results of this study suggest that DCP stabilization in organic geosorbents results from a combination of sorption and cross-coupling of DCP and precipitation of DPP; in inorganic soils, precipitation of DPP macromolecules is the dominant process. HRP-mediated stabilization of DCP in soils was effective and independent of the solution ionic concentration. The amount of DCP stabilized in the mineral soil was comparable to that stabilized in humin-mineral geomaterials. The research reported in this dissertation demosntrates the potential of HRP enzyme to stabilize DCP in subsurface geomaterials under variable contaminant and salt concentrations, thereby restricting its transport in the environment.

Table of Contents

List of Figures

List of Tables

Acknowledgements

First, I want to thank my family: Edwin my beloved husband and Verónica and Isabel my *little princesses*. Edwin, thank you for believing in me at all times and for encouraging me and filling me up with energy during those moments when I thought I had nothing more to give. This work would not be possible without your supporting hand always holding mine. You have taught me that patience is one of the most tresureable possessions. Thanks for sacrificing all those moments when you wanted to hang out together, but I was gone working in the lab or studying at home. Verónica and Isabel, *mis pedacitos de amor*, thank you both for giving me nine months of company through the toughest times of my lab work. I will always remember how, during the long hours of my lab work, your little kicks inside my tummy would fill me up with energy and would put a cheek-to-cheek smile on my face. Thank you *botoncitos* for the way you looked at me with your shinny stars every time I came to pick you up from school. Thank you Verónica and Isabel for the time you have given up so mommy could accomplish her dream. Without your lovely smiles, warm hugs and your magical voice everytime you called me *mama* I would have never been able to complete this work.

I want to thank my parents, for believing in me and for supporting all my crazy ideas about making a difference in the world. I would not be here if it were not for you. *Mamichi*, you taught me that loving what I do would make me happy and it would lead me to be successful. Through your example I have learned that the greatest things can be achieved with excellence and constant work. *Papichi,* your passion for your tunnels and all your machines inspired me to become an engineer. You are the best engineer I have ever known.

Mimis and Guer, my dear sisters, I also want to thank you because you gave up a lot so I could materialize my dreams. This work would not be possible without your love and support.

I also want to thank my major advisor: Dr. Alok Bhandari. Thank you for trusting on my work and for supporting my ideas. Thank you for sharing all your professional experiences, (as researcher, teacher, and consultant) and for encouraging me to participate in many different acitivites which made me grow professionally and personally. Thank you for being the best advisor!

I want to thank my committee members: Dr. Gary Pierzynski, Dr. Gerard Kluitenberg,

and Dr. Om Prakash for teaching me to see the world from a new wonderful perspective, as well as for the time and dispossiton allotted to help me improving the quality of this work.

I want to thank to all my K-State friends that made my graduate program really special: Heather, Fang, James, Larry, Karen, Sailaja, Aruna, Ashwini, Sujatha, Josh, Wongee, Long, Mbaki, Farhana, Steve, Chandra, Daba. Thank you for your company, advises and friendship.

I want to thank Mrs. Laverne Bitsie-Baldwin for her constant support and for her continuous encouragement to participate in different activites that enriched my experience at KSU.

Special thank to Danita, Peggy, Angie and Debbie, whose inconditional support and help facilitated my graduate student life.

Finally, but most importantly, I want to thank God. Dear Father, thank you for giving me the strength to overcome all the challenges that I have found, thank you for the energy provided to keep my family happy while satisfying the demands of my work. Thank you for being present in my life through every person around me. Than you for all the blessings you have brought to my life.

Dedication

A mis pedacitos de amor: Verónica e Isabel que con su sonrisa y sus dulces besos me llenan de energía para seguir adelante…

A mi gran amor y mejor amigo: Edwin, tu noble y gran corazón me llena de calma y alegría…

A mi mami, la mujer mas dulce y admirable de este mundo…

A mi papi, el mejor ingeniero de minas…

CHAPTER 1 - GENERAL LITERATURE REVIEW

1.1 Sorption Process and Sorption Models

A thorough knowledge of the soil matrix composition is important for understanding sorption and transport of organic chemicals in the soil environment. In general, the components of the soil matrix are grouped into organic and inorganic domains. The inorganic or mineral components are clay, silt, and sand. These particles are characterized on the basis of their size and mass, and they constitute the bulk of the soil. Soil inorganic components include crystalline, semicrystalline and amorphous oxyhydroxyde minerals (Laird et al., 1994). In SOM-containing soils, the mineral particles are often covered by an organic matter layer. Even though the organic matter represents a small weight fraction of the bulk soil mass, the mineral surface that is covered is usually very large. SOM controls the sorption of nonionic organic compounds when organic C levels are higher than 0.1 %, while interactions with mineral surfaces are more significant below this SOM value (Pignatello, 1989).

Sorption of organic contaminants by natural geosorbents is a significant process controlling the fate and transport of pollutants in the environment. Adsorption and absorption are two different physical processes that can occur when an organic contaminant interacts with a solid phase. These two processes can take place at the same time. However, in a majority of environmental systems the distinction between adsorption and absorption is rarely clear. Adsorption refers to the interaction of the solute molecules at the surface of the sorbent or between phases; while absorption is a three-dimensional process, where the solute molecules penetrate and occupy intraparticle pore spaces within the soil matrix. Due to the difficulty of discrimination between these two processes, the term sorption is typically utilized to describe the overall retention of a given organic chemical by a sorbent (Weber and DiGiano, 1996; Essington, 2004). The following sections of this chapter present a comprehensive disscusion of the most common approaches and models used by environmental researchers to study the sorption phenomena of organic chemicals. The aim of the literature review is to present the methods that have been adapted and utilized to investigate the sorption of xenobiotics to natural sorbents, like soils, sediments and organic matter.

1.1.1 The Sorption Isotherm

The most common approach to study the sorption behavior of an organic pollutant is by employing isotherms. An isotherm is a graphical representation of the relationship between the amount of chemical sorbed to the solid phase and its aqueous concentration under equilibrium conditions, at a given temperature, pressure and solution chemistry. The term "equilibrium" is defined as the thermodynamic equilibrium between two phases such as solid/soil organic matter (SOM) and water. In other words, a system is in equilibrium when the concentration of the compound of interest remains constant with time in the phases present in the system.

The shape of the isotherm indicates the strength of the interaction between the solute and sorbent. Four shapes are commonly used to describe the solute phase distribution relationship between the sorbent and sorbate: L-curve, S-curve, H-curve and C-curve (Figure 1). Low affinity of soil for the adsorbate at low solute concentrations is represented by the S-curve. However, as the solute concentration increases more solute is forced onto the soil modifying its surface and increasing the affinity between sorbent and adsorbate. A L-curve (Type I) indicates a relatively high affinity between the solute and sorbent at low solute concentration. In a C-curve (Type II), known as a linear isotherm, the slope remains constant since affinity of sorbent and adsorbate is independent of surface coverage or initial aqueous solute concentration. Finally, the H-curve results when the solute exhibits a very high affinity for the solid phase.

The occurrence of true "thermodynamic equilibrium" in a complex sorbent like soil can take from days to years. Phase-distribution relationships (PDRs) have been proposed to describe the time-dependent sorption of organics under non-equilibrium conditions (Weber and Huang, 1996). When equilibrium has not been reached, the solute concentration in both aqueous and solid phases continues to change over time. PDRs that describe solute distribution at equilibrium represent the sorption isotherm. Figure 2 shows graphical representations of a system in equilibrium (isotherms) and a system under non-equilibrium conditions (PDRs).

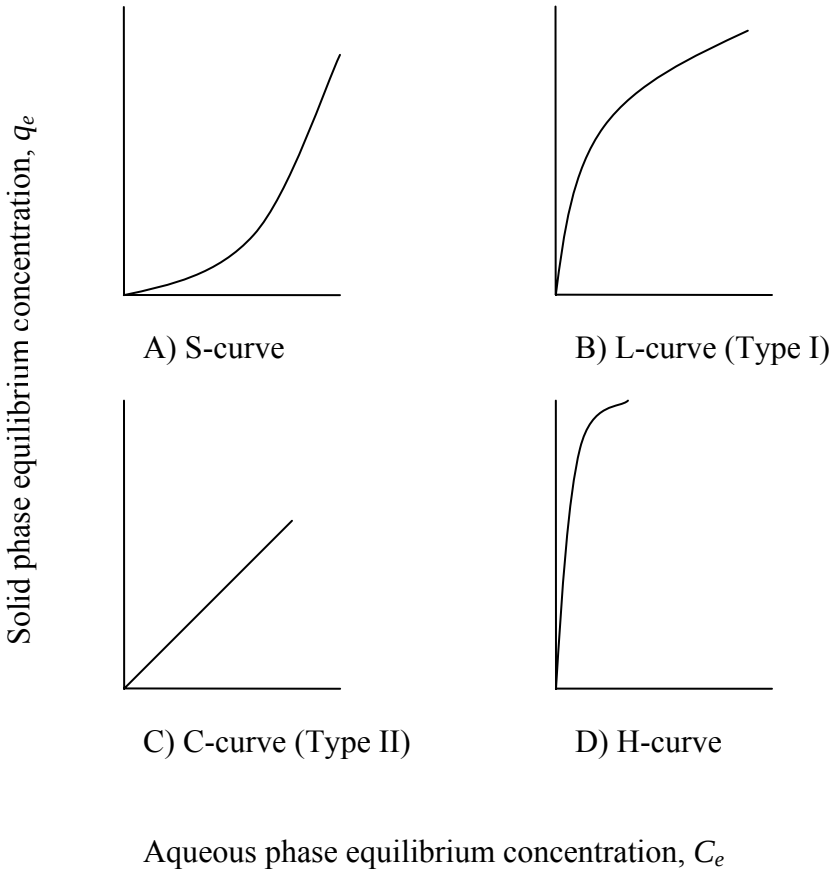

Figure 1.1. Illustration of sorption isotherm types. Adapted from Essington, 2004.

In some cases despite achievement of "apparent" sorption equilibrium, i.e., a constant aqueous concentration of solute, true "thermodynamic equilibrium" may not have been attained. Palomo and Bhandari (2006) reported apparent sorption equilibrium of 2,4-dichlorophenol on surface soil within 7 days, in a study that lasted up to 84 days. However, upon studying the distribution of solute among different SOM phases they observed that solute mass transfer between the humic acid, fulvic acid and humin fractions continued to proceed over longer intervals of time, suggesting that true sorption equilibrium had not been reached even after 84 days of sorption contact time.

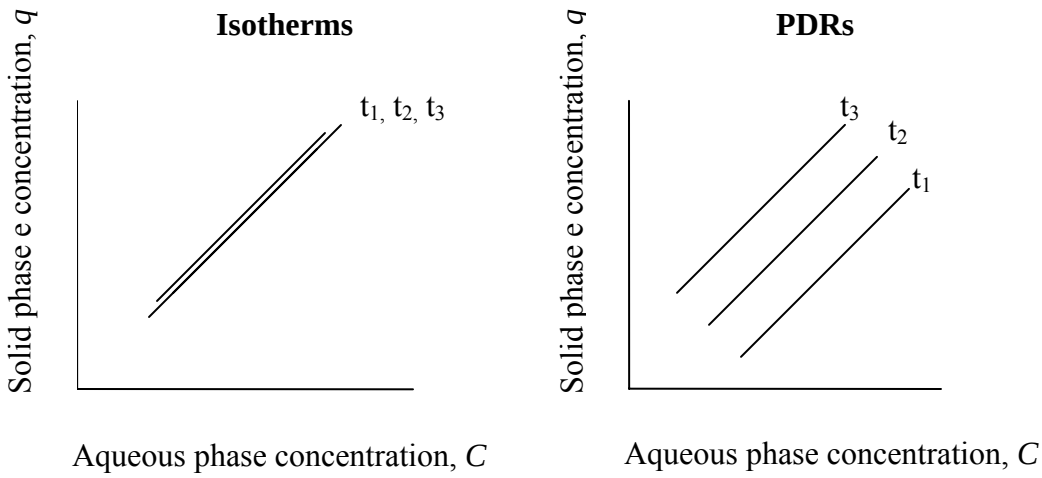

Figure 1.2. Graphical representation of a system under equilibrium and non-equilibrium sorption conditions at 3 different times ($t_1 < t_2 < t_3$)

1.1.2 Sorption Models

There are many different mathematical models to quantitatively describe the sorption behavior of solutes when in contact with geosorbents. The most common models are the linear partitioning model, the Freundlich model and the Langmiur model. Out of these three models the ones most frequently used to explain the sorption phenomena of xenobiotics in soils are the linear partitioning and Freundlich models. More complicated models, like the distributed reactivity model (DRM) and the dual-mode model (DMM) have been proposed to explain more accurately the sorption behavior of the organic pollutants in complex soil systems (Weber et al., 1992; Xing et al., 1996). The work presented here utilizes the Freundlich model to describe the experimental data. Since the linear model is considered a special case of the Freundlich model, these two models are briefly explained in the following sections.

1.1.2.1 Linear Partitioning Model

The linear partitioning model is often used to characterize the sorption of hydrophobic organic chemicals on geosorbents. Some assumptions for this model are 1) the sorption of solute molecules is directly proportional (linear) to the aqueous phase solute concentration, 2) the presence of a second compound does not affect the sorption of the studied chemical, 3) the magnitude of sorption is dependent on the SOM content of the geosorbent, 4) all adsorption sites

are similar (same energy) and 5) initial sorption does not enhance or hinder subsequent sorption. The linear partitioning model can be described mathematically as follows:

$$q_e = K_D C_e \qquad \text{(Eq. 1.1)}$$

where:

$q_e =$ mass of solute per unit mass of sorbent at equilibrium (mass/mass),

$C_e =$ solute aqueous concentration at equilibrium (mass/volume),

$K_D =$ distribution coefficient (volume/mass)

When the sorbent is a soil or sediment, K_D can be normalized to the fraction of organic carbon or SOM content to obtain the K_{OC} or K_{OM}, respectively (Essington, 2004; Weber and DiGiano, 1996).

1.1.2.2 Freundlich Model

The Freundlich model has been broadly used by scientists to describe the sorption behavior of numerous hydrophobic organic contaminants in environmental samples. This model is empirically based. The Freundlich model assumes an unlimited number of adsorption sites with a wide energy distribution associated with them. Solute molecules occupy surface sites according to the energy associated with them. Thus, higher adsorption energy sites are occupied first, followed by the ones possessing lower energy (Montgomery, 1985).

The Freundlich model can be mathematically described as:

$$q_e = K_F C_e^{\,n} \qquad \text{(Eq. 1.2)}$$

where:

$q_e =$ solid-phase concentration of solute at equilibrium (mass/mass),

$C_e =$ equilibrium aqueous phase concentration of solute (mass/volume),

$K_F =$ sorption capacity, empirical constant

(mass of solute solid phase /(mass of solid × mass of solute in aqueous phase)^n

$n =$ linearity constant, empirical constant.

The empirical constants n and K_F, depend on the chemical and physical properties of the sorbent and organic solute. The value of K_F is a measure of the sorbent sorption capacity at 1μM. The parameter n describes the diversity of sorption site energies. Graphically, n is a measure of the inflexion of the curve fitted to the experimental data (or isotherm data). Generally, n has a value close to 1. A broad distribution of site energies is indicated when n <1, and the isotherms produced are convex, L-shaped or Type I. Surface homogeneity is expressed by n approaching unity or n=1. The isotherm corresponds to the C-curve of Type II, and represents linear sorption behavior, which is analogous to the linear partitioning model. Finally, if n>1, it suggests that sorbed molecules modify the surface of the sorbent in a way that enhances subsequent sorption of more solute molecules, i.e., an S-curve- (Essington, 2004; Weber and DiGiano, 1996).

The linearized form of Freundlich equation is often utilized to graphically illustrate the sorption isotherms. The linearized form is especially helpful when there is a need to observe the whole range of concentrations over which the isotherm has been generated. The linearized form is described as:

$$\log q_e = \log K_F + n \log C_e \qquad \text{(Eq. 1.3)}$$

1.2 Soil Organic Matter

1.2.1 Formation of Soil Organic Matter

The soil organic matter (SOM) formation process is known as humification or humus formation. One of the most common reactions in the humification process is the oxidative coupling of naturally occurring phenolic acids (Sjoblad and Bollag, 1981). This reaction allows integration of the abundant natural phenolic acids in soil into the SOM structure via covalent linkages, thus forming a complex and heterogeneous SOM (Bollag, 1992). In nature, oxidative coupling can take place in the presence of abiotic catalysts, such as mineral oxides, or biological catalysts, like enzymes (Bollag, 1992; Pal et. al., 1994). The rate of humification depends, among other factors, on soil pressure and temperature, pH of the site, soil management practices, vegetation, soil microbial population, soil enzymes and the input of fresh litter or other organic material to the soil surface. Thus, the degree of humification of SOM varies from site to site and it changes with soil depth (Huc and Duran, 1973; Hatcher et al., 1983). While the general characteristics of SOM are well known, obtaining information related to SOM from a particular

site of interest requires a detailed soil analysis.

Formation of SOM has been explained by various theories including the lignin theory, sugar-amine theory, polyphenol theory and self aggregation theory (Essington, 2004). Perhaps, the most widely accepted argument for SOM synthesis is put forward by the polyphenol theory. According to this theory, humus formation is a multistage process, where humic substances (HS) are derived from lignin and other aromatic biopolymer, where lignin is the major source of phenolic substances. First, all the plant and microbial biopolymers are decomposed to polyphenol molecules. Biopolymers are transformed into phenolic substances that are converted to quinones. Humic substances are formed by enzymatically induced repolymerization of the quinones with amino compounds. The polyphenol theory considers that HS formation is a sequential polymerization process where formation of fulvic acids (FA) occurs first, followed by humic acids (HA) and finally the humin (HM) component. Humin is considered the most humified HS fraction. The complexity of the HS increases with time since more monomeric degradation products are incorporated to the HS macromolecule (Stevenson, 1982; Essington, 2004). A study performed by Huc and Duran (1973) supported the theory that FA and HA are the first step of the geological cycle for the stabilization of C in soils and sediments. While, studying HA and kerogens, these researchers observed that the difference in the functional groups that contained oxygen related well to the CO_2 loss.

1.2.2 Composition of Soil Organic Matter

Soil organic matter plays a very important role in surface soils and sediments. Despite the fact that on a mass basis SOM is not the dominant component of soil, its significance arises from the properties it gives to the soil. These properties include water holding capacity, control and regulation of soil temperature, storage of inorganic and organic nutrients for plants and microorganisms, buffering capacity, soil structure, and cation exchange capacity. The hydrophobic and highly reactive functional groups of SOM are important in the determination of the fate, transport and remediation of contaminants in the soil environment (Stevenson, 1982).

SOM has traditionally been believed to be composed of two classes of substances, the non-humic and the humic substances. Non-humic substances have been conventionally defined as those with identifiable molecular structures such as: lipids, carbohydrates, peptides, proteins, amino acids, amino sugars, nucleic acids, and their derivatives. These substances can be

degraded by soil microorganisms, and mineralized to CO_2 and H_2O; or they can be incorporated into the macromolecular structure of humic substances.

Humic substances have more complex structures with no unique molecular structure or chemical formula (Stevenson, 1982; Essington, 2004). However, several models have been developed to elucidate the structural characteristics of humic substances (Kononova, 1966; Schnitzer and Khan, 1972; Buffle, 1977; Stevenson, 1982; Schulten and Schnitzer, 1997). These models have described a unique type of macromolecule that is formed naturally in soil and sediments and is different from known biomolecules. Figure 1.3 shows the molecular model developed by Buffle (1977) for a fulvic acid. Macromolecular structures for two humic acids were developed by Stevenson (1982) and by Schulten and Schnitzer, (1997). These models show structures formed by phenolic units with some degree of aliphatic character. The models, however, are unique to the type of HS studied.

While Stevenson (1982) defined HS as high molecular weight material that are formed naturally in soil as a result of secondary synthesis of biomolecules, MacCarthy (2001) has described humic substances as complex, reactive and heterogeneous molecules formed as a product of the decay of animal and plant residues without making reference to their molecular size. Other rsearchers have utilized hollow fiber filtration (Christl et al., 2000), high performance size exclusion chromatography (HPSEC), and NMR spectroscopy (Piccolo et al., 2001 a and b) to conclude that HS are formed by supramolecular association of small heterogeneous molecules that are primarily associated by weak hydrophobic interactions (Tandford, 1991; Israelachvili, 1991) and weak dispersive forces (van der Waals, Π-Π, CH- Π) (Piccolo, 1996 a and b). These findings challenge the traditional belief that HS are polymers of high molecular size.

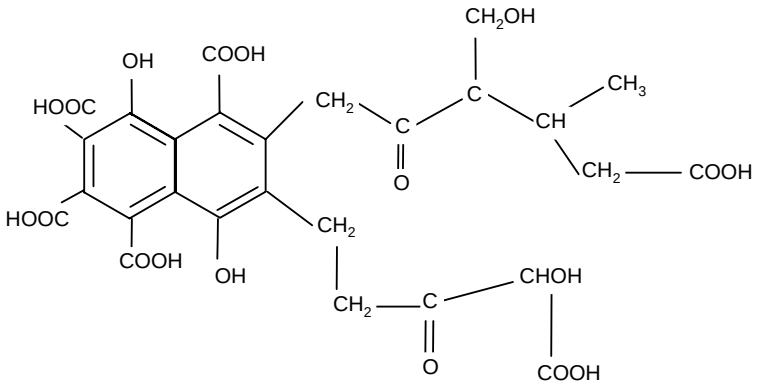

Figure 1.3. Macromolecular model of a fulvic acid. Adapted from Buffle 1977.

State-of-the-art technologies, such as nuclear magnetic resonance (NMR), have allowed researchers to study the conformation of SOM in a greater detail. One, two and three dimensional NMR has provided new information about SOM conformation. One of the most recent and novel studies was done by Kelleher and Simpson (2006). These researchers tried to find the NMR signal corresponding to the unique HS molecule. They identified the signals of the principal biopolymers in microbes and plants that could contribute to the formation of SOM and performed an NMR analysis of IHSS peat humic acid and HS isolated from a surface soil identifying the signals corresponding to the biopolymers. The intensive NMR analysis revealed that, contrary to what was recognized in the past regarding the uniqueness of the HS chemical structure, humic substances were primarily a complex mixture of biopolymers and their degradation products derived from plant and soil microbial residues. The authors stated that the chemical distinction among humic acids resides in the proportion of biopolymers to residual products. This proportion is determined by the conditions in the soil micro-environment.

Sutton and Sposito (2005) recently proposed a modified definition of humic substances. Humic substances were defined as "supramolecular association of low-molecular mass organic molecules". This definition includes all the molecules that are strongly associated with HS, i.e., including recognizable biopolymers that are connected to HS molecules (through hydrophobic interaction, H-bond or even through covalent bounds) and which provide important physical-chemical characteristics. This new definition of HS erases the distinction between biopolymers and HS and attributes more complexity to the SOM than the traditional definition.

One of the most important characteristics of HS is that they are refractory, i.e., they are transformed and degraded by soil microorganism very slowly. The persistence and stability of SOM in the soil/sediment environment is one of the characteristics that had led researchers to think that a novel unique HS structure existed in the soil environment. The heterogeneous, complex, and undefined mixture of organic molecules is responsible for the slow degradation of SOM. Only a large consortium of enzymes could possibly rapidly mineralize the complex SOM mixture (Swaby and Ladd, 1962).

Haider and Martin (1975) attributed the slow microbial turnover of SOM to the oxidative coupling reactions between a model humic acid and phenolic compounds. These researchers suggested that covalent linkages protect or stabilize the phenolic compounds from microbial attack. Meyer (1994) proposed that the roughness (tiny pores smaller than 10 nm) of the minerals

surface was responsible for the preservation of the SOM. SOM located in such small pores could be unreachable and protected from hydrophobic enzymes responsible for the biological attack. Kelleher and Simpson (2006) did not find a novel cross-linkage in the HA's they studied and suggested that the physical-chemical associations among the biopolymers/degradation products, the biodegradation resistance of the different biopolymers, and their associations with clay particles might be more likely responsible for the recalcitrant behavior rather than the oxidative coupling reactions.

McCarthy (2001) proposed a "two-compartment" model where most of the HS is stable or resistant to biodegradation and a second fraction is labile and contributes significantly to the C turnover rate. Sutton and Sposito (2005) unified the "two-compartment" model with their HS definition to explain the recalcitrance of some biomolecules in soils. These researchers argued that strongly associated biomolecules could be part of either compartment. While a majority is part of the transient portion of the HS, since they are easily degraded; a minority is protected from degradation through the linkages with HS

1.2.3 Fractions of Soil Organic Matter

The organic components or fractions of soil organic matter are defined according to the extraction method utilized to separate them. When the separation is performed based on the solubility properties of the organic fractions in alkali and acid solutions, the fractions are defined as humic acids (HA), fulvic acids (FA) and humin (HM). FA is the fraction that becomes soluble under alkaline conditions and remains soluble in aqueous solutions regardless of the pH. HA is soluble under alkaline conditions but insoluble at acidic pHs. Humin is the SOM fraction that is insoluble in aqueous solution at any pH. These fractions are also classified by their degree of humification. According to the phenolic theory, humin is the most humified fraction (oldest), followed by HA, and the least humified fraction is FA (fresher). The three SOM fractions are chemically and structurally different because of the variation of their formation process (climate, vegetation, topography, age, water content and subsurface pressure), and interact differently with organic contaminants in contact with them (Stevenson, 1982).

The predominant elements in HA and FA are carbon and oxygen. FAs have predominantly less carbon and greater oxygen than HAs, thus the O/C ratio is higher for FAs than for HAs. Higher O/C and higher oxygen content is associated with the presence of more

oxygen containing functional groups and carbohydrates. The solubility of FA at any pH is related to its highly polar and acidic functional groups that are rich in oxygen (COOH-, phenolic-, enolic-OH, ester and ether linkages and ketone groups). The relatively hydrophobic portions of the FA molecule are also highly oxygenated. The H/C molar ratio is used to characterize the degree of aromaticity and saturation of carbon chains. High aromaticity is characterized by a low H/C, while high H/C values are indicative of low aromaticity or the dominance of aliphatic carbon chains. Generally, FAs have a more aliphatic nature than the HA (H/C_{FA})>(H/C_{HA}) (Stevenson, 1982; Essington, 2004; Tan, 2003). On average FAs contain less nitrogen than HA (Rice and MacCarthy, 1991). Elemental composition of humin has been described as that of HAs. Humin will be discussed in more detail in the following section of this chapter.

1.3 Humin Fraction of the SOM

Humin is defined as the SOM fraction that is strongly associated with inorganic soil fractions, and is a key component of SOM-clay aggregates (Kononova, 1966; Shah et al., 1975; Theng, 1979). Humin is believed to be a stabilized form of HA through the mineral-linkages (Kononova, 1966). Vanderbrouke (1985) suggested that HA lose their solubility properties through reactions that cause loss of functional groups. This idea has been supported by the fundamental composition of humin reported to be similar to that of HA (Stevenson, 1982). Shah et al., (1975) attributed the loss of ability of humin to dissolve in alkali solutions to the tight nature of its association with the mineral part of the soil (humic acid-clay complexes). These researchers pointed out that in some soils, the insoluble humin includes some carbonized plant residues that are formed by restricted aeration in the soil environment. Anderson et al. (1974) also reported the occurrence of a clay-associated humin and attributed the low degree of humification of the clay-humin complex to protection provided by the sorption of humin to fine mineral colloids. Other researchers have described humin as a high molecular weight polymer (Stevenson, 1982), as bio-molecule like lignoproteins, whose recalcitrant behavior is due to its structure (Somani and Saxena, 1982), as melanines (Russell et al., 1983) or as plant residues in varying states of decay (Kononova, 1966; Anderson et al., 1974; Stevenson, 1984). Humin has been compared to protokerogen, a term used in petroleum chemistry to make reference to the insoluble organic matter isolated from unlithified sediments (Rice, 2001).

Humin typically represents 40% or more of the total organic carbon in most soils or

unlithified sediments (Kononova, 1966, Huc and Durand, 1977; Hedges and Keil, 1995). Somani and Saxena (1982) found in the six different soils they studied that the humin varied from 42 to 49 % of total organic carbon. Hatcher et al. (1983) reported that for a surface sapropel (term in marine geology describing dark-colored sediments rich in organic matter) the humin material represented 20 % of total carbon. While at greater depths, the humin content increased up to 60% of SOM. Ishiwatari, (1985), studied sediments taken from six different lakes in Japan and found that the humin fraction ranged between 43 to 76 % of total carbon.

Humin has been reported as the more dominant component of humic substances relative to FA and HA with soil/sediment burial depth (Huc and Duran 1973; Hatcher et al., 1983; Ishiwatari, 1985; Vanderbrouke, 1985). The reduced quantities of FA and HA fractions have been attributed to aging process or loss of oxygen-containing groups during condensation reactions (Huc and Duran 1977). Vanderbrouke (1985) observed that nitrogen-containing functional groups disappeared more quickly compared to oxygen-containing groups. Hatcher, et al. (1983) attempted to explain the enrichment of humin with depth and proposed the theory of the "selective preservation" of the more refractory humin. Where the more labile molecules of carbohydrates, proteins and lipids are biodegraded and humin is preserved and concentrated. These researchers utilized ^{13}C NMR and stable carbon isotope methods to prove their hypothesis. They observed that with depth the degradation of carbohydrates, proteins and lipids correlated with the increase in humin. In addition, the big differences in isotopic composition between the parent marine algal sapropel and the extracted-humin in near-surface locations diminished as depth increased. The difference in isotopic composition between extracted humin and sapropel correlated with the loss of carbohydrates that were more likely to be hydrolyzed and were ^{13}C-enriched.

It has been reported that humin rapidly sorbs organic compounds and it often binds them irreversibly (Kohl and Rice, 1998). However, the interaction of humin with organic compounds will be discussed in a later section.

1.3.1 Physical and Chemical Characteristics of Humin

Radiocarbon dating studies have reported that the typical age of humin is 1000 years or more and its age increases with soil depth. Skujins and Klubek (1982) found that the value of humin residence time varied from 920 to 1050 yrs. Gouveia et al. (1999) found that the age of

the humin fraction extracted from different Brazilian soil materials depended on the soil depth and the age ranged from 2,500 to 10,000 years. Goh and Malloy (1978) studied the radiocarbon dating of humin extracted from two paleosols using three different isolation procedures. Humin age was found to be dependent on the extraction method and it ranged between 600 to 800 years.

The insolubility of humin has been one of the main limitations to study its physical-chemical characteristics in the past. The isolation of humin, to study its properties, has been achieved by utilizing aggressive treatments like dissolution of the mineral fractions and extraction of humic and fulvic acids. Traditionally to isolate humin, soil/sediment material is treated by alkali-extraction to remove FA and HA and then the remaining soil is digested by HF/HCl solution to dissolve the mineral fraction. However, researchers have varied the operational definition of humin with the objective of getting a closer look at its properties and structure. Anderson et al. (1974) utilized the alkali-pyrophospahte extraction method combined with insonation to promote soil dispersion and breakage of the soil aggregates. Goh and Molloy (1978) utilized a sequential extraction method with $Na_4P_2O_7$/NaOH and then HCl or HNO_3 to separate the humic and humin fraction. Russell et al. (1983) isolated humin from the top 15 cm of a profile of the Boyndie series. First, the material was refluxed with ethanol/toluene to remove waxes and fats. Next seven sequential NaOH extractions (18-hour long) were performed, and finally the material was subjected to a water washing-digestion (HF)-water washing-drying cycle. Ishiwatari (1985) utilized the traditional alkali extraction with a temporal variation, where the first lasted six hours while the second 1-month. Hatcher et al. (1985) defined humin as the residue left after extractions with benzene/methanol and NaOH; and treated with HF/HCl. Preston et al. (1989) employed the traditional alkali extraction method with NaOH under N_2 atmosphere to prepare four operationally different humin fractions. The residue remaining after NaOH-extraction was washed with deionized water to neutralize its pH (humin-1). Humin-2 was obtained by digesting the washed residue three times with a dilute solution of HCl/HF. To produce humin-3, humin-2 was contacted with a more concentrated solution of HCl/HF for 90 days, at room temperature. Finally a portion of humin-3 was treated again in the same manner to generate humin-4. These researchers concluded that extensive de-ashing of the humin caused minimal structural changes. Gouveia et al. (1999) utilized a combination of HCl-NaOH-distilled water to remove minerals, FA, and HA from the material.

Rice and MacCarthy (1989) proposed a novel liquid-liquid extraction technique to isolate

humin from soil/sediment and to separate humin from its organic components. The technique consisted of partitioning humin components between an aqueous phase and methyl-isobutyl-ketone (MIBK) as a function of pH. For separation of humin from the minerals, the MIBK method provided a gentler alternative and avoided possible modifications of the organic matter. Based on the MIBK method, a model of humin was proposed by Rice and MacCarthy (1990). These researchers observed that disaggregation of humin led to four different fractions: bitumen (lipids extracted by soxhlet extraction), bound-lipid fraction (isolated by MIBK), bound-humic acid (brown fraction, alkali-soluble and precipitated under acid conditions) and the insoluble residue or mineral matter. They proposed that humin is an aggregate of those four fractions that can be separated by the MIBK procedure. The hydrophobic character of humin was attributed to the bitumen and bound-lipid fraction, while acidity, metal complexation, and some degree of CEC were attributed to the bound-humic portion and to the mineral residue.

1.3.1.1 Elemental Composition of Humin

The development of modern analytical techniques has allowed researchers to study humin characteristics more exhaustively. These techniques include IR, solid-state NMR, and ESR spectroscopy; and X-ray scattering (Hatcher et al., 1985). Employment of solid state ^{13}C NMR and X-ray scattering are currently the most important nondestructive analytical techniques that provide information of the composition of humin.

Russell et al. (1983) observed that the IR spectrum of a hydrolyzed humin was similar to that obtained for a hydrolyzed HA. Hydrolysis removed proteins and polysaccharides from humin. For both samples a COOH and conjugated H-bonded COOH bands were identified. Some researchers have demonstrated that humin is formed by aliphatic units and bound-HA components (Hatcher, 1980; Preston and Newman, 1992; Rice and MacCarthy, 1989). A comparison between an aquatic and a terrestrial humin was presented by Hatcher et al (1980). Large amounts of aliphatic structures were common components of both humins. By employing a CPMAS technique, they provided evidence that terrestrial HS were predominantly aromatic (lignin incorporated to its structure) while aquatic HS was more aliphatic. The dominance of humin with depth has been attributed to the loss of oxygen bearing groups due to condensation reactions and to selective preservation of aliphatic units (Hatcher, 1983). Hatcher et al. (1985) acknowledged that oxygen availability, drainage, and sediment deposition were factors that determine the composition of humin. A near-surface (0-5 cm) non-hydrolyzed humin was

reported to be composed predominantly of polysaccharides and paraffinic groups. Aromatic signal was dominant especially at 130 ppm indicating some abundance of carbon rings not substituted by electron withdrawing groups. Lignin was negligible in the sample since the phenolic (150 ppm) or methoxyl-substituted (55 ppm) signals were minor. The ^{13}C NMR study also showed that a hydrolyzed humin, from an aerobic soil, was mainly composed of paraffinic structures which originated from algal and microbial organisms. No significant peaks were observed for carbohydrates (due to hydrolysis), and although the aromatic region was predominant (100- 180 ppm) only a small shoulder was observed for phenolic carbons (150 ppm).

Two humin samples from different depths were compared and while the polysaccharides peak decreased in intensity with soil depth, the signal at 30 ppm increased in relative intensity. A signal in this region is usually attributed to proteinaceous material. However, the authors attributed the signal to highly cross-linked paraffinic molecules containing ether, carboxyl, and amide linkages; rather than to proteinaceous materials, which are labile and expected to be degraded as a function of depth. These researchers compared the HA and humin composition and noticed that the greatest difference between HA and humin was the larger relative concentration of paraffinic carbons in humin. The comparison provided evidence to state whether HA is a product of humin decomposition or is unrelated to the latter; concluding that humin is not a HA-clay complex or a product of HA condensation.

Additionally, Hatcher et al. (1985) discussed the ^{13}C NMR characterization of two peat materials. The spectra for both showed significant amounts of polysaccharides that decreased with depth; however, the remaining C signals had similar intensities at all depths. Lignin was found to be a major component of humin (well defined peaks at 55, 130 and 150 ppm) at all studied depths. A major component of the peat were the paraffinic carbons that were observed to be selectively preserved with increasing depth. Finally, a humin isolated from aquatic sediments was reported as dominated by paraffinic structures (at 30 ppm) and minor amounts of C-O carbons of ethers or carbohydrates (72 ppm), aromatic carbons (130 ppm), and carboxylic or amides carbons (175 ppm). It was observed that humin from the aquatic materials was extremely resistant to degradation by hydrolysis.

Vanderbroucke et al. (1985) studied FA, HA, and humin by means of infrared spectroscopy. These researchers observed that FA and HA contained more oxygenated functional

groups (O-H from alcohols; C=O from quinones, ketones and carboxylic acids; and C-O from alcohols, esters and ethers) than humin. Aliphaticity was reported to decrease from humin to HA and FA. Rice and MacCarthy, (1989) observed that the extracted organic part of humin consisted of aliphatic bound-lipids and a bound humic acid-like component. The O/C ratio of humic substances was observed to decrease in the order: FA > HA > humin (Rice and MacCarthy, 1991). Preston and Newman (1992) studied de-ashed humin samples by means of ^{13}C CPMAS NMR and they were able to obtain spectra of carbons in different spatial domains with separations of tens of nanometers or more. Methyl chains were predominant in the slower relaxing protons (rigid aliphatic carbons), while the faster relaxing protons (mobile carbons) contained more aromatic and *o*-alkyl carbon.

Rice and MacCarthy (1992) characterized the elemental composition of the bound-humic fraction of humin. These investigators found that bound-humic acids had higher O and H contents and less C and N than HA and FA obtained from the same source with the MIBK method. The high H/C ratio suggested a higher degree of aliphaticity in the bound-humic portion. Bound-humic acids were reported to be more aliphatic compared to the HA and FA fractions.

More evidence of the aliphatic nature and preservation of humin was provided by Almendros et al. (1996). From three different terrestrial samples, two types of humin were prepared, 1) by ultrasonic disruption of soil aggregates and separation by flotation and 2) by the MIBK method. Pyrolysis and on-line GC-MS along with ^{13}C NMR were used to characterize the prepared humin fractions. It was reported that the flotation method removed less humin (12 to 35 % of total insoluble organic matter) than the MBIK method (47 to 64%). NMR results showed a predominantly aliphatic nature of carbon (0-46 ppm) with a maximum intensity at 33 ppm, while aromatic carbon represented only 24% of the total signal intensity. The signal at 33 ppm was attributed to polymethylene chains whereas the signal at 172 ppm was assigned to carbonyl groups that could have been influenced by free carboxylic, esters and amino groups. The *o*-alkyl region showed a peak at 73 ppm and it was assigned to carbons in cellulose and hemicellulose. Thus, it was suggested that the *o*-alkyl region was composed of recalcitrant carbohydrates (crystalline cellulose and polysaccharides bound to the mineral fraction) and/or altered carbohydrates. Products of pyrolysis consisted of alkyl molecules (alkenes, alkanes and fatty acids) cyclic molecules (aromatic and hydroaromatic), fluorenes, phenols, catechols and methoxyphenolic compounds (lignin). Humin characteristics were reported to be different from

SOM because of the dominance of alkyl molecules and low proportion of alkylbenzenes and hydroaromatic groups. These researchers explained that the recalcitrant humin was formed not only due to selective preservation of aliphatic bio-molecules, but also due to the formation of aggregates by fixation of reactive lipids on mineral fractions and their compartments.

Fabbri et al. (1996) utilized pyrolysis GC-MS and pyrolysis/methylation GC-MS to characterize a humin from an agricultural soil. Based on the structural importance and identification reliability, they reported 36 products of pyrolysis. A large number of the products were described as carbohydrates. The *n*-alkenes/*l*-alkenes molecules arising mainly from samples containing polymethylene structures (such as lipids with long aliphatic units and paraffinic materials) were observed to be the most important products of pyrolysis. Lignin precursors, guaiacyl, and syringyl, were scarce in the pyrolyzates of humin. The proportion of N-containing compounds and aromatic hydrocarbons in humin was similar to that observed for a HA from the same source. Due to polarity, reactivity of the fragments and/or their sorption to the pyrolysis unit, these researchers also utilized the pyrolysis/methylation GC-MS. Methylation allows detection of the methyl derivative of compounds with exchangeable hydrogen (like carboxylic acids). A total of 46 products were reported when pyrolysis/methylation was employed. The dominant signals were attributed to carbohydrates. Some of the compounds corresponded to alkanoic acids (linear saturated fatty acids, unsaturated and branch-chained fatty acids, fumaric acid, and succinic acid), aromatic acids (permethylated derivatives of *p*-coumaryl, guaiacyl, and syringyl), methyl esters of benzoic acid, 3-methyoxy-benzoic acid, 4-methoxybenzoic acid and phthalic acid among others.

Grasset and Ambles (1998 a) isolated soil humin to study its properties. The aliphatic nature of the humin was revealed by the H/C ratio (1.86) and by the FT-IR spectrum bands that showed hydroxyl and methoxyl groups from cellulose and lignin. Some bands were inferred as hydrolysable groups (C=O and C-O). Phase transfer catalyzed hydrolysis (PTC-hydrolysis) was employed to characterize humin chemical conformation. Two sequential hydrolysis steps of humin were able to solubilize more than 87% of the initially insoluble humin. Considerable amounts of short-chain dicarboxylic acids were extracted in the first hydrolysis, while in the second hydrolysis long-chained (with even number of carbon) molecules were observed. They also found monocarboxylic acids and alkanols. The origin of short linear molecules (fatty acids and alkanols) was suggested to be bacterial, while long-chained molecules and aromatic acids

were believe to originate from plants. Analysis of the hydrolysis products led these researchers to conclude that the structure of humin includes cross-linking of macromolecular chains bound through ester groups to humin matrix. Thus, low molecular weight molecules are trapped by complex polymers.

One of the more common polymers in soil is cellulose. The ability of soil cellulose to retain organic molecules was studied by Grasset and Ambles (1998 b). These researchers applied a method to hydrolyze the cellulose from a humin sample with the objective of releasing lipids trapped by macromolecules. The hydrolysis released C_9-C_{20} fatty acids, C_{12}-C_{20} fatty acids methyl esters, C_{16}-C_{35} *n*-alkenes; and C_{16}, C_{18} and C_{20} *n*-alkenes. The occurrence of free lipids (directly extractable by PTC-hydrolysis) and trapped lipids (extracted by hydrolysis of cellulose) was observed but their origin was considered different. While free lipids were reported to have a plant origin (Grasset and Ambles, 1998 a), trapped lipids were attributed to a microbial source since there was not an even/odd tendency in the *n*-alkane distribution. These researchers suggested that these two different types of lipids were not mixed in the humin arrangement but each type formed large independent aggregates that were adjacent to each other contributing in this way to the heterogeneity of humin configuration.

Incubation of sedimentary humin for 60 days in the absence of biocide showed the conservation of paraffinic and aromatic functional groups, while the ^{13}C NMR signal indicated loss of alcohols and carbohydrates (Guthrie et al., 1999). Hu et al. (2000) observed the occurrence of crystalline and noncrystalline (mobile) poly(methylene) regions $-(-CH_2-)_n-$ in humins extracted from two different peat materials. These researchers suggested that some of the soil specific characteristics were due to the presence of poly(methylene) crystallites. Both types of poly(methylene) domains were reported to form large aggregates. Semicrystalline domains in humin were on average more than 10 nm in diameter. Mobile amorphous domains were compared to alkane solvents because they exhibited hydrophobicity and relatively low density. While the crystalline domain was suggested to be responsible for resistance to biological degradation, the mobile and amorphous domain was suggested to provide sorption sites that could interact with nonpolar compounds.

Kang and Xing (2005) studied the structural properties of HA and humins that were progressively alkali extracted from the same soil material. After alkali extraction, the humin residue consisted of two layers. The top layer, dark brown colored, (HuH) and the lower layer

with a lighter brown color (HuL) were separated and used as different humin fractions in the sorption experiments. Elemental analysis showed C/H values of 0.68 and 0.63 for HuH and HuL, respectively (or 1.47 and 1.59 H/C). The H/C values suggested the aliphatic nature of both humin fractions. Both humins were less polar than the extracted HA fractions (0.42 and 0.40 for HuH and HuL, respectively). The increase in aliphatic groups with increase in the number of extractions was suggested by elemental analysis and it was confirmed by Diffuse Reflectance Infrared Fourier-Transform Spectroscopy (DRIFTS). Polarity was reported to decrease with the number of extraction. Some peaks were attributed to unknown mineral compounds observed in both humin fractions. The ratio of aliphatic/aromatic carbon (1.97 for both humins) indicated a decrease in aromaticity compared to that observed for the HA fractions (1.6 to 1.89). NMR analysis also confirmed the increase of aliphatic nature, with 68 and 67% of total carbon for HuH and HuL, respectively. Amorphous (30 ppm) and crystalline (33 ppm) aliphatic poly(metheylene) groups were detected by clearly separated and defined peaks in the humin NMR spectra. The origin of these insoluble, hydrophobic groups was attributed to selective preservation of cutin or suberin due to their resistance to biological and chemical attack. The researchers also suggested that aliphatic carbon sorbs preferentially to clay minerals, thus humin organic carbon should be mainly composed of paraffinic groups with less predominance of polar functional groups.

Two peat soils (Amherst and Florida peat) were subjected to four different extraction methods to concentrate four different humin fractions (Wang and Xing, 2005). After extracting HA and FA six times with 0.1 M $Na_4P_2O_7$ the four methods used to obtain humin were: 1) –HF– treating soils four times with 1.0 M HF for 24 hrs, 2) –HF/HCl– treating soil with 6 M HF/HCl at 60 C for 24 hrs, 3) –NaOH– exhaustive extraction of soil with 0.1 M NaOH until no color was left in supernatant and 4) –NaOH- HF– exhaustive NaOH extraction followed by de-ashing with 1.0 M HF, four times for 24 hours. For both Florida and Amherst humins, NaOH humin had the highest amount of ashes and lowest percent of carbon, while the HF/HCl humin had the highest C and lowest ash content. ^{13} C NMR spectra showed that humins treated with NaOH and NaOH-HF were relatively richer in aliphatic groups and poorer in aromatic regions. The researchers alleged that exhaustive NaOH extraction removed all the FA and HA that are rich in aromatic carbon, thereby enriching the humin with aliphatic carbon. HF/HCl treatment removed half of the carbohydrate content of the soils and proportionally enriched the aromatic and aliphatic

carbons in the humin residue. HF/HCl treated humin showed higher aromatic content than the NaOH humin. Polarity index was defined by these authors as the ratio of polar C (carbohydrate, phenolic, and carboxylic) to total organic C (0-220 ppm). Polarity indexes showed that HF/HCl humin had the lowest polarity while HF extracted humin had the highest. Alteration of the poly(methylene) carbon conformation was observed since amorphous poly(methylene) carbons were enlarged when HF/HCl treatment was used but the crystalline poly(methyle) carbons were dominating in the samples treated by the other methods. The researchers hypothesized that enrichment of the amorphous nonpolar aliphatic domains (considered to be more mobile) in humin could favor the sorption of nonionic organic chemicals. The increase in the mobile, aliphatic carbon was attributed to the breakage of linkages between carbohydrates and SOM functional groups, and between humic material and minerals by the HF/HCl treatment which caused humin conformation to become loose and more flexible. Amherst humin was observed to contain higher amount of mobile carbon compared to Florida humin.

The review of literature suggests that there is sufficient scientific evidence to support the aliphatic nature of the humin fraction of SOM. The following section will discuss how the aliphatic character of the humin fraction is important since aliphatic structures have been reported to sorb appreciable quantities of organic contaminants (Salloum et al, 2002).

1.3.2 Interaction of Humin with Organic Chemicals

The importance of studying interactions between organic contaminants and humin residue is that it represents the most aged of the three SOM fractions (Huc and Duran, 1977; Stevenson, 1982, Vanderbrouke, 1985), and interacts directly with the mineral soil components, thus acting as a bridge between the inorganic and organic phases of the soil. Organic compounds tend to sorb rapidly and often irreversibly to humin. The high affinity of organic solutes to humin has been explained by the reactive surface of humin components and their insolubility (Hayase and Tsubota, 1983; Guetzolff and Rice, 1994; Malekani et al., 1997). In addition, it has been reported that the humin components (humic and lipids) have mesoporous surfaces, i.e. more than 60% of their surface area in <10 nm-wide pores (Malekani et al., 1997). The sub-nanometer scale pores have been suggested to significantly influence the sorption kinetics of nonionic organic chemicals (De Jonge and Mittelmeijer-Hazeleger, 1996). This section summarizes the most important results of studies where sorption behavior of humin was studied.

Recent technological advances of spectroscopy have facilitated and originated numerous studies that have focused on the sorption of organic solutes on concentrated (isolated) humin or humin-mineral fractions. Other researchers have conducted experiments consisting of sorption of chemicals into bulk soils or sediments, where later the soil has been fractionated into the three main SOM fractions for determination of the mass of solute associated with each organic fraction (Capriel et al., 1985; Dec et al., 1997; Kohl and Rice, 1998; Loiseau et al., 2000; Xu and Bhandari, 2003 a and b; Lesan and Bhandari, 2004; and Palomo and Bhandari, 2006).

Capriel et al. (1985) reported that the atrazine bound residue associated with humin fractionated from a methanol extracted soil, increased from 22% of total bound residue to 44% in 9 years of contact time. The affinity of toluene and trichloroethylene (TCE) to organic sorbents, soils and soil fractions including alkali extracted humin and the residue left after oxidation with H_2O_2, was evaluated by Garbarini and Lion (1986). The alkali extracted humin contained 0.88 % of total carbon while the oxidized humin had only 0.14%. While the K_d values for toluene and TCE for the whole soil were 11.3 and 7.95, the K_d s for alkali extracted humin were significantly smaller (2.36 and 1.28 for toluene and TCE, respectively). For oxidized humin the K_d values were noted to reduce dramatically (0.49 and 0.40 for toluene and TCE, respectively). However, when K_ds were normalized by soil carbon contents, the K_{OC} s for alkali extracted humin (268 and 145 toluene and TCE, respectively) and oxidized humin (348 and 287 toluene and TCE, respectively) were bigger than those reported for the whole soil (151 and 106 for toluene and TCE, respectively). The largest K_{OC}s were reported for the oxidized humin. The researchers attributed this phenomenon to a different type of SOM that might be found in mineral matrices with low organic carbon but is capable of binding organic solute more effectively. They also suggested that the organic matter in a soil with high SOM content should be physically and chemically different from that found in a mostly mineral soil. The difference was mainly attributed to the degree of interaction of the SOM with minerals, the source of SOM, and the extent of diagenesis.

The MIBK method was utilized to isolate the humin portion and three humin fractions (bound-humic acid, bound lipid and mineral component) from a soil that was previously contaminated with ^{14}C-labeled pesticides-DDT, 2,4-D and atrazine (Xie et al., 1997). The percent of bound residue in humin ranged from 15 to 63% of the total chemical associated with the whole soil. The bound-humic acid was observed to contain significant portions of bound

pesticides. However, for DDT, the bound-lipid fraction also represented a major sink for the bound residue. These researchers explained that the hydrophobicity of the lipids in humin favored the association of DDT. Some radioactivity was associated with the mineral portion, but these researchers attributed the result to a small amount of SOM that could not be removed from the mineral fraction by the MIBK method. Dec et al. (1997) observed that a soil contaminated with ^{14}C-labeled cyprodinil and sequentially extracted retained 18.1 % of the initially added radioactivity. Whereas, the isolated humin fraction contained 4.9% of the bound activity, 5.7 % and 7 % were associated with HA and FA, respectively.

The formation of bound residues of 5 different chemicals (naphthalene, phenanthrene, benzo(a)pyrene, 4,4'-dichlorobiphenyl and 2,2',55'-tetrachlorobiphenyl) associated with humin isolated from three different soil was studied by Kohl and Rice (1998). These researchers observed that the humin fraction contained 50% or more of the total bound residue. In absolute basis, the humin fractions showed higher contaminant binding (humin was the most abundant SOM fraction), however, in some cases the chemicals showed more affinity for FA or HA. The low aqueous solubility of benzo(a)pyrene was used to explain its high affinity towards humin. The affinity of the contaminants to the SOM fractions was dependent on the characteristics of the sorbent. For two of the soils, the humin fraction showed a high affinity for phenanthrene, while for the third soil, the affinity decreased in the order HA>humin>FA. Naphthalene exhibited higher affinity to FA and HA than to humin. The humin portion of each soil was fractionated into bound-humic acids, bound-lipids and insoluble residue components to study the distribution of the contaminants within the humin structure. Although the bound-lipid fraction showed the highest relative affinity for all solutes studied, the bound-humic acid portion contained the most bound residue because it was the largest fraction of humin. On the other hand, low affinity was observed for the insoluble residue. In a later study Kohl and Rice (1999) observed that removal of naturally occurring lipids from bulk soil increased the uptake of PAHs, suggesting that lipids were competing for hydrophobic sorption sites.

Louseau et al. (2000) observed that the amount of atrazine and its degradation products bound to SOM fractions depended on the soil pH. Thus, humin fraction (left after HF and/or alkali extractions) of two alkaline soils, was associated with the higher amount of chemical (more than 75% of total chemical added), while for the acidic soil, the amount of chemical associated with humin decreased to 54%. These researchers concluded that, the bound residue

formation was more favorable in the humin fraction for alkaline soils, while at acidic soil pHs the FA fractions were favored. In general, these investigators reported that organic chemicals associated preferentially with FA and or humin and finally with HA.

To study the sorption properties of humin, some investigators have removed HA and FA from soil or sediments using a variety of methods, leaving the humin-mineral fraction as the solid phase to conduct the sorption experiments (Guthrie et al., 1999; Chiou et al., 2000; Chefetz et al., 2000; Mao et al., 2002). Other researchers isolated and concentrated humin material by alkaline extraction of HA and FA followed by mineral dissolution (Shih and Wu, 2002; Kang and Xing, 2005; Wang and Xing, 2005). These studies had the common objective of elucidating structural properties of humin capable of influencing the binding of organic compounds. Guthrie et al. (1999) studied sorption of pyrene to sedimentary organic matter. Bulk (NaOH extracted residue) and concentrated humin (HF/HCl digested residue) fractions were isolated at the end of the solute-sorbent contact period. The mass of pyrene associated with concentrated humin increased over the course of 60 days from 0 to 306 μg. In the bulk humin, the mass of chemical increased from 14 to 189 μg for 0 and 45 days, respectively and later decreased to 89 μg at 60 days. Although humin sorbed pyrene efficiently, pyrene binding was not attributed to the formation of covalent bonds but to hydrophobic interactions. Pyrene was not released by solvent extraction but recovered after pyrolysis. Soil biological activity was investigated and it was observed that it increased pyrene sequestration, while the addition of a biocide significantly reduced the pyrene retention. High affinity between pyrene and humin was attributed to the soil diagenesis which selectively removes hydrophilic SOM while making available the hydrophobic sites for non-covalent interactions.

The sorption of 1-naphthol to humin isolated from soils and geological samples was studied by Salloum et al. (2001). Humin was defined as the insoluble material remaining after exhaustive NaOH extraction. Humin samples showed H/C values ranging from 1.2 to 4.8. High H/C values were attributed to the H present in water or hydroxyl molecules associated to the mineral fraction in the humin. The H/C humin values were higher than those observed for parent soils and HAs. Carbon analysis revealed that for all geosorbents, the humin contained the majority of the carbon (53 to 94 % of the total carbon in the sample). The reported K_{OC} values varied from 387 ml/g, for Pahokee peat-humin, to 7660 ml/g, for the Brown Chernozemic surface soil-humin. These authors suggested that the high K_{OC} values could have resulted from

changes in the SOM physical conformation due to sequential alkali extractions. Surface soils with higher mineral fraction demonstrated higher K_{OC} values suggesting that minerals to some extent also determined the magnitude of K_{OC}. The researchers utilized the K_{OC} for sorption to FA, HA, and humin fractions to reconstitute the overall K_{OC} for the six parent materials. The K_{OC}s for the parent materials were significantly smaller in most cases when compared to values obtained from reconstituted K_{OC}s. To explain the difference in the K_{OC} values, the researchers suggested that the accessible carbon rather than the total carbon in a sorbent was a factor in the retention of any contaminant.

In contrast to conventional belief that high aromaticity is related to high retention of nonionic compounds, the contribution of aliphatic groups to the sorption of pyrene and phenanthrene was demonstrated by Chefetz et al. (2000), Mao et al. (2002), and Kang and Xing (2005). CPMAS ^{13}C NMR and H/C ratio were used to confirm the predominant aliphaticity of a humin extracted from an algal deposit (Chefetz et al., 2000). The H/C had a value of 1. 7 and the NMR spectrum showed the most dominant peak at 30 ppm, which corresponded to methylene carbons. Carbohydrates (72 and 105 ppm), carboxyl and amides groups (128 and 172 ppm) were also identified by well defined peaks. Sorption behavior of pyrene to the humin fraction was studied by means of an isotherm. The isotherm resulted in an L-shaped (nonlinear) curve and the calculated K_{OM} and K_{OC} had values of 148,300 ml/g and 313,800 ml/g, respectively. Humin structure was predominantly aliphatic. Total aromaticity and aliphaticity were 8.8 and 91.2 % of the total carbon, respectively. These researchers concluded that aliphatic structures, especially paraffinic and o-alkyl C, were also important in the sorption of nonionic compounds.

Sorption isotherms for ethylene dibromide (EDB), diuron, and 3,5-dichlorophenol were generated by Chiou et al. (2000). One of the sorbents utilized was a humin extracted from peat. These researchers hypothesized that soil or sediments that were subjected to intense alkali extraction were enriched with high-surface carbonaceous material. Thus, they proposed that the high-surface carbonaceous material was responsible for nonlinear sorption of nonpolar compounds, while for ionic solutes, specific interaction between SOM active groups and the solute were responsible for their significant nonlinear sorption. For EDB sorbed to humin, the isotherm was nonlinear at low aqueous concentrations and became linear as the solute concentration increased. The nonlinear capacity was higher for EDB sorbed to humin (0.5 mg/g) than the parent peat (0.18 mg/g). For humin the Freundlich *n* value was smaller (0.88) than for

peat (0.91). For diuron and 3,5-dichlorophenol, the sorption isotherms showed higher uptake and more nonlinear behavior in the case of humin than for whole peat.

Comparable C contents of peat and humin (49.3% and 51.4%, respectively) yet significantly different surface areas (1.8 and 4.5 m^2/g, respectively), for peat and humin respectively, were used to support the idea of enrichment of high-surface carbonaceous material in humin, eliminating the idea of mineral or ash as accountable for the higher sorption. The nonlinear sorption of EDB was attributed to the surface area of humin available. The positive correlation between surface area and sorption capacity of EDB led the researchers to conclude that for nonpolar compounds, sorption capacity was strongly influenced by the presence of high-surface carbonaceous material. While for polar solutes, sorption was not significantly affected by the polarity of the sorbent. Therefore, nonlinear sorption was attributed to specific interactions with the reactive moieties of SOM rather than to surface area related interactions (nonlinear sorption capacity was higher than the estimated surface adsorption capacity).

Hu et al.'s (2000) contention that poly(methylene) regions favored the retention of hydrophobic organic compounds was further evaluated by Mao et al. (2002) who studied the sorption of phenanthrene in the presence of various geosorbents, including humin materials. Phenanthrene sorption isotherms were produced. To solve the known dimensional problem with the Freundlich parameter K_F, these authors utilized a modified version of the model and utilized the unit equivalent K_F', where the equilibrium aqueous concentration was normalized by dividing its value by the aqueous solubility of the sorbate (Carmo et al., 2000). It was observed that while Amherst humin, with a high content of poly(methylene), exhibited a higher sorption capacity (K_F' =26.8 mg/g) and sorption linearity (n=1.0); the Florida humin, with low content of poly(methyelene), had lower sorption capacity (K_F' =11.4 mg/g) but showed nonlinear sorption behavior (n=0.69). Weight fractions of amorphous and crystalline poly(methyelene) regions were quantified and they were converted to a fraction of the total sample weight. Sorption of phenanthrene was suggested to be controlled by the amorphous poly(methylene) since a positive correlation was observed between amorphous poly(methylene) weight fraction and the K_F' value. On the other hand, there was no significant correlation between K_F' and the weight fraction of aromatic carbons, and K_F' and the polarity index. Polarity index was determined by these authors by the ratio of carbohydrate C, phenolic C, and carboxylic C to the total C in the region of -10 to 260 ppm, then multiplied by the fraction of organic carbon of the sample weight.

The polarity index and aromatic groups in the humins were not significant factors in determining the sorption of phenanthrene, while amorphous poly(methylene) and branched nonpolar aliphatic domains appeared to be responsible for the sorption of phenanthrene.

Shih and Wu, (2002) evaluated the sorption behavior of VOCs using sorption-desorption kinetics of toluene in contact with the de-mineralized humin. Humin was compressed and placed on disks or thin films for gas-phase sorption experiment. Diffusivity of toluene into humin ranged from 10^{-8} to 10^{-9} cm^2/s. Toluene sorption to humin was completely reversible since no residual toluene was detected in the humin after desorption. No strong interactions between toluene and humin were observed by IR analyses. The authors concluded that sorption of toluene was controlled by diffusion and that the mechanism governing toluene sorption was simple partitioning.

The effect of humin hydration on the sorption of acetophenone, nitrobenzene, benzyl alcohol and *m*-nitrophenol was investigated by Borisover and Graber (2004). The humin was alkali extracted from a peat material. In general it was observed that hydration of humin could increase (benzyl alcohol and *m*-nitrophenol) and decrease (nitrobenzene and acetophenone) the sorption of organic compounds. The difference in the hydration effects were attributed to the sorbent-sorbate interaction in the dry state. In dry humin systems, the interaction strength followed the order nitrobenzene >*m*-nitrophenol>> acetophenone> benzyl alcohol. The researchers suggested that dry humin interacted preferentially with low polarity compounds than with polar compounds. Hydration assisted the sorption of compounds that formed strong specific interactions with organic matter like benzyl alcohol and *m*-nitrophenol. It was suggested that these compounds could interact with hydrated sorption sites or compete with water for originally accessible sorption sites or sites at disrupted SOM contacts.

Kang and Xing (2005) studied the effect of the structural properties of HA and humins, progressively alkali extracted from the same soil material, on the sorption of phenanthrene as a model HOC. Phenanthrene sorption isotherms were fitted to the Freundlich model. The K_{OC} values for HuH (f_{OC} = 0.231) and HuL (f_{OC} = 0.053) were 24,700 ml/g and 64, 200 ml/g, respectively. While HA K_{OC}'s ranged from 8,400 ml/g to 19,400 ml/g. The nonlinear sorption behavior was evident from the Freunlich *n* values (0.880 and 0.895 for HuH and HuL, respectively). The nonlinear behavior was attributed to conformational changes in SOM due to association with the mineral fraction. Organic-mineral associations were suggested to lead to a

more condensed condition of the humin matter. The researchers suggested that amorphous aliphatic carbon was responsible for the sorption of phenanthrene since a positive correlation was observed between log K_{OC} and aliphaticity. The negative correlation between polarity and log K_{OC} suggested that hydrophobic rather than polar interactions favored phenanthrene sorption to humin. The researchers concluded that the polarity of the sorbent was a key characteristic regulating HOC sorption rather than the predominance of aromatic or aliphatic groups.

The effect of different humin isolation methods on the carbon structure and on the sorption of phenanthrene was evaluated by means of ^{13}C NMR and sorption isotherms (Wang and Xing, 2005). Two peat soils (Amherst and Florida peat) were subjected to four different extraction methods to concentrate four different humin fractions. For humin fractions from both soils, all phenanthrene sorption isotherms showed nonlinear sorption. The NaOH extracted humin exhibited the highest nonlinear behavior in both soils (n = 0.553 for Florida and 0.869 for Amherst humins). Values of n were reported to be correlated to the ash content of the humin fractions. HF and HF/HCl treated humin exhibited higher n values. The increase in sorption linearity was attributed to de-ashing that was able to free humin components making them available to interact with phenanthrene. This increased the available sorption sites and as a consequence increased the value of n. Phenanthrene sorption behavior was more linear in the Amherst humin and the n values were lower for Florida humin. These results were consistent with the NMR data that showed that Florida humin was less mobile, with fewer aliphatic domains and more aromatic carbons. Large K_{OC} were obtained for both Florida and Amherst humins. The general trend was HF<NaOH≈ NaOH-HF<HF/HCl. The high K_{OC} values were explained by the relative increase in aliphatic nonpolar carbons, due to NaOH extraction, and by the enrichment of poly(methylene) groups and a reduction in polarity due to digestion of carbohydrates. The researchers concluded that the structural conformation of humin (mineral-humin interactions, ash content) and its chemical composition were the primary factors affecting the nonlinear sorption behavior of nonpolar organic compounds. Similar conclusions were derived in a recent study that reported sorption isotherms for 1-naphthol in six humin samples (Simpson and Johnson, 2006) and of phenanthrene in four humin fractions and their parent soils (Bonin and Simpson, 2007). In both studies the sorption of the solute in the whole soils and the humin fractions was investigated. The researchers reported that K_{OC} values for humin were consistently higher than those obtained for the parent soils. Some of the bulk soils and humins

were common in both studies. In the case of 1-naphthol the K_{OC} values ranged from 387 to 7,660 mL/g (Simpson and Johnson, 2006). In the study of Bonin and Simpson (2007), two types of humin fractions were used: alkali extracted and de-ashed fractions. The K_{OC} values for de-ashed humin were higher than those for alkali extracted humin. K_{OC} values ranged between 14,400 to 53,700 ml/g including both types of humin. Sorption isotherms showed higher affinity of phenanthrene for the alkali extracted humin isolated from peat. For the common sorbents used in these two studies the K_{OC} values obtained for phenanthre sorption were always higher than those obtained for 1-naphthol. However, none of the studies provided discussion of the linearity of the isotherms.

1.4 Enzymatic Polymerization of Phenols

Several soil enzymes (and transition metal oxides) have been shown to catalyze the oxidative polymerization of aromatic compounds such as phenols. Oxidative coupling reactions can also occur spontaneous by alkaline pHs in the presence of oxygen (Bollag, 1992 b). Enzymes that catalyze the oxidative polymerization of phenols are classified as polyphenol oxidases (phenoloxidases) and peroxidases. Polyphenol oxidases are oxidoreductases that are classified into two subgroups: laccases and tyrosinases. These enzymes require molecular oxygen to catalyze the oxidation of phenols, and their activities do not depend on the presence of coenzymes or cofactors. Peroxidases, on the other hand, require the presence of peroxides (like hydrogen peroxide) to catalyze oxidative polymerization of phenols (Bollag, 1992 a and b; Duran and Esposito, 2000).

Tyrosinases contain copper in their active site and are produced by microorganisms, plants and animals. The two types of reactions performed by tyrosinases include: oxidation of *o*-diphenols to *o*-quinones (catecholase or diphenolase) and the *o*-hydroxylation of monophenol (monophenolase or cresolase). Benzoic acid, L-mimosine, benzohydroxamic acid and sulfhydryl compounds inhibit the activity of tyrosinases (Bollag, 1992-b; Duran and Esposito, 2000).

Laccases are produced by basidiomycetes and are copper-containing polyphenol oxidases. Laccases are remarkably non-substrate specific, so the variety of utilizable substrates (phenols, phenolic dyes, chlorophenols, lignin-related diphenylmethanes, benzopyrenes, N-substittued *p*-phenylenediamines, organophosphorus) vary from one laccase to another. The oxidation efficiency among catalases broadly varies, and typical inhibitors for these enzymes

include azide, thioglycolic acid, and diethyl-dithiocarbonic acid. The oxidation efficiency among a diversity of catalases broadly varies (Bollag, 1992 b; Duran and Esposito, 2000).

Oxidative polymerization of chlorinated phenols and anilines in the presence of phenoloxidases occurs in two stages. The first stage consists of oxidation of substrates into free radicals or quinones. In the second stage the products are subjected to chemical coupling or polymerization (Park, 1999). While laccases oxidize the pollutants into free radicals, tyrosinases oxidize the organic contaminants directly into quinones. Nucleophilic addition or coupling of free radicals is the mechanism responsible for the polymerization of phenolic substrates.

Peroxidases are hemoproteins produced by microorganisms such as *Bacillus, Pseudomonas, Arthobacter* and *Streptomyces* as well as by plants. Peroxidases are enzymes that oxidize the phenolic compounds into phenolic radicals. All types of peroxidases (horseradish peroxidase (HRP), chloroperoxidase (CPO), lignin peroxidase (LiP), manganese peroxidase MNP)) contain an iron porphyrin ring (Bollag, 1992 b and Bollag, 1992 a). The most widely studied peroxidase enzyme is that derived from horseradish. The properties and characteristics of horseradish peroxidase are discussed in detail in the following section.

1.4.1 Horseradish peroxidase

Horseradish peroxidase (HRP) catalyzes the polymer formation of a broad range of phenols, anilines, biphenols, and related heteroaromatic compounds (Bollag, 1992 a; Duran and Esposito, 2000). HRP has over 40 identified isoenzymes, with the isoenzyme A (acidic), isoenzyme C (neutral or lightly basic) and a strong basic HRP, as three most important utilized HRP isoenzymes in experimental work (Dunford, 1991).

The dominance of the isoenzyme C in horseradish roots has been reported. The HRP C is characterized by a heme-prosthetic group, with two Ca^{2+} and 308 amino acids residues (that include four disulfide bridges in a single polypeptide chain that contains eight neutral carbohydrate side-chains). The molecular weight of HRP C has been reported as approximately 44 KDa (Welinder, 1979).

The enzymatic reaction cycle involved in the oxidative polymerization of phenols is illustrated by the following set of equations. (Dunford, 1991; Ibrahim et al., 2001):

$$E + H_2O_2 \rightarrow E - I + H_2O \qquad \text{(Eq. 1.4)}$$

$$E - I + AH_2 \rightarrow E - II + AH\bullet \qquad \text{(Eq. 1.5)}$$

$$E - II + AH_2 \rightarrow E + AH\bullet + H_2O \qquad \text{(Eq. 1.6)}$$

The overall reaction of the reaction cycle is:

$$2AH_2 + H_2O_2 \rightarrow 2AH\bullet + 2H_2O \qquad \text{(Eq. 1.7)}$$

where E is the enzyme, E-I and E-II are the reactive enzyme intermediate compounds I and II, respectively. AH_2 is a reducing substrate such as phenol and $AH\bullet$ is a free radical product. A graphical representation of the HRP cycle during the oxidative polymerization reaction is shown in Figure 1.5.

The chemistry of the free radical product determines its ultimate fate, which may include co-oxidation by attaching to another free radical or diffusion from the enzyme's active site into solution followed by oxidative coupling with another substrate molecule to form dimer, trimers, tetramer or larger oligomers (Dunford, 1991). The cross coupling oxidation can result in C-C and C-O bonds linking phenolic molecules and C-N and N-N bonds involving aromatic amines (Bollag, 1992 a).

Peroxidases oxidize the phenols to free radicals, and the subsequent coupling of free radicals is the mechanism responsible for the polymerization of the phenolic substrates or xenobiotics. Phenol polymerization products generally have high molecular weights and lower solubility than the parent solutes. Thus, the newly formed polymerization products are removed from solution by sorption to sorbent or by precipitation.

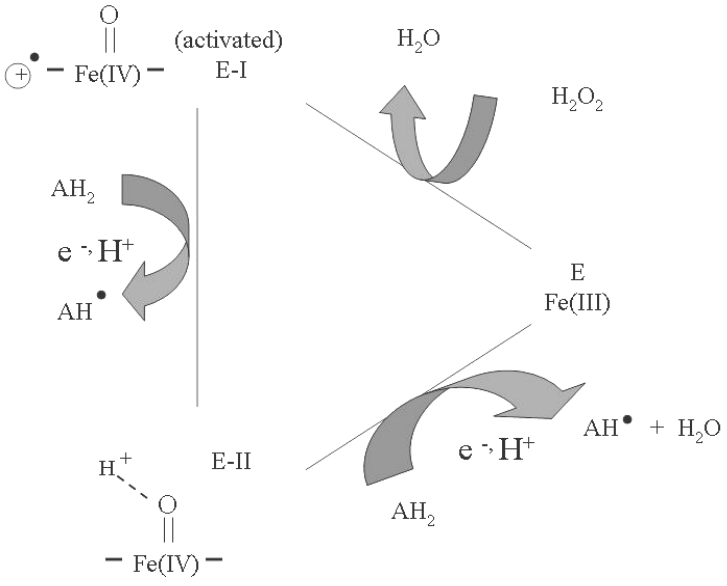

Figure 1.4 Horseradish peroxidase oxidizing cycle. Adapted from Berglund et al., 2002.

1.4.2 Use of Enzymes to Remediate Contaminated Environmental Media

The use of enzymes to enhance polymerization of phenolic contaminants has been considered for water treatment, wastewater treatment and for remediation of contaminated soil The low solubility of the resulting phenolic polymerization products allows the use of conventional treatment processes like coagulation, sedimentations and filtration for subsequent removal from the aqueous phase (Minard et al., 1981; Nanniperi and Bollag, 1991).

For example, Klibanov et al. (1983) reported the removal of phenols from a coal-conversion effluent during polymerization with HRP. Ferrer et al. (1991) utilized HRP to remove color in wood-pulp bleaching process effluent caused by the presence of phenols. Roper et al. (1996) used minced horseradish roots to treat water containing phenols and anilines. Maximum precipitation of 2,4-dichlorophenol (DCP) occurred at pH values ranging from 5 to 7 and when initial substrate concentrations were between 7 and 8 mM. Cooper and Nicell (1996) studied the enzyme-catalyzed removal of phenols from foundry wastewaters and reported an overall removal efficiency of 98% for total phenols. HRP enzymatic reaction was not affected by the presence of iron and other organic compounds. HRP catalyzed polymerization and precipitation of pentachlorophenol (PCP) in aqueous solution was observed by Zhang and Nicell (2000). The optimum pH range for PCP removal ranged from 4 to 5. The predominant products of the oxidative polymerization of PCP were dimers. The treatment of foul condensates, generated from a hardwood Kraft pulp and paper mill, with HRP and H_2O_2, resulted in a reduction of total phenols to levels below 1 mg/L (Wagner and Nicell, 2001).

Incorporation of phenolic and anilinic compounds to soil humic substances has also been extensively studied and reported in the scientific literature (Bollag et al., 1980; Thorn et al., 1996; Bhandari et al., 1996; Weber et al., 1996; Bhandari et al., 1997; Flanders et al., 1999; Park et al., 2000; Dec and Bollag, 2000; Bhandari and Xu, 2000; Xu and Bhandari, 2003 a and b; Palomo and Bhandari, 2005 and 2006).

Bhandari et al. (1996) attributed the formation of non-extractable 4-monochlorophenol residues (nearly 15% of the chemical initially applied) in surface soils to indigenous soil enzymes and transition metal oxides. Minced HRP roots were found to enhance the binding of ^{14}C-dichlorophenol to soil (Flanders et al., 1999). Huang et al. (2002) investigated the rate of HRP-mediated phenol non-extractable product formation in the presence of natural geosorbents. Xu and Bhandari (2003) reported the sorption and non-extractable residue formation of phenol,

o-cresol and DCP in the humic (HA) and fulvic (FA) acid fractions of two surface soils. Palomo and Bhandari (2006) reported the effect of contact time on the formation of nonextractable DCP residues formed in the presence of HRP in two surfaces soils upon employment of the oxidative polymerization process. More recently, Kim (2007) reported the deposition of HRP-generated phenol polymerization products in a simulated aquifer column system free of organic matter.

1.5 Oxidation of Soil Organic Matter

The removal of SOM from soil and sediments is necessary to conduct mineralogical analyses like particle size-distribution and X-ray diffraction. The objective of the methods used to remove SOM is to achieve minimum alteration of the inorganic fraction. The use of hydrogen peroxide (H_2O_2) digestion is among the most common and accepted methods for removal of organic carbon from soil samples. Acidification of samples to be treated with H_2O_2 is important since effective removal of SOM by H_2O_2 is achieved under acidic soil conditions. Efficient removal of SOM allows complete dispersion of the inorganic aggregates of the soil material under study.

The H_2O_2 method has been reported to significantly impact the integrity of the mineral soil fractions by causing dissolution of Si, Al, Fe (Lavkulich and Wiens, 1970) alkaline earth carbonates (Bourget and Tanner, 1953; Anderson, 1963) and Mn oxides (Jackson, 1956; Lavkulich and Wiens, 1970). Formation of calcium oxalate in soils treated by H_2O_2 has also been reported (Martin, 1954). Alteration of properties of the residues remaining after the H_2O_2 digestion includes loss of phosphate sorption capacity (Williams et al., 1958). Consequently, some researchers have utilized alternate SOM-oxidizing methods that allow higher preservation of the inorganic fractions of the soil sample.

Bourget and Tanner (1953) studied the effectiveness of sodium hypobromite (NaOBr) in digesting organic matter. These researchers compared the removal SOM from non-calcareous and calcareous soils (with and without addition of acetic acid, respectively). The NaOBr treatment consisted of contacting 20 grams of soil with 100 mL of NaOBr solution for a period of two hours at room temperature. The suspension was then heated with no boiling and an extra 200 mL NaOBr solution was added to the hot suspension. The mixture was stirred and kept over-night with no further heating. For non-calcareous soils, the NaOBr method showed a comparable SOM digestion capacity as observed with H_2O_2. However, when calcareous soils

were used, the H_2O_2 method removed calcium and magnesium carbonates, while the NaOBr technique did not.

Anderson (1963) proposed the utilization of a pH 9.5 sodium hypochlorite (NaOCl) solution to oxidize the SOM. A pH of 9.5 was suggested for complete sodium saturation without causing dissolution of aluminum. The objective of their work was to determine a method that could provide complete SOM removal without removal or complexation of sesquioxide coatings, silica or carbonates. The oxidation method consisted of adding a 20 ml aliquot of pH 9.5 NaOCl solution to 10 g of soil in a test tube. The tube was placed in boiling water for 15 minutes followed by centrifugation to separate the aqueous and solid phases. The supernatant was decanted and the treatment repeated three times. While the H_2O_2 method was ineffective in removing SOM when calcareous soils were used and no sample acidification was performed, when carbonate destruction was done, the H_2O_2 oxidation efficiency resulted in comparable SOM removal as NaOCl treatment. Three consecutive treatments with NaOCl solution were sufficient to reduce the organic content to values as low as 0.03 %. Thus, the NaOCl digestion method was able to achieve rapid and efficient oxidation of SOM without removal of carbonates or sesquioxide or silica coatings.

Lavkulich and Wiens (1970) evaluated the destruction of SOM in different soils by means of the NaOCl (as proposed by Anderson, 1963) and H_2O_2 oxidation methods. The amounts of Si, Al, Fe, and Mn extracted from different soils were determined. The NaOCl digestion method was modified from that proposed by Anderson (1963) since the suspension was heated in boiling water for 15 minutes. The NaOCl method was found to be more effective in removing organic matter than the H_2O_2 method with less alteration or destruction of the mineral oxides. The higher removal of organic matter was attributed to the higher oxidation potential of NaOCl at pH of 9.5 when compared to the oxidation potential of H_2O_2. The amounts of SiO_2, Mn, Fe, and Al removed from the samples with NaOCl treatment were lower than the amounts removed when the H_2O_2 method was utilized. Five successive NaOCl treatments performed on 16 soil samples reduced the carbon by 98%. These researchers also evaluated the effect of pH on the oxidation of organic matter and noted that as the pH of the NaOCl solution was reduced the amount of organic matter removed decreased, while the concentration of dissolved Fe increased. A pH of 9.5 was the optimum pH for the destruction of the organic matter with minimum removal of oxides.

While the NaOCl utilized in the previously described studies was reagent grade, Omueti (1980) employed commercial bleach solution that was adjusted to a pH of 9.5 immediately before use. The oxidation with commercial bleach (NaOCl) was more effective in oxidizing SOM than the H_2O_2 method. Other researchers have also reported using the NaOCl method along with other additives to extract metals from soil organic and inorganic phases (Shuman, 1983; Hettiarachchi et al., 2003).

1.6 Sorption of Organic Contaminants and Macromolecules to Mineral Soil Surfaces

Soils with no or negligible SOM content can be classified as inorganic or mineral soils. The interactions of organic contaminants with mineral soils are mechanistically different than those with organic soils. These interactions, however, play a significant role in the fate and transport of organic solutes in the subsurface environment.

An extensive collection of studies on sorption of organic contaminants and pesticides to mineral phases is available in the literature (Sanchez Martin and Sanchez Camazano, 1984; Micera et al., 1988; Laird et al., 1992; Barriuso et al., 1994; Hatzinger and Alexander, 1995; Huang et al., 1996; Sannino et al., 1997; Bhandari and Weber, 1998; Gonález – Pradas et.al., 1999; Ran et al., 2005). Ionic and neutral pesticides species have also been reported to be adsorbed in clay particle interlayers (Sanchez Martin and Sanchez Camazano, 1984; Ainsworth et al., 1987, Micera et al., 1988; Huang et al., 1996; Sheng et al., 2001; Sheng et al., 2002).

Protonated species of pesticides have been shown to sorb preferentially on smectite surfaces over nonprotonated species (Laird et al., 1992; Barriuso et al., 1994; Sannino et al.; 1997). Adsorption of neutral species is enhanced by an increase of the organic surface coverage (surface density), solution concentration, and higher solution pH (Ainsworth et al., 1987). Micera et al. (1988) investigated the sorption and interactions of fluazifop acid, a metabolite of the pesticide fluazifop-butyl, on bentonite. Interlayer sorption of fluazifop was attributed to two interrelated mechanisms: 1) mineral surface deprotonation and 2) coordination of COOH groups with exchangable metals. Briefly, formation of pyridinium ion by consumption of surface protons resulted in a decrease of surface acidity, enhancing dissociation of carboxylic protons and their coordination with the mineral surface metals. Thus, these researchers proposed that fluazifop acid associated with bentonite by proton transfer from the water associated with

exchangeable cations to the pyridinic nitrogen, and by the coordination of carboxylate with the exchange metal ions. The proposed chemical species formed were: fluazifop with protonated N and non- dissociated COOH's, fluazifop with protonated N and dissociated COOH's associated to exchangeable cations, and adsorbed molecules with protonated N and cation-COOH's bounds. The formation of the previously mentioned chemical species was observed to be dependent on the exchanged metal.

Laird et al. (1992) studied sorption of atrazine to fourteen smectitic clays and reported an increase in sorption with a decrease of the surface charge density of the clay. The sorption capacity of the clays was observed to be inversely related to their CEC. The low-charge-density siloxane surfaces had higher affinity to atrazine than those with a high-charge density. The results suggested that atrazine was preferentially sorbed as neutral species. These researchers suggested that smectite's high adsorption potential for pesticides is more likely due to the abundance of the clay in the soil, high surface area, and clay surface chemistry. Barriuso et al. (1994) observed that atrazine adsorption decreased at high surface charge densities and its adsorption was a reversible process. Thus, the formation of weak van der Waals or H-bonds between atrazine and smectites was suggested. However, some degree of hysteresis was reported and atrazine was more strongly retained by smectities holding high surface charge density.

A study of atrazine sorption into four particle size fractions separated from soil clay was presented by Laird et al. (1994). Inorganic components of the soil comprised 89% of the mass but accounted for the 32% of atrazine's soil clay affinity. Clay fractions were treated to remove free iron compounds and the effect of atrazine sorption was evaluated. Results for the coarse fraction indicated that free Fe compounds interacted with silicate clays by coating quartz particles, thus suppressing atrazine sorption affinity. While for the fine clay fraction, atrazine affinity was reduced by the removal of the free Fe compounds. In general, it was concluded that silicate minerals had a moderate affinity for atrazine and its retention was due to physical sorption.

Bhandari et al. (1996) investigated the association of 4-chlorophenol (4-CP) with a soil oxidized to reduce the SOM to 0.52 %. The binding of 4-CP to the soil with such low SOM content was attributed to the participation of the metal oxides present on the mineral surfaces, and to the availability of the internal sorption surfaces in mineral micropores.

Specific interactions of nonpolar compounds with inorganic solids have also been studied

in presence of different model sorbents (Huang et al., 1996). These researchers hypothesized that internal mineral surfaces have a small contribution in the sorption process of organic compounds. Experimental results indicated that the surface geometry favored water adsorption and hindered the organic molecules from accessing internal surfaces. Pore diffusivity was suggested as the process controlling the slow and nonlinear sorption of phenanthrene on bentonite.

Adsorption-desorption of the pesticide 2,4-dichlorphenoxiacetic acid (2,4-D) in the presence of a pure montmorillonite and chlorite-like complexes [$Al(OH)_x$- montmorillonite] was evaluated by Sannino et al. (1997). These researchers postulated two types of processes as responsible for the measured adsorption: 1) Physical adsorption through electrostatic interactions between COO^- moieties of the herbicide and the positive $Al(OH)^{2+}$ groups on the clay surfaces and 2) chemisorption through ligand exchange of COO^- groups with surface –OH or water.

Removal of atrazine, isoproturon (nonionic herbicides) and imidacloprid by sepiolite was addressed by González–Pradas et al. (1999). Sepiolate was selected since it is a silicate mineral known for its interaction with organic solutes, with a characteristic high surface area, and with a fibrous texture that contains molecular-sized channels. These authors reported a medium affinity of sepiolite for isoproturon and imidacloprid, while high adsorption affinity was reported for atrazine. Achivement of complete monolayer surface coverage was suggested by the isotherm's plateau for the three solutes. Adsorption of the solutes was explained by acid-base equilibrium reactions of the solutes, where the protonated base formation (BH^+) would compete with the adsorption of the base form (B) of the solutes on the acidic centre of sepiolite. The order of the adsorption affinity of the sepiolite for the three solutes was explained by the Lewis-base character of the three herbicides. Thus, isoproturon exhibited a poor Lewis- base character that increased for imidacloprid, and being highest for atrazine that showed the highest adsorption affinity. These authors assumed a similar Lewis base character of the solutes and that the dominant adsorption mechanism was the neutralization of acid Lewis sepiolite sites by solute molecules.

The sorption behavior of soil aggregates is dependent on the physical-chemical characteristics of the aggregate surface that remains available for interaction with organic compounds (Celis et al., 1999). Celis et al. (1999) studied sorption of 2,4-D in binary and ternary model systems formed by montmorillonite, ferrihydrite, and humic acid. These authors reported a reduction in the sorption of 2,4-D by Ca and K-saturated montmorillonites. At the end of the

sorption process, the concentration of the pesticide in the aqueous phase increased and it was explained by the fact that the two clays sorbed water preferentially resulting in an increased solute concentration. The anionic form of 2,4-D was predominant at the pH of the experiment (pH > 7) and sorption was not likely to happen since the clay particles were negatively charged.

Sorption isotherms on ferrihydrate were of the S-type, indicating that at low concentrations the solute most likely competed for sorption sites with water and/or ionic species present in solution. The authors confirmed through IR spectroscopy that the Fe oxide surfaces interacted with the $–COO^-$ groups of the pesticide, while the aromatic ring was oriented toward the solution. Thus, sorption occurred through Coulombic interactions between the anion form of 2,4-D and the positively charge surface of the Fe oxides. The formation of van der Waals interactions between the aromatic rings of the sorbed molecules, and competition between the pesticide and Cl^-, OH^- and water molecules for the iron oxide surface were also suggested as factors impacting 2,4-D sorption.

When 2,4-D was in contact with humic acid, minimal competition between solute and water molecules for sorption sites was suggested by the L-type isotherms. While sorption of the pesticide by iron oxides was observed to be important at high solute concentration, sorption by humic acids was significant at low solute concentrations. Sorption and precipitation of Fe oxides on Ca-saturated Wyoming montmorillonite (Ca-SWy clay) significantly increased the sorption of the pesticide compared to sorption observed for the clay alone. However, the low pH (≈ 4) of the montmorillonite-adsorbed Fe produced higher sorption of pesticide. Higher charge density was noted to have reduced the sorption of the solute, while low surface charge density promoted the interactions of the anionic species of the solute with the clay surface.

The binary association of Fe-HA particles showed similar sorption as that observed for the ferrihydrate alone. It was proposed that the available area of the ferrihydrate was provided by micropores that were not available to large HA molecules and that continued to be accessible to the solute after the association of the soil particles. While in the ternary systems, HA coating reduced the sorption of 2,4-D. This behavior indicated that some of the available sites were blocked by the HA molecules. Thus, these authors concluded that the utilization of sorption parameters obtained for model sorbents or isolated soil fractions (like K_{OC}, K_{OW}, $K_{mineral}$ or K_{FE}) could lead to results that do not agree with real conditions. The 2,4-D sorption was observed to depend on the nature of the surface available which, in turn, is a function of the degree of

association of the individual soil particles rather than on the summation of its interactions with individual soil fractions.

Adsorption of pesticides in major components of aquifer material was investigated by Clausen et al. (2001). These authors used calcite, quartz, kaolinite, and α-alumina, to investigate the sorption of ionic (2,4-D, mecoprop and bentazone) compounds. Sorption of the ionic pesticides on quartz occurred only when the solution pH was below or close to the point of zero charge. The adsorption of ionic pesticides by calcite and α-alumina was attributed to the interaction between the COO- groups with the positively charged surfaces groups. Adsorption decreased in the order mecoprop> 2,4-D> bentazon. At a pH of 2.4, the quartz $=SiOH_2^+$ and $+SiO^-$ sites were assigned as responsible for the sorption of the anionic pesticides and the positively charged atrazine, respectively. While in calcite the $=Ca(OH_2)^+$ sites, and in α-alumina, the electrostatic interactions between the anionic molecules and the $=AlOH_2^+$ sites were suggested as responsible for the adsorption of the anionic pesticides.

Addition of $CaCl_2$ to the background solution enhanced adsorption of 2,4-D and mecoprop by kaolinite. Adsorption of anionic pesticides was found to reduce with increasing ionic strength. Thus, through the variation of the electrolyte concentration in solution (ionic strength), these researchers demonstrated that phenoxy acids are adsorbed to mineral surfaces through outer sphere interactions (nonspecific adsorption) by means of electrostatic bonding. These researchers concluded that anionic pesticides are weakly bonded to the mineral surfaces through electrostatic linkages, while nonionic pesticides are adsorbed through hydrophobic interactions. Thus, the characteristics of the pesticide and of the mineral, govern the adsorption process of organic contaminants to mineral soils.

The importance of the iron oxides present in aquifer sediments was evaluated by Clausen and Fabricius (2001) using the same nonionic and anionic pesticides as Clausen et al. (2001) and SOM-free iron oxides (ferrihydrite, goethite and lepidocrocite). While no adsorption was reported for the nonionic pesticides, adsorption of the anionic pesticides by the iron oxides was considerable. The carboxyl-bearing pesticides exhibited the highest adsorption. The adsorption behavior of the iron oxides was different (ferryhydrate>goethite>lepidocrocite) and the differences were attributed to the density of singly coordinated hydroxyl groups.

Sheng et al. (2001) studied the contribution of smectite clays and SOM on the rentention of seven different pesticides, including 4,6-dinitro-o-cresol. A K-saturated clay (SWy-2) was the

only clay utilized for the adsorption study. The K-saturated SWy-2 clay was found to be more effective than SOM in the sorption of 4,6-dinitro-o-cresol. These authors observed that basal spacing of K-saturated SWy-2 clay increased with increasing 4,6-dinitro-o-cresol adsorption. Results from the X-ray diffraction analysis showed that the pesticide should have been randomly stratified in the interlayers of the clay and that a fraction of the clay domains showed increased *d*-spacings from 10 to 13 Å.To explain the high adsorption of 4,6-dinitro-o-cresol , these authors suggested that the characteristics of the molecule like the planarity, aromaticity and the strongly electron-withdrawing nitro groups, favored the formation of electron-donor acceptor complexes, where the aromatic pesticide accepted the electrons donated by the negatively charged siloxanes surfaces.

Sorption mechanisms identified by Sheng et al. (2001) occurring between the organic molecules and the clays were electron donor-acceptor interactions, water bridges between polar substituents and hydrated exchangeable cations, interaction of negatively charged substituents with exchangeable cations, and hydrophobic interactions between the pesticide molecules and the siloxane surfaces. The authors also observed that low surface charge densities caused an increase of the adsorption domains, thus enhancing adsorption of organic solutes.

In a later work, Sheng et al. (2002) utilized FTIR, XRD, and molecular dynamic simulations to study the adsorption of two dinitrophenol herbicides (4,6-dinitro-o-cresol and 4,6-dinitro-o-sec-butyl phenol) to two smectite clays (SWy-2 and SAz-1) that differed in their charge density. Adsorption of the herbicides was also evaluated when the clays were saturated with cations with different hydration energies. Interlayer cations with low hydration energy resulted in more herbicide adsorption compared to cations with higher hydration energies. These researchers determined that the adsorption of both herbicides occurred when both solutes were present in neutral form (lower pHs). The FTIR analyses demonstrated a clay-surface's parallel arrangement of the molecules of 4,6-dinitro-o-cresol.

Adsorption of 4,6-dinitro-o-cresol was favored at the optimum *d*-spacing of 12.5 Å, where contact with water was minimal and the 4,6-dinitro-o-cresol molecules interacted with clay surfaces in opposite sides of the molecule. The optimum interlayer spacing was achieved upon saturation of the clay with the low hydration energy exchangeable cations (K^+ ad Cs^+). Direct association of $–NO_2$ groups with exchangable cations was enhanced at lower hydration energies.

In general, adsorption of the herbicides was affected by the increase of the size of adsorption domains due to the presence of exchangeable cations with high hydration energy and by the high surface charged density. The large size of organic molecules was also identified as a factor that hindered adsorption of organic molecules. Similar conclusions for the adsorption 4,6-dinitro-o-cresol and dichlobenil have been presented elsewhere (Li et al., 2003).

The study of the sorption of 2,4-D to ten different aquifer sediments was reported by Madsen et al. (2000). Sorption of 2,4-D was observed to decrease at higher pHs. The researchers suggested that the process responsible for sorption was the electrostatic interactions between carboxylic anions and the positively charged sites like iron minerals and/or clay minerals. In general, the researchers suggested that the specific surface area, pH and TOC were the conditions that determine the magnitude of the sorption affinity.

Sorption of phenols and nitrophenols onto aquifer material with an organic content of 0.038% was presented by Amiri et al. (2004). Five of the phenols under study (phenol, 4-nitrophenol, 2-chlorophenol, 3-chlorophenol, and 2,4-dimethylphenol) did not show significant sorption to the aquifer material, in contrast to 2-methyl-4,6-dinitrophenol (MDNP), 2,4,6-trichlorophenol (TCP) and pentachlorophenol (PCP) exhibited significant affinity for the material. The study utilized flow-through column sorption experiments to evaluate the sorption of neutral or ionic species by varying the pH of the solution.

Lower solution pH enhanced the sorption or retardation of MDNP, TCP and PCP in the aquifer material. In general, enhanced sorption at low pH indicated that the neutral species of the solutes were responsible for the retardation rather than the ionized species. However, utilization of a linear model, developed based on the degree of protonation of the solutes allowed the authors to differentiate the species being sorbed. The anion species of MDNP and TCP did not sorb significantly while ionized PCP sorption was as high as that observed for the neutral species of TCP.

1.7 Phenol Stabilization in Mineral Soils via Oxidative Polymerization

The study of engineered humification as an effective process to stabilize phenolic contaminants in mineral soils, by the formation of nonextractable or bound residues has not been broadly addressed in the literature (Shannon and Bartha, 1988; Kim, 2007). Some studies have described the immobilization of enzymes that catalyze the oxidative coupling of phenolic

compounds like HRP and laccase, on mineral surfaces of clays and on soil, as a method to protect and preserve the activity of the enzyme and extend its catalytic life (Ruggiero et al., 1989; Gianfreda and Bollag, 1994; Pal et al., 1994; Park et al., 1999; Ahn et al., 2002). Other studies have addressed abiotic polymerization of phenols by means of minerals (birnessite), transition metal oxides (MnO_2, ZnO, CuO) and dissolved cations of Mn^{2+} and Fe^{3+} (Larson and Hufnal, 1980; Stone and Morgan, 1984; Kung and McBride 1988; Colareti et al., 2002; Selig et al., 2003).

The impact of the presence of inorganic solids like silica gel, silica sand and clays on the peroxidase-catalyzed polymerization process of phenol has also been discussed in the scientific literature (Wagner and Nicell, 2003; Huang and Weber, 2004). The impact of the presence of silical gel, kaolin and bentonite on the HRP-mediated treatment of aqueous phenol was investigated by Wagner and Nicell (2003). Silica gel had no effect on the conversion of phenol. All studied solids enhanced phenol transformation at pH of 5 and 7, at all solids concentration studied (100 to 10,000 mg/L). Enhancement in phenol polymerization by kaolin increased with increasing solids concentration, however, the effect of bentonite was greater at 1000 mg/L and decreased at higher solid conconcentrations. At pH 9, none of the solids had a positive effect on phenol transformation. To study the stability of HRP and H_2O_2, both chemicals were incubated for 1 hr at pH 7 and 10000 mg/L of soil in fresh suspension, before initiating the reaction. When silica gel and kaolin were used, HRP and H_2O_2 were stable, (i.e., in presence of those solids no effect on the level of phenol transformation was observed). Fresh suspensions of bentonite inactivated the HRP thus, lowering the phenol polymerization process. H_2O_2 pre-incubated with bentonite had not effect on phenol removal. Exposure of bentonite to aqueous phase prior to addition of HRP eliminated its ability to inactivate the HRP's activity.

Wagner and Nicell (2003) observed that solutions treated in the presence of bentonite and kaolin were less toxic than the controls with no solids added. However, kaolin and bentonite were not able to adsorb reaction products that were formed in solid-free aqueous solutions. These results suggested that these clays were able to interact with and partially deactivate precursors of toxic reaction products. Similar adsorption interaction of the phenol reaction products with talc, resulted in protection of the activity of the enzyme from inactivation by the enzymatic products, and in higher phenol conversion and removal of the products from the aqueous phase (Arseguel and Baboulene, 1994)

No discussion on the formation of nonextractable or solid bound residue formation was presented in this study since the authors developed their work in the context of treatment of water and wastewater contaminated with phenol (Wagner and Nicell, 2003). However, their results provide important information that could also be applied to the soil environment, where the contaminated soil-solution could exist in contact with inorganic solid phases, which could play a positive effect on the application of the enzymatic polymerization process by modifying the chemical character and amount of the residual polymerization products.

The effect of silica sand on the formation of nonextractable products was studied by Huang and Weber (2004). Silica sand adsorbed the enzyme, forming enzyme-solid associations that reduced HRP inactivation. These researchers suggested that HRP associated preferentially with hydrophilic materials that contain abundant surface oxygen atoms. Formation of nonextractable products was slightly enhanced by increasing silica sand solid/water ratios up to 200 g/L.

Only two studies have reported data about phenol stabilization in mineral soils through enzymatic polymerization (Shannon and Bartha, 1988; Kim, 2007). A study to determine the feasibility of employing chemical stabilization through enzymatic polymerization processes in a column aquifer system was performed by Shannon and Bartha, (1988). Polymerization of 4-methylphenol (4-MP) and DCP induced in a sand-column system was found to enhance retention of both chemicals in Lakewood sand material with 90% sand and 1.3 % SOM content. Leaching of chemical in both control (no enzyme) and treated (with enzyme) columns decreased with increasing contact time. In untreated columns, from time zero to 21 days, the amount of leached MP decreased from 98.1 to 70.7%, respectively. Upon acidification of the leachate, humic precipitate was formed and ranged between 5.2 and 8.1% of the total ^{14}C activity added. While the solvent extractable chemical ranged from 0.9 to 3.7 % and consisted mainly of parent MP and minimal amounts of polymer products. Formation of bound residue was observed and it varied from 3.6 to 19.5 % within the 21 days of study. Minimal CO_2 evolution was measured throughout the experimental time.

The activity of MP in the leachate from the treated column decreased more with contact time than in the leachate from the control column. Thus, the MP activity decreased from 9.1 to 6.8%. Less humic precipitate was formed and it decreased with the progression of the experiment (from 4.1% at time zero to 2.3% at the conclusion of the experiment). Most of the ^{14}C activity

was solvent extractable (59 to 74 %), predominantly in the form of polymerization products with trace amounts of the unreacted MP. Bound residue formation was observed, however, it decreased with increasing time from 17.7 to 10 %. The production of $^{14}CO_2$ was lower in the treated columns and it was attributed to the slower degradation rate of the polymerization products.

In the case of DCP, the leachate from the control column accounted for 91.6 and 63 % of total added chemical at zero and 21 days, respectively. After 21 days of experiment, half of the activity was associated with parent DCP while the other half was identified as water-soluble complexes of DCP. Thus, MP and DCP experienced autooxidation in the controls columns, although, the researchers did not provide further discussion. There was some humic precipitate formation (0.7- 6.1%), the solvent extractable fraction ranged from 5.2 to 19.3 %, and a very small amount of bound residue was produced (0.5 to 3.1 %).

In the case of the treated column, the leaching out of DCP decreased from 48.5 to 10 % from one to 28 days. As time progressed, the free-DCP activity in the leachate decreased to as low as 10 %, while the remaining activity corresponded to water-insoluble extractable polymers. Humic precipitate, resulting from acidification of the leachate, increased from 5.3 to 34.6 %, while the solvent extractable activity did not change significantly over time (26.9 to 23.1 %). Most of the activity detected in the solvent extractable fraction was associated with polymerization products and trace amounts to unreacted DCP. The soil bound fraction increased with time from 8.5 to 17.9 %. A further incubation study revealed that even after four more weeks no remobilization of the bound products occurred. The authors concluded that the enzymatic polymerization of organic compounds was a convenient method to prevent and control groundwater pollution, indicating that the efficiency of the process depended on the characteristics of the pollutant.

Kim (2007) evaluated the ability of the HRP enzymatic polymerization process to remove phenol from aqueous solution under simulated aquifer conditions. The aquifer conditions were simulated by utilizing a saturated column, packed with Ottawa sand, and no organic carbon present in the porous media. The study included the effect of solution pH, ionic strength and enzyme dose on the polymerization efficiency and removal of phenol within the saturated porous media. The variation of aqueous solution conditions resulted in different effects on the polymerization process.

While the removal efficiency at HRP doses of 0.5 and 1 AU/mL were similar and corresponded to a 70% reduction of the total phenol concentration, at 2 AU/mL, the removal increased up to 90 %. At all HRP concentrations, unreacted phenol and soluble polymers were measured in the column effluent. The increase of the HRP dose resulted in higher production of soluble polymers while it lowered the concentration of unreacted phenol exiting the column.

Polymerization of phenol resulted in precipitation and accumulation of the insoluble polymers in the porous media. The amount of phenol mass converted to polymers that were accumulated at 0.5, 1 and 2 AU/mL of HRP were reported to be 51, 59 and 66 % of the total phenol entering the column system, respectively. Precipitation and accumulation of the phenolic polymers changed the pore volume of the media thus changing the hydraulic properties of the system. The effect was observed to be more pronounced at higher enzyme concentrations. Thus, the higher the enzyme dose, the greater the reduction of the pore volume since greater deposition of polymers was observed. Pore volume was reduced by 5.8 and 7.8 % at HRP doses of 0.5 and 2 AU/mL, respectively.

The change of solution pH (5, 7 and 9) affected the amount of soluble products exiting the column. The results did not indicate a consistent trend corresponding to the pH changes. The highest concentration of soluble polymers exiting the system and insoluble polymers accumulated in the media was observed at pH 7, while the lowest concentrations were detected at pH 9. The author suggested that at pH 9 there was minimal deposition of polymers in the media, thus modification of its hydraulic properties were negligible.

The removal of phenol was observed to be higher at the ionic strength of 20 mM
The increase of solution ionic strength (5, 20 and 100 mM) was observed to decrease the amount of soluble polymers produced and exiting the system. Deposition of the polymers also increased with increasing ionic strength. At 5 mM of ionic strength the system experienced 5% decrease of pore volume, while at 20 and 100 mM, the porosity decreased by 8%.

1.8 Statistical Analysis

This section describes the stadistical analysis done for the data presented in Chapter 3, Chapter 4 and Chapter 5. R-squared values of the Freundlich sorption parameters (K_F and n) for the PDRs were obtained in Microsoft Excel. The standard error and 95% confidence interval for

both K_F and n Freundlich parameters were obtained by utilizing the procedure indicated by Ott and Longnecker (2001).

$$\beta_0 \pm t_{\alpha/2} S\varepsilon \sqrt{\frac{\sum x^2}{nS_{xx}}} \qquad \text{(Eq. 1.4)}$$

$$\beta_1 \pm t_{\alpha/2} \frac{S\varepsilon}{\sqrt{S_{xx}}} \qquad \text{(Eq. 1.5)}$$

where

$$S\varepsilon = \sqrt{\frac{SSE}{n-2}} \qquad \text{(Eq. 1.6)}$$

$$SSE = \sum (y - \hat{y})^2 \qquad \text{(Eq. 1.7)}$$

The β_0 and β_1 stand for the sorption Freundlich parameters log K_F and n, respectively. The log C_e and log q_e are represent the x and y, respectively. $S\varepsilon$ is the standard deviation and SSE is the sum of squares. The Freundlich parameters β_0 and β_1 were calculated using the following equations.

$$\hat{\beta}_1 = \frac{S_{xy}}{S_{xx}} \qquad \text{(Eq. 1.8)}$$

$$\hat{\beta}_0 = \bar{y} - \hat{\beta}_1 \bar{x}_1 \qquad \text{(Eq. 1.9)}$$

$$S_{xx} = \sum x^2 - \frac{(\sum x)^2}{n} \qquad \text{(Eq. 1.10)}$$

$$S_{xy} = \sum xy - \frac{(\sum x)(\sum y)}{n} \qquad \text{(Eq. 1.11)}$$

In the equations above *n* is the number of replicate data points. The *n* values were usually 15, unless test tubes were lost due to breakage. The value of $\alpha/2$ used for the 95% confidence interval was 0.025. The $t_{\alpha/2}$ values was obtained from a standard t-distribution table Ott and Longnecker (2001). All other averages and standard deviations values of replicates were obtained in Microsoft Excel

1.9 Literature Cited

Ahn, Mi-Youn; Dec, Jerzy; Kim, Jang-Eok; and Bollag, J. M. Treatment of 2,4-dichlorophenol polluted soil with free and immobilized laccase. *J.Environ.Qual.* 2002, 31: 1509-1515.

Ainsworth, C. C., Zachara, J. M.; and Schmidt, R. L. Quinoline sorption on Na-montmorillonite: contributions of the protonated and neutral species. *Clays & Clay Miner*. 1987, 35: 121-1128.

Almendros, G.; Guadalix, M. E.; Gonzalez-Vila, F. J. and Martin F. Preservation of aliphatic macromolecules in soil humins. *Org. Geochem*. 1996, 24(6/7): 651-659.

Amiri, F.; Rahman, M. M.; Bornick, H.; and Worch, E. Sorption behaviour of phenols on natural sandy aquifer material during flow-through column experiments: the effect of pH. Acta Hydrochim. Hydrobiol. 2004, 32(3): 214-224.

Anderson, D. W.; Paul, E. A. and Arnard, R. J. St. Extraction and characterization of humus with reference to clay-associated humus. *Can. J. Soil. Sci*. 1974, 54:317-323.

Anderson, J. U. An improved pretreatment for mineralogical analysis of samples containing organic matter. *Clays & Clay Miner*. 1963, 10: 380-388.

Arseguel, D.; and Baboulene, M. Removal of phenol from coupling of talc and peroxidases. Application for depollution of waste water containing phenolic compounds. J. Chem. Technol. Biotechnol. 1994, 61: 331-335.

Barriuso, E.; Laird, D. A.; Koskinen, W. C.; and Dowdy, R. H. Atrazine desorption from smectites. *Soil Sci. Soc Am. J*. 1994, 58: 1632-1638.

Berglund, G. I.; Carlosson, G. H.; Smith, A. T.; Szoke, H.; Henriksen, A.; and Hajdu, J. The catalytic pathway of horseradish peroxidase at high resolution. *Nature*. 2002, 417: 463-468.

Bhandari, A.; Novak, J. T. and Berry, D. F. Binding of 4-monochlorohenol to soil. *Environ. Sci. Technol.* 1996, 30:2305-2311.

Bhandari, A.; Novak, J. T.; Burgos, W. D.; and Berry, D. F. Irreversible binding of chlorophenols to soils and its impact on bioavailability. *J. Environ. Engineering*. 1997, 506-513.

Bhandari, A. and Weber, W. J. Sorption of 1,2,4-trichlorobenzene to natural geosorbents. In: *Proceedings Of The Thirieth Mid –Atlantic Industrial And Hazardous Waste Conference*. July 12-15, 1998 Villanova University, Villanova, Pennsylvania. 1998.

Bhandari, A. and Xu, F. Impact of peroxidase addition on the sorption-desorption behavior of phenolic contaminants in surfaces soils. *Environ. Sci. Technol.* 2001, 35:3163-3168.

Bollag. J. M.; Liu; S.-Y.; and Minard, R. D. Cross coupling of phenolic humus constituents and 2,4-dichlorophenol. *Soil Sc. Soc. America J.* 1980, 44: 52-56.

Bollag, J. M. Enzyme catalyzing oxidative coupling reactions of pollutants. *Metal Ions in Biological Systems*. 1992-a, 28: 205-217

Bollag, J.-M. Decontaminating the soil with enzymes. *Environ. Sci. Technol.* 1992 b, 26:1876-1881

Bonin, J; and Simpson, M. J. Variation in phenanthrene sorption coefficients with soil organic matter fractionation: the result of structure or conformation? *Environ. Sci. Technol.*2007, 41: 153-159.

Borisover, M.; and Graber, E. R. Hydration of natural organic matter: effect of sorption of organic compounds in humin and humic acids vs original peat material. *Environ. Sci. Technol.* 2004, 38: 4120-4129.

Bourget, S. J.; and Tanner, C. B. Removal of organic matter with sodium hypobromite for particle-size analysis of soils. *Can. J. Agr. Sci*. 1953, 33: 579- 585.

Buffle, J. A. E. Les substances humiques et leurs interactions avec les ions mineraux. *Conference Proceedings de la Commission d'Hydrologie Apppliquee de l'A.G.H.T.M.* l' Universite d'Orsay, 3-10, 1977.

Capriel, P.; Haisch, A. and Khan, S. U. Distribution and nature of bound (nonextractable) residues of atrazine in a mineral soil nine years after the herbicide application. *J. Agric. Food Chem.* 1985, 33, 567-569.

Carmo, A. M,; Hundau, L. S. and Thompson, M. L. Sorption of hydrophobic organic compounds by soil materials: application of unit equivalent Freundlich coefficients. *Environ. Sci. Technol.* 2000, 34:4363-4369.

Celis, R.; Hermosín, M. C.; Cox, L.; and Cornejo J. Sorption of 2,4-dichlorophenoxyacetic acid by model particles simulating naturally occurring soil colloids. *Environ. Sci. Technol.* 1999, 33: 1200-1206.

Chefetz, B.; Deshmukh, A. P.; Hatcher, P. G.; and Guthrie, E. A. Pyrene sorption by natural organic matter. *Environ. Sci. Technol.*2000, 34:2925-2930.

Chiou, C. T; Kile, D. E.; Rutherford D. W.; Sheng, G.; and Boyd, S. A. Sorption of selected organic compounds from water to a peat soil and its humic-acid and humin fractions: potential sources of the sorption nonlinearity. *Environ. Sci. Technol.* 2000, 34:1254-1258.

Christl, I; Knicker, H.; Kogel_Knabner, I.; and Kretzschmar, R. chemical heterogeneity of humic substances: characterization of size fractions obtained by hollow fiber ultrafiltration. *Eur. J. Soil Sci*. 2000, 51: 617-626.

Clausen, L.; Fabricius, I.; and Madsen, L. Adsorption of pesticides onto quartz, calcite, kaolinite and α-alumina. *J. Environ. Qual.* 2001, 30: 846-857.

Colareti, M. L.; Toscano, G.; and Greco, G. Jr. Soil-catalyzed polymerization of phenolics in polluted waters. *Water Res*. 2992, 36: 3015-3022.

Cooper, V. A.; and Nicell J. A. Removal of phenols from a foundry wastewater using horseradish peroxidase. *WaterRes.*1996; 30 (4): 954- 964.

De Jonge, H and Mittelmeijer-Hazeleger, M. C. Adsorption of CO_2 and N_2 on soil organic matter: nature of porosity, surface area and diffusion mechanisms. *Environ. Sci. Technol.* 1996, 30:408-413.

Dec, J.; Haider, K.; Benesi, A.; Rangaswamy, V.; Schäffer, A.; Plücken, U.; and Bollag, J.M. Analysis of Soil-Bound Residues of ^{13}C-Labeled Fungicide Cyprodinil by NMR Spectroscopy. . *Environ. Sci. Technol.*1997, 31, 1128-1135.

Dec, J. and Bollag, J. M. Phenoloxidase-mediated interactions of phenols and anilines with humic materials. *J. Environ. Qual.*2000, 29(3): 665-676.

Duran, N. and Esposito, E.. Potential applications of oxidative enzymes and phenoloxidase-like compounds in wastewater and soil treatment: a review.*Applied Catalysis B. Enviromental*, 2000, 28:83-99.

Dunford, H. B. Horseradish peroxidase: structure and kinetic properties. In: *Peroxidases 21in Chemistry and Biology*, Volume II, CRC Press, Inc. Editors: Everse, J.; Everse, K. E. and Grishman, M. B. 1991.

Essington, M. E. *Soil and water chemistry. An integrative approach.* CRC Press, 2004.

Fabbri, D.; Chiavari, G.; and Galletti, G. C. Characterization of soil humin by pyrolysis(/methylation)-gas chromatography/mass spectrometry:structural relationships with humic acids. *J. Anal. App. Pyrol*. 1996, 37:161-172.

Flanders, C.; Dec, J.; and Bollag, J. M. Horseradish-mediated binding of 2,4-dichlorophenol to soil. *Bioremediation Journal*. 1999, 3(4): 315-321.

Ferrer, I.: Dezotti, M.; and Duran, N. Decolorization of kraft effluent by free and immobilized lignin peroxidase and horseradish peroxidase. *Biotechnol. Lett.* 1991, 13:577.

Garbarini, D. R.; and Lion, L. W. Influence of the nature of soil organics on the sorption of toluene and trichloroethylene. *Environ. Sci. Technol.*1986, 20:1263-1269.

Giafreda, L. and Bollag, J-M. Effect of soils on the behavior of immobilized enzymes. *Soil Sci. Soc.Am. .J*. 1994, 58: 1672-1681.

Goh, K. M.; Malloy, B. P. J. Radicarbon dating of paleosols using soil organic matter components. *J. Soil Sci*. 1978, 29, 567-573.

Gonzáles-Pradas, E.; Villafranca-Sánzchez, M.; Socías-Viciana, M.; Fernández-Pérez, M.; and Urena-Amate M.D. Preliminary studies in removing atrazine, isoproturon and imidacloprid from water by natural sepiolite. *J. Chem. Technol. Biotechnol*. 1999, 74: 417-422.

Gouveia, S.E M; Pessenda, L. C. R.; and Aravena, R.Dating of the humin fraction of soil organic matter and its comparison with ^{14}C ages of fossil charcoal. *Quimica Nova*. 1999, 22 (6):810-814.

Grasset, L.; and Ambles, A. Strcuture of humin and humic acid form an acid soil as revealed by phase transfer catalyzed hydrolysis. *Org. Goechem*. 1998 a, 4: 881-891.

Grasset, L.; and Ambles, A. Aliphatic lipids released from a soil humin after enzymatic degradation of cellulose. *Org. Goechem*. 1998 b, 4: 893-897.

Guetzloff, T. F.; and Rice, J. A. Does humic acid from a micelle? *Sci. Total Environ*. 1994, 152:31-35

Guthrie, E. A.; Bortiatynski, J. M. ; Van Heemst, J. D. H. ; Richman, J.E.; Hardy, K. S.; Kovach, E. M.; and Hatcher, P. G. Determination of [^{13}C]Pyrene sequestration in sediment microcosms using flash pyrolysis-GC-MS and ^{13}C NMR. *Environ. Sci. Technol.*1999, 33:119-125.

Haider, K,; and Martin, J. P. - Decomposition of specifically carbon-14 labeled benzoic and cinnamic acid derivatives in soil. *Soil Sci. Soc. Am. Proc*. 1975, 39:657-662.

Hatcher, P. G.; Vanderhart, D. L. and Earl, W. L. Use of solid-state ^{13}C NMR in structural studies of humic acids and humin from Holocene sediments. *Org. Geochem*. 1980, 2: 87-92.

Hatcher, P. E. G.; Spiker, E. C.; Szeverenyi, N. M. and Maciel, G. E. Selective preservation and origin of petroleum-forming aquatic kerogen. *Nature*. 1983, 305:498-501.

Hatcher, P; Breger, I.; Maciel, G.; and Szeverenyi, N. Geochemistry of humin. In *Humic Substances in Soils, Sediments, and Water: geochemistry, isolation, and characterization*. G. R. Aitken, D. M. MczKnight, R. L. Wershaw, and P. MacCarthy (eds) John Wiley, New York. 1985, 275-302.

Hatzinger, P. B. and Alexander, M. Effect of aging of chemicals in soil on their biodegradability and extractability. *Environ. Sci. Technol*. 1995, 29:537-545.

Hayase, K. and Tsubota, H. Sedimentary humic acid and fulvic acid as surface active substances. *Geochimica et Cosmochimica Acta*. 1983, 47:947-952.

Hedges, J. I.; and Keil, R.G.Sedimentary organic matter preservation: An assessment and speculative synthesis. *Mar. Chem*. 1995, 49:81-115.

Hettiarachchi, G. M.; Ryan, J. A.; Chaney, R. L.; and La Fleur, C. M. Sorption and desorption of

cadmium by different fractions of biosolids-amended soils. *J. Environ. Qual*. 2003, 32: 1684-1693.

Hu, W-G.; Mao, J.D.; Xing, B.; Schmidt-Rohr, K. Poly(methylene) crystallites in humic substances detected by nuclear magnetic resonance. *Environ. Sci. Technol*. 2000, 34:530-534.

Huc, A.; and Durand, B. Etudes des acides humiques er de L'humine de sediments. Recents consideres comme precurseurs des kerogens. In *Advances in Organic Geochemistry*, 1973, Tissot and F. Bienner (eds). Editions Technip, Paris, 53-72.

Huc, A.; and Durand, B. Occurrence and significance of humics acids in ancient sediments. *Fuel*.1977, 56:73-80

Huang, W., Schlautman, M. A. and Weber, W. J. Jr. A distributed reactivity model for sorption by soils and sediments. 5. The influence of near-sruface characteristics in mineral domains. *Environ. Sci. Technol.* 1996, 30 : 2993-3000.

Huang, Q.; Selig, H.; and Weber, W. J. J. Peroxidase-catalyzed oxidative coupling of phenols in the presence of geosorbents: rates of non-extractable product formation. *Environ. Sci. Technol.*2002, 36: 596-602.

Huang, Q. and Weber, W. J. Jr. Peroxidase-catalyzed coupling of phenol in the presence of model inorganic and organic solid phases. *Environ. Sci. Technol.* 2004, 38: 5238-5245.

Ibrahim, M.S.; Ali, H. I.; Taylor, K. E. Biswas, N.; and Bewtra, J. K. Enzyme-catalyzed removal of phenol from refinery wastewater: feasibility studies. *Water Environ. Res.* 2001, 73(2): 165-172.

Israelachvili, J. *Intermolecular and surface forces*. Academic Press, San Diego, 1991.

Ishiwatari, R. Geochemistry of humic substances in lake water. *In* Humic Substances in Soils, Sediments, and Water: geochemistry, isolation, and characterization. G. R. Aitken, D. M. MczKnight, R. L. Wershaw, and P. MacCarthy (eds) John Wiley, New York. 1985, 147-180.

Jackson, M. L. *Soil Chemical Analysis*. Advance course. Published by author, Madison WI.

Kang, S.;and Xing, B. Phenanthrene sorption to sequentially extracted soil humic acids and humans. *Environ. Sci. Technol.* 2005, 39: 134-140.

Kelleher, B. P.; and Simpson, A. J. Humic substances in soils:are they really chemically distinct? *Environ. Sci. Technol.* 2006, 40:4605-4611.

Kim, W. Phenol removal from saturated porous media using horseradish peroxidase mediated oxidative polymerization process. Ph.D. dissertation, Department of Civil Engineering, Kansas State University, 2007.

Klivanov, A., M.; Tu, T.-M.; and Scott, K. P. Peroxidase-catalyzed removal of phenols from coal-conversion wastewater. *Science.* 1983, 21:259-260.

Kohl, S. D. and Rice, J. A. The binding of contaminants to humins: a mass balance. *Chemosphere*. 1998, 36(2):251-261.

Kohl, S. D. and Rice, J. A. Contribution of lipids to the nonlinear sorption of polycyclic aromatic hydrocarbons to soil organic matter. *Organic Geochem*. 1999, 30: 929-936.

Kononova, M. *Soil organic matter*. Second Edition. Pergamon Press Oxford New York, 1966.

Kung, K-H; and McBride, M. B. Electron transfer processes between hydroquinone and hausmannite (Mn_3O_4). *Clays & Clay Miner*.1988, 36(4): 297-302.

Laird, D. A.; Barriuso, E.; Dowdy, R. H and Koskinen, W. C. Adsorption of atrazine on smectites. *Soil Sci. Soc. Am. J*. 1992, 56 62-67.

Laird, D. A.; William, P. Y. Y.; Koskinen, C.; Steinhelmer, T. R.; and Dowdy, R. H. Sorption of atrazine on soil clay components. *Environ. Sci. Technol*. 1994, 28: 1054-1061.

Lavkulich, L. M.; and Wiens, J. H. Comparison of organic matter destruction by hydrogen peroxide and sodium hypochlorite and its effects on selected mineral constituents. *Soil Sci. Soc. Amer. Proc*. 1970, 34: 755-758.

Larson, R. A. and Hufnal J. M. Jr. Oxidative polymerization of dissolved phenols by soluble and insoluble inorganic species. *Limnol. Oceanogr*. 1980, 25(3): 505-512.

Lesan, H.M.; Bhandari, A. Contact-time dependent atrazine residue formation in surface soils. *Water Res*. 2004, 38:4435-4445.

Li, H.; Sheng, G.; Johnston C. T.; Teppen, B. J.; Johnston, C. T.; and Boyd, S. A.Sorption and desorption of pesticides by clay minerals and humic acid-clay complexes. *Soil Sci. Soc. Am. J.* 2003, 67: 122-131.

Loiseau, L.; Barriuso, E.; Zegouagh, Y.; Largeau, C. And Mariott , A. Release of the atrazine non-extractable (bound) residues of two soils using degradative techniques. *Agronomie*. 2000, *20*, 513–524.

MacCarthy, P. *The principles of humic substances*. *Soil Sci*. 2001-a, 166:738-751.

MacCarthy, P. *The principles of humic substances*.An introductrion to the first principle. In *Humic Substances: Structures, Models and Functions*: Ghabbour, E. A.; Davies, G.; Eds: Royal Society of Chemistry. Cambridge U.K., 2001-b; 10-30.

Madsen, L.; Lindhardt, B., Rosenberg, P.; Clausen, L.; and Fabrocous, I. Pesticide sorption by low organic carbon sediments: a screening for seven herbicides. *J. Environ. Qual.* 2000, 29:

1488-1500.

Malekani, K.; Rice, J. A.; and Lin, J-S. The effect of sequential removal of organic matter on the surface morphology of humin. *Soil Sci*. 1997, 162(5):333-342.

Mao, J.D.; Hundal, L. S.; Thompson, M. L. and Schmidt-Rohr, K. Correlation of poly(methylene)-rich amourphopus aliphatic domains in humic substances with sorption of a nonpolar organic contaminant, phenanthrene. *Environ. Sci. Technol.* 2002, 36,929-936.

Martin, R. T. Calcium oxalate formation in soil from hydrogen peroxide treatment. *Soil Sci.* 1954, 77: 143-145.

Micera, G.; Pusino, A.; Gessa, C. and Petretto, S. Interaction of fluazifop with Al-,Fe^{3+}, and Cu^{2+}- saturated montmorillonite. *Clays & Clay Miner*. 1988, 36:354-358.

Minard, R. D.; Liu, S-Y; and Bollag J. M. Oligomers and quinones from 2,4-dichlorophenol.. *J. Agricul.Food Chem*. 1981, 29:250-253.

Montgomery, J. M. *Water Treatment Principles and Design*. John Wiley & Sons, Inc. New York, 1985.

Nanniperi, P.and Bollag, J. M. Use of enzymes to detoxify pesticide-contaminated soils and waters." *J. Environ. Qual*. 1991, 20: 510-517.

Omueti, J. A. I. Sodium hypochlorite treatment for organic matter destruction in tropical soils of Nigeria. *Soil Sci. Soc. Am. J*. 1980, 44: 878-880.

Pal, S.; Bollag, J.-M.; and Huang, P. M. Role of abiotic and biotic catalyst in the transformation of phenolic compounds through oxidative coupling reactions. *Soil Biol. Biochem.* 1994, 26(7): 813-820.

Palomo, M.; and Bhandari, A. Time-dependent sorption-desorption behavior of 2,4-dichlorophenol and its polymerization products in surface soils. *Environ. Sci.Technol*. 2005, 39: 2143-2151.

Palomo, M.; and Bhandari, A.Impact of aging on the formation of bound residues after peroxidase-mediated treatment of 2,4-DCP contaminated soils. *Environ. Sci. Technol*. 2006, 40:3402-3408.

Park, J-W; Dec, J. Kim, J-E. and Bollag, J. M. Effect of humic constituents on the transformation of chlorinated phenols and anilines in the presence of oxidoreductive enzymes or birnessite. *Environ. Sci. Technol*. 1999, 33:2028-2034.

Park, J.-W.; Dec, J.; Kim, J.-E. and Bollag, J. M. Transformation of chlorinated phenols and anilines in the presence of humic acid. *J. Environ. Qual*. 2000, 29:214-220.

. . . .
Piccolo, A; Nardi, S; and Cancheri, G. Macromolecular changes of soil humic subtances induced by interaction with organic acids. *Eur. J. Soil Sci*. 1996 a, 47:319-328.

Piccolo, A; Nardi, S; and Cancheri, G. Micelle-like conformation of humic substances as revealed by size-exclusion chromatography. *Chemosphore*. 1996 b, 33:595-600.

Piccolo, A. The supramolecular structure of humic substances. *Soil Sci*. 2001 a, 166, 810-832.

Piccolo, A.; Conte, P.; and Cozzolino, A. Chromatographic and spectrophotometric properties of dissolved humic substances compared with macromolecular polymers**.** *Soil Sci.* 2001 b, 166(3):174-185.

Pignatello, J. J. Sorption dynamics of organic compounds in soils and sediments. In *B.L. Sawhney and K. Brown (ed). Reactions and movement of organic chemicals in soil.* SSSA Spec. Publ. 22. SSSA and ASA, Madison, WI, pg. 45-80,1989.

Preston, C. M.; Schnitzer, M.; an Ripmeester, A. A spectroscopic and chemical investigation on the de-ashing of a humin. *Soil Sci. Soc. Am. J.* 1989, 53:1442-1447.

Preston, C. M; and Newman, R. H. Demonstration f spatial heterogeneity in the organic matter of de-ashed humin samples by solid-state ^{13}C CP-MAS NMR. *Can. J. Soil Sci*. 1992, 72:13-19.

Ran, Y.; Xing, B.; Suresh, P.;Rao, C.; Sheng, G.; and Fu, J. Sorption kinetics of organic contaminants by sandy aquifers and its kerogen isolate. *Environ. Sci. Technol.* 2005, 39: 1649-1657.

Rice, J. A.; and MacCarthy, P. Isolation of humin by liwuid-liqud partitioning. *The Science of Total Environment*. 1989, 81/82: 61-69.

Rice, J. A.; and MacCarthy, P. A model of humin. *Environ. Sci. Technol*. 1990, 24(12):1875-1877.

Rice, J. A.; and MacCarthy, P. Statistical evaluation of the elemental composition of humic substances. *Org. Geochem*. 1991, 17:635-648.

Rice, J. A.; and MacCarthy, P. Disaggregation and characterization of humin. *The Science of the Total Environment*. 1992, 117/118:83-88.

Rice, J.A Humin. *Soil Sci*. 2001, 166 (11):848-857.

Roper, C. J.; Dec, J.; and Bollagn J. M. Using minced horseradish roots for the treatment of polluted waters. *J. Environ. Qual.* 1996, 25:1242-1247.

Ruggiero, P; Sarkar, J.M and Bollag J-M. Detoxification of 2,4-dichlorophenol by laccase

immobilized on soil or clay. *Soil Sci.* 1989, 147(5): 361-370.

Russell, J.D.; Vaughan, D. Jones, D. and Fraser, A.R. An IR spectroscopic study of soil humin and its relationship to other humic substances and fungal pigments. *Goederma*, 1983, 29:1-12.

Salloum, M. J.; Dudas, M. J. and McGill, W. B. Variation of 1-napththol sorption with organic matter fractionation: the role of physical conformation.*Org. Geochem*. 2001, 32: 709-719

Salloum, M. J.; Chefetz, B; and Hatcher, P. G. Phenanthrene sorption by aliphatic-rich natural organic matter. *Environ. Sci. Technol*. 2002, 36:1953-1958.

Sanchez Martin, J. M. and Sanchez Camazano, M. Aspects of the adsorption of azinphos-methyl by smectites. *J. Agric. Food Chem*. 1984, 32,: 720-725.

Sannino, F.; Violante, A.; and Gianfreda, L. Adsorption-desorption of 2,4-D by hydroxyl aluminium montmorillonite complexes. *Pest. Sci.* 1997, 51: 429- 435.

Shannon, M. J. R.; and Bartha, R. Immobilization of leachable toxic soil pollutants by using oxidative enzymes. *Appl. Environ. Microbiol*. 1988, 54 (7): 1719-1723.

Sheng, G.; Johnston C. T.; Teppen, B. J. and Boyd, S. A. Potential contributions of smectite clays and organic matter to pesticide retention in soils. *J. Agric. Food Chem.* 2001, 49: 2899-2907.

Sheng, G.; Johnston C. T.; Teppen, B. J. and Boyd, S. A.Adsorption of dinitrophenol herbicides from water by montmorillonites. *Clays & Clay Miner*. 2002, 50 (1): 25-34

Schnitzer, M; and Khan, S. U. Humin *substances in the environment.* Marcel Dekker, New York, 1972.

Schulten, H-R; and Schnitzer, M. Chemical model structures for soil organic matter and soils. *Soil Science*. 1997,162 (2):115-130.

Selig, H.; Keinath II, M. T.; and Weber, W. J. Jr. Sorption and manganese-induced oxidative coupling of hydroxylated aromatic compounds by natural geosorbents. *Environ. Sci. Technol.* 2003, 37: 4122-4127.

Shuman, L. M. Sodium hypchlorite methods for extracting microelements associated with soil organic matter. *Soil Sci. Soc. Am. J*. 1983, 47: 656-660.

Shah, R. K; Choski, M. R.; and Joshi, B. C. Development studies of soil organic matter: humin. *Chem. Era*. 1975, 6:1-3.

Shih, Y-h; and Wu, S-c.; Sorption kinetics in humin under two different levels of relative humidity. *J. Environ. Qual.* 2002, 31:970-978.

Simpson, M. and Johnson, P. C. E. Identification of mobile aliphatic sorptive domains in soil humin by solid-state ^{13}C nuclear magnetic resonance. *Environ. Toxicol. Chem*. 2006, 25 (1): 52-57.

Sjoblad, R. D. and Bollag, J. M. Oxidative coupling of aromatic pesticide intermediates by a fungal phenol oxidase. *Appl. Environ. Microbiol.*1977, 906-910.

Skujins, J. and; Klubek, B. Soil biological properties of a montane forest sere: corroboration of Odum's postulates. *Soil Biol. Biochem.* 1982, 14 (5): 505-513

Somani, L.L and Saxena, S. N. Distribution of humus fractions in some soil groups of Rajasthan. *Agrochim.* 1982, 26: 95-103

Stevenson F.J. *Humus chemistry: genesis, composition, reactions*. New York: Wiley, 1982.

Stone, A. T. and Morgan, J. J. Reduction and dissolution of manganese (III) and manganese (IV) oxides by organics: 2. Survey of the reactivity of organics. *Environ. Sci. Technol.* 1984, 617-624.

Sutton, R. and Sposito G. Molecular structure in soil humic substances: the new view. *Environ. Sci. Technol*. 2005, 39 (23):9009-9015.

Swaby, R. J.; and Ladd, J. N. Chemical nature, microbial resistance, and origin of soil humus. In *Int. Soc. Soil. Sci. Trans.,* IV and V Comm. G. J. Neale edit. Palmerson North, New Zeland. 1962: 197-202. 1962

Tan, Kim H. *Humin matter in soil and the environment. Principles and controversies*. Marcel Dekker, Inc. New York, 2003.

Tandford, C. *The hydrophobic effect: formation of micelles and biological membranes*. Krieger, Malabar, 1991.

Theng, B. K. G. *Formation and properties of clay-polymers complexes.* Elsevier, Amsterdam, 1979, page 362.

Thorn, K. A.; Pettigre, P. J.; Goldenberg, W. S. and Weber, E. J. Covalent binding of aniline to humic substances. 2. ^{15}N NMR studies of nucleophilic addition reactions. *Environ. Sci. Technol.*1996; 30:2764-2775.

Vanderbrouke, M.; Pelet, R.; and Debyser, Y. Geochemistry of humic substances in marine environments. In *Humic Substances in Soils, Sediments, and Water: geochemistry, isolation, and characterization.* G. R. Aitken, D. M. MczKnight, R. L. Wershaw, and P. MacCarthy (eds) John Wiley, New York. 1985, 249-273.

Wagner, M. and Nicell, J. Treatment of a foul condensate from Kraft pulping with horseradish peroxidase and hydrogen peroxide. *Water Res*. 2001, 35 (2): 485-495.

Wagner, M., and Nicell J. Impact of the presence of solids on peroxidase-catalyzed treatment of aqueous phenol. *J. Chem. Technol. Biotechnol*. 2003, 78: 694-702.

Wang, K.; and Xing, B. Chemical extractions affect the structure and phenanthrene sorption of soil humin. *Environ. Sci. Technol.* 2005, 39: 8333-8340.

Weber, W. J.; and DiGiano, F. A. *Process Dynamics in Environmental Systems*; Wiley-Interscience: New York 1996.

Weber, W. J. J;. and Huang, W. A distributed reactivity model for sorption by soils and sediments.4. Intraparticle heterogeneity and phase-distribution relationships under nonequilibrium conditions. *Environ. Sci.Technol*. 1996, 30:881-888.

Weber, W. J. J.;McGinley, P. M. and Katz, L. E. A distributed reactivity model for sorption by soils and sediments.1.Conceptual basis and equilibrium assessments. *Environ. Sci .Technol*. 1992, 26:1955-1962.

Williams, E. G; Scott N. M; and McDonald M. J. Soil properties and phosphate sorption. *J. Sci. Food Agr.* 1958, 9: 551-559.

Xie, H.; Guetzloff, T. F.; and Rice, J. A. Fractionation of pesticides bound to humin. *Soil Sci*. 1997, 162 (6):421429.

Xing, B.; Pignatello, J. J. and Gigliotti, B.. Competitive sorption between atrazine and other organic compounds in soils and model sorbents. *Environ. Sci.Technol.* 1996, 30:2432-2440.

Xu, F.; and Bhandari, A. Retention and distribution of 1-naphthol and naphthol polymerization products on surface soils. *J. Environ .Eng*. 2003a, *129,* 1041-4050.

Xu, F.; and Bhandari, A. Retention and extractability of phenol, cresol, and dichlorophenol exposed to two surfaces soils in the presence of horseradish peroxidase. *J. Agric. Food Chem.* 2003b, *51*, 183-188.

Zhang, G. and Nicell, J. A. Treatment of aqueous pentachlorophenol by horseradish peroxidase and hydrogen peroxide. *Water Res*. 2000, 34 (5): 1629- 1637.

CHAPTER 2 - RESEARCH GOAL AND HYPOTHESES

The overall objective of this research was to assess the feasibility of utilizing horseradish peroxidase (HRP) in the chemical stabilization of phenolic contaminants in soils and aquifer materials. The research utilized 2,4-dichlorophenol (DCP) as a model phenolic contaminant. An alkali-extracted humin-mineral soil matrix and a SOM-free mineral soil matrix were used as model geosorbents. The specific hypotheses evaluated in this research are presented in this chapter. Also listed (in brackets) are the chapters of this document that present and discuss the experimental results used to test each hypothesis.

Hypothesis 1 (Chapter 3)
HRP-mediated oxidative coupling of DCP in the presence of a humin-mineral geomaterial, results in the formation of dichlorophenol polymerization products (DPP).

Hypothesis 2 (Chapter 3)
DPP macromolecules are removed from the aqueous phase as a result of sorption and retention on humin-mineral particle surfaces.

Hypothesis 3 (Chapter 3)
DPP macromolecules associate strongly with humin-mineral surfaces and are resistant to extraction by water and methanol.

Hypothesis 4 (Chapter 3)
Post-polymerization contact time between the DPP-containing aqueous phase and humin-mineral particles enhances the formation of nonextractable DPP residues.

Hypothesis 5 (Chapter 4)
HRP-mediated oxidative coupling of DCP in the presence of SOM-free geomaterial results in the formation of DPP.

Hypothesis 6 (Chapter 4)

DPP macromolecules are removed from the aqueous phase as a result of sorption and deposition on mineral particle surfaces.

Hypothesis 7 (Chapter 4)
DPP molecules associate strongly with mineral surfaces and are resistant to extraction by water and methanol.

Hypothesis 8 (Chapter 4)
Post-polymerization contact time between the DPP-containing aqueous phase and mineral particles enhances the formation of nonextractable DPP residues.

Hypothesis 9 (Chapter 4)
HRP-mediated removal of aqueous DCP is influenced by the presence/absence of SOM in the geosorbent particles.

Hypothesis 10 (Chapter 5)
Precipitation of soluble DPP in aqueous solution can be enhanced by increasing the solution ionic strength.

Hypothesis 11 (Chapter 5)
HRP-mediated polymerization of DCP in the presence of a geosorbent is affected by the solution ionic strength.

Hypothesis 12 (Chapter 5)
Redissolution of precipitated DPP occurs when the solution ionic strength is lowered.

CHAPTER 3 - SORPTION AND PEROXIDASE-MEDIATED STABILIZATION OF 2,4-DICHLOROPHENOL IN A MODEL HUMIN-MINERAL SOIL

3.1 Abstract

Soil-water distribution of 2,4-dichlorophenol (DCP) and 2,4-dichlorophenol polymerization products (DPP) generated from peroxidase-catalyzed oxidation was studied in the context of two surface soils that were modified by removing humic and fulvic acids. Alkali-extractable soil organic matter (SOM) was sequentially removed from agricultural and woodland soils to produce geomaterials containing humin and mineral domains. Reduction in aqueous DCP concentrations resulted from DPP formation and the subsequent sorption of DCP to soil and DPP. While sorption of DCP to the agricultural humin-mineral (AHM) was complete in one day, it took about a week to reach sorption equilibrium with the woodland humin-mineral (WHM). Phase distribution equilibrium for solute in peroxidase amended slurries required one day in both geomaterials. The 24-hour water extraction data indicated that aqueous extractability of sorbent-associated DCP and DPP was reduced as a function of solute-sorbent contact time. The amount of solute stabilized in WHM at the end of the study was significantly higher than that bound to AHM. However, more unreacted DCP sorbed to the humin-mineral geomaterials in peroxidase-amended systems than in controls without enzyme, thus alluding to the possibility of slow, long-term release of some DCP from the solid phase in treated soils. Results from this work illustrate the potential of utilizing peroxidase-mediated oxidation for the transformation and *in situ* stabilization of agrochemicals, such as DCP, in geomaterials rich in humin SOM, such as those found below the root zone in soils.

3.2 Background

3.2.1 Enzymes Catalyzing Oxidative Polymerization of Phenols

Several enzymes produced by plants, bacteria, and fungi are able to catalyze the oxidative coupling of aromatic compounds like phenols. These enzymes are classified as polyphenol oxidases (phenoloxidases) and peroxidases. Polyphenol oxidases are classified are oxidoreductases that can be further classified into two subgroups: laccases and tyrosinases.

Polyphenol oxidases require molecular oxygen to catalyze oxidation of phenolic compounds, and their acitivities do not depend on the presence of coenzymes or cofactors. Peroxidases require the presence of peroxides (like hydrogen peroxide) to catalyze oxidative polymerization of phenols (Bollag, 1992 b; Duran and Esposito, 2000).

Peroxidases are hemoproteins produced by microorganisms such as *Bacillus, Pseudomonas, Arthobacter* and *Streptomyces* as well as in plants. These enzymes oxidize phenolic compounds into phenolic radicals. All peroxidases including horseradish peroxidase (HRP), chloroperoxidase (CPO), lignin peroxidase (LiP), and manganese peroxidase (MnP) contain an iron porphyrin ring (Bollag, 1992 b and Bollag, 1992 a). CPO from the fungus *Caldariomyces fumago* is capable of oxidizing several phenolic compounds (Bollag, 1992 a); LiPs from different sources can oxidize a broad variety of aromatics, polycyclic aromatics and phenolic compounds (Bollag, 1992 a); and oxidation of monoaromatic phenols and dyes is catalyzed by MnP (Bollag, 1992 a).

HRP is perhaps the most widely studied peroxidase. The properties and characteristics of HRP will be discussed in detail in the following section.

3.2.1.1 Horseradish Peroxidase

Horseradish peroxidase catalyzes the oxidative polymerization of a broad range of phenols, anilines, biphenols, and related heteroaromatic compounds (Bollag, 1992 a; Duran and Esposito, 2000). HRP has over 40 identified isoenzymes, with the isoenzyme A (acidic), isoenzyme C (neutral or lightly basic) and a strong basic HRP, as the three most utilized HRP isoenzymes in experimental work (Dunford, 1991).

Dominance of the isoenzyme C in the horseradish roots has been reported (Welinder, 1979). HRP C is characterized by a heme-prosthetic group, two Ca^{2+}, and 308 amino acids residues (that include 4 disulfide bridges in a single polypeptide chain that contains 8 neutral carbohydrate side-chains). The molecular weight of HRP C has been reported as approximately 44 kDa (Welinder, 1979). HRP retains its activity over a broad pH range (Wagner and Nicell, 2001).

The reaction cycle involved in the oxidative polymerization of phenols is presented by the following set of reactions. (Dunford, 1991; Ibrahim et al., 2001).

$$E + H_2O_2 \rightarrow E - I + H_2O \qquad \text{(Eq. 3.1)}$$

$$E - I + AH_2 \rightarrow E - II + AH\bullet \quad \text{(Eq. 3.2)}$$

$$E - II + AH_2 \rightarrow E + AH\bullet + H_2O \quad \text{(Eq. 3.3)}$$

The overall reaction of the reaction cycle is:

$$2AH_2 + H_2O_2 \rightarrow 2AH\bullet + 2H_2O \quad \text{(Eq. 3.4)}$$

where E is the enzyme, E-I and E-II are the reactive enzyme intermediate compounds I and II, respectively. AH_2 is a reducing substrate such as phenol and $AH\bullet$ is a free radical product.

The chemistry of the free radical determines its ultimate fate, which may include co-oxidation by attaching to another free radical or diffusion from the enzyme active site into solution followed by oxidative coupling with another substrate molecule to form polymers (dimer, trimers or larger oligomers) (Dunford, 1991). The oxidative coupling process results in C-C and C-O bonds linking phenolic molecules and C-N and N-N bonds involving aromatic amines (Bollag, 1992 a). The phenolic polymerization products generally have high molecular weights and lower solubility than the parent solutes. The newly formed polymerization products can be removed from solution by sorption to sorbent material or by precipitation.

3.2.2 Humin

3.2.2.1 Characteristics of Humin

Several studies described in section 1.3.2 illustrated that sorption of organic compounds to humin is fast and considerably high due to the high affinity of contaminants for the humin. These studies also argued that the high affinity was often attributable to the abundance of hydrophobic aliphatic carbon in the humin structure and its interactions with the mineral matrix. It can, therefore, be said that the predominantly humin-type SOM present at greater depths in the soil profile can exert significant control over the fate and transport of organic contaminants in soils.

Humin has been defined as the SOM fraction that is strongly associated with inorganic soil fractions, and a key component of SOM-clay aggregates (Kononova, 1966; Anderson et al., 1974; Shah et al., 1975; Theng, 1979). Radiocarbon dating studies have reported soil humin to have a typical age of 1000 years or more with its age increasing with soil depth (Skujins and Klubek, 1982; Gouveia et al., 1999; Goh, and Malloy, 1978). Humin is believed to be a

stabilized form of huminc acid (HA) through mineral-linkages (Kononova, 1966). Other researchers have described humin as a high molecular weight polymer (Stevenson, 1982), as lignoprotein type biomolecule, whose recalcitrant behavior is due to its structure (Somani and Saxena, 1982), as melanines (Russell et al., 1983) or as plant residues in varying states of decay (Kononova, 1966; Anderson et al., 1974; Stevenson, 1984). Humin has been compared to protokerogen, a term used in petroleum chemistry to refer to the insoluble organic matter isolated from unlithified sediments (Rice, 2001).

Humin typically represents 40% or more of the total organic carbon in most soils or unlithified sediments (Kononova, 1966, Huc and Durand, 1977; Hedges and Keil, 1995) becoming the more dominant form of soil organic matter relative to fulvic acids (FA) and HA with increasing soil/sediment burial depth (Huc and Duran 1973; Hatcher et al., 1983; Ishiwatari, 1985; Vanderbrouke, 1985). The reduced quantities of the FA and HA fractions have been attributed to the loss of oxygen-containing groups during condensation reactions (Huc and Duran, 1977).

Hatcher, et al. (1983) proposed the theory of the "selective preservation" of the more refractory humin to explain the enrichment of humin with depth. According to this theory, the more labile molecules of carbohydrates, proteins, and lipids are biodegraded and humin is preserved and concentrated. These researchers utilized ^{13}C NMR and stable carbon isotope methods to report that with depth, the degradation of carbohydrates, proteins and lipids correlated with higher humin content. Differences in isotopic composition between a marine algal sapropel and an extracted-humin in near-surface locations diminished as depth increased. The difference in isotopic composition between extracted humin and sapropel correlated with the loss of carbohydrates that were more likely to be hydrolyzed and were ^{13}C-enriched.

Rsearchers have operationally defined humin in a variety of ways while describing its properties and structure. However, the insolubility of humin has been one of the main limitations to study its physical-chemical characteristics in the past. Isolation of humin has required aggressive treatments like dissolution of the mineral fractions and extraction of the HA and FA. Humin is traditionally isolated by treating the soil/sediment material by alkali-extraction to remove FA and HA and digesting the remaining soil with HF/HCl to dissolve the mineral fraction.

The development of modern analytical techniques has allowed researchers to study

humin characteristics more exhaustively. These techniques include: infrared (IR) spectroscopy, solid-state NMR spectroscopy, electron spin resonance (ESR) spectroscopy, and X-ray scattering (Hatcher et al., 1985). Employment of solid state ^{13}C NMR and X-ray scattering are currently the most important nondestructive analytical techniques that provide information of the composition of humin.

Russell et al. (1983) observed that the IR spectrum of a hydrolyzed humin was similar to that obtained for a hydrolyzed HA. Hydrolysis removed proteins and polysaccharides from humin. For both samples a COOH and conjugated H-bonded COOH bands were identified. Some researchers have established that humin is formed by aliphatic units and bound-HA components (Hatcher, 1980; Preston and Newman, 1992; Rice and MacCarthy, 1989). Others contend that humin is formed by aliphatic units and bound-HA components with a predominance of polymethylene carbon chains (Hatcher, 1980; Vanderbroucke et al., 1985; Rice and MacCarthy, 1989; Rice and MacCarthy, 1991; Preston and Newman, 1992; Almendros et al., 1996; Fabbri et al., 1996, Grasset and Ambles, 1998 a; Hu et al., 2000; Kang and Xing, 2005).

Almendros et al. (1996) explained that the recalcitrant humin was formed not only from selective preservation of aliphatic biomolecules, but also aggregation caused by fixation of reactive lipids on mineral fractions and their compartments. Wang and Xing, (2005) hypothesized that the enrichment of amorphous nonpolar aliphatic domains in humin favored sorption of nonionic organic solutes. The increase in aliphatic carbons resulting in the modified humin was attributed to the breakage of linkages between carbohydrates and SOM functional groups, and between humic material and minerals during HF/HCl treatment.

3.2.2.2 Interaction of Humin with Organic Contaminants

It has been reported that humin rapidly sorbs organic compounds and it often binds them irreversibly (Kohl and Rice, 1998). The aliphatic character of the humin fraction is considered important since aliphatic structures have been reported to present preferential domains for the sorption of organic contaminants (Salloum et al, 2002).

The high affinity of organic solutes to humin has been explained by the reactive surface of humin components and their insolubility (Hayase and Tsubota, 1983; Guetzolff and Rice, 1994; Malekani et al., 1997). In addition, it has been reported that the humin components (humic and lipids) have mesoporous surfaces, i.e., more than 60% of their surface area consists of <10 nm-wide pores (Malekani et al., 1997). The sub-nanometer scale pores have been suggested to

significantly influence the sorption kinetics of nonionic organic chemicals (De Jonge and Mittelmeijer-Hazeleger, 1996).

Several studies have evaluated the distribution of sorbed solutes among the three main SOM fractions (Capriel et al., 1985; Dec et al., 1997; Kohl and Rice, 1998; Loiseau et al.; 2000; Xu and Bhandari, 2003 a and b; Lesan and Bhandari, 2004 and Palomo and Bhandari, 2006). Researchers have selectively removed HA and FA from soil or sediments to study the role of the chemistry and structure of humin material on sorption and binding of organic solutes. These methods results in a humin-mineral material which has been utilized as the solid phase to conduct the sorption experiments (Guthrie et al., 1999; Chiou et al., 2000; Chefetz et al., 2000; Mao et al., 2002). Other researchers have isolated and concentrated humin material by alkaline extraction of HA and FA followed by mineral dissolution (Shih and Wu, 2002; Kang and Xing, 2005; Wang and Xing, 2005).

Sorption isotherms for ethylene dibromide, diuron, and 3,5-dichlorophenol using humin were generated by Chiou et al. (2000). These researchers argued that soil or sediments that were subjected to intense alkali extraction exhibited higher sorption capacities because they were enriched with high-surface carbonaceous material. Enhanced sorption of pyrene and phenanthrene to humin has also been attributed to the contribution of aliphatic carbon (Chefetz et al. 2000; Mao et al., 2002; Kang and Xing, 2005).

Hu et al.'s (2000) contention that poly(methylene) regions favored the retention of hydrophobic organic compounds on geosorbents was tested by Mao et al. (2002). These researchers studied the sorption of phenanthrene to various geosorbents, including humin materials, by generating sorption isotherms. Positive correlation between the sorption capacity of phenanthrene and the weight fraction of amorphous, nonpolar aliphatic poly(methylene) rich domains confirmed the idea that these domains played important roles in controlling the interactions between organic contaminants and SOM in contaminated soils. Shih and Wu (2002) evaluated sorption-desorption kinetics of toluene in contact with humin. The researchers detemined that sorption of toluene was controlled by diffusion and that the mechanism governing toluene sorption was simple partitioning. Borisover and Graber (2004) studied the effect of humin hydration on sorption of acetophenone, nitrobenzene, benzyl alcohol, and *m*-nitrophenol. It was reported that humin hydration increased benzyl alcohol and *m*-nitrophenol sorption while decreasing sorption of nitrobenzene and acetophenone. These researchers suggested that dry

humin interacted preferentially with low polarity compounds than with polar compounds. While hydration assisted sorption of compounds that formed strong specific interactions with organic matter like benzyl alcohol and *m*-nitrophenol

More recently, sorption of 1-naphthol in six humin samples (Simpson and Johnson (2006) and of phenanthrene in four humin fractions and their parent soils (Bonin and Simpson, 2007) was reported. K_{OC} values for humin were consistently higher than those obtained for the parent soils. K_{OC} values for naphthol ranged from 387 to 7,660 mL/g (Simpson and Johnson, 2006) while those for phenanthrene ranged between 14,400 to 53,700 mL/g (Bonin and Simpson, 2007).

3.2.2.3 Enzymatic Polymerization of Organic Compounds in the Humin Fraction

Polymerization and incorporation of phenolic contaminants and anilines to bulk soil have been broadly studied (Simmons et al., 1987; Liu et al., 1987; Weber et al., 1996; Bhandari et al., 1996; Thorn et al., 1996; Bhandari et al., 1997; Park et al., 2000; Dec and Bollag, 2000; Bhandari and Xu, 2001; Xu and Bhandari, 2003 a; Palomo and Bhandari, 2005). Some of these studies have evaluated the polymerization of phenols in bulk soils and the distribution of the bound solute among the HA, FA and humin components (Bhandari et al., 1996; Xu and Bhandari, 2003; Palomo and Bhandari, 2005). Till date, the majority of the work reported in the humin literature has focused on the sorption of nonpolar organic solutes such as toluene, pyrene and phenanthrene. However, it is also important to investigate the interactions between humin and polar solutes such as phenols because of their wide presence in the soil environment. Sorption of such solutes to isolated humin-mineral fractions has not been studied except by Chiou et al. (2000) and Simpson and Johnson (2006). Furthermore, peroxidase-mediated stabilization of phenolic solutes in model humin-mineral soils representing geomaterials found at greater depths has not been reported.

3.3 Research Goal and Hypotheses

The work presented in this chapter focuses on the interaction of DCP with two alkali-extracted surface soils. The purpose of the alkali extractions was to remove the FA and HA fractions, leaving the insoluble humin as the only organic fraction associated with the inorganic soil component. The research also explores peroxidase-mediated stabilization of DCP as a potential remediation technique for contaminated geomaterials. The significance of studying

polymerization of DCP in humin-mineral domains is the fact that phenolic contaminants can be present in the subsurface where SOM is dominated by the humin fraction.

Thus, the overall goal of this work is to gain an improved understanding of peroxidase-mediated stabilization of DCP in humin-mineral soils. This work represents the first time that a humin-mineral fraction is utilized to evaluate peroxidase-mediated stabilization of phenolic contaminants. The work is also distinctive since it addresses the effect of contact time on the extent of contaminant stabilization.

Four key hypotheses are evaluated in this work. These are:

Hypothesis 1

HRP-mediated oxidative coupling of DCP in the presence of a humin-mineral geomaterials results in the formation of dichlorophenol polymerization products (DPP).

Hypothesis 2

DPP macromolecules are removed from the aqueous phase as a result of sorption and retention on humin-mineral particle surfaces.

Hypothesis 3

DPP macromolecules associate strongly with humin-mineral surfaces and are resistant to extraction by water and methanol.

Hypothesis 4

Post-polymerization contact time between the DPP-containing aqueous phase and humin-mineral particles enhances the formation of nonextractable DPP residues.

3.4 Material and Methods

3.4.1 Sorbent Preparation and Characterization

The two soils utilized in this study were collected near the city of Gardner in Johnson County, KS, from an agricultural field and a nearby wooded area. They belong to the Woodson series and were classified as silty loam (Lesan and Bhandari, 2003). Both soils were sterilized by three sequential cycles of autoclaving and incubation, and stored in a freezer until used.

Humic acid (HA) and fulvic acid (FA) were extracted from the two soils with 0.1 N NaOH solution (pH>11) under a nitrogen atmosphere. A total of 42 sequential fill-withdraw cycles were required to obtain a clear supernatant while extracting the HA and FA from both soils. The alkali extractions raised the pH of the soil while the excess of sodium produced dispersion of clay particles. To reduce the pH to neutral and to control soil dispersion the following washes were performed: 10 washes with distilled-deionized water (to remove excess of OH), 10 washes with pH 7 phosphate buffer (2.82 mM K_2HPO_4, 1.8 mM KH_2PO_4, and 500mg/L NaN_3) to neutralize pH, and finally 10 washes with 1.5 g/L $CaCl_2$ to saturate the soil with Ca^{2+}. Soil samples were dried at 78 °C. Dried soil material was homogenized by means of mortar and pestle and stored in the refrigerator at 1 °C until further use. The final alkali extracted woodland and agricultural soils utilized in the experiments contained a total of 1.8 and 1.1 % SOM as humin, respectively. Texture and organic matter content analyses of the geosorbents were performed by the Kansas State University Soil Testing Laboratory (Department of Agronomy). Surface area analysis for the original soil and the humin-mineral geomaterial was conducted in the Department of Chemical Engineering, at Kansas State University. The pH of the soil was measured by using continuously mixed soil slurry. Selected properties of the two geomaterials are summarized in Table 3.1.

Organic carbon characterization of the humin samples was performed by Spectral Data Services, Inc, Champaign, IL with solid-state ^{13}C NMR, utilizing cross-polarization magic angle spinning (CP-MAS). Solid-state NMR spectra were obtained at 91 MHz and 7 KHz CP-MAS on a 360-1 instrument using > 25 000 scans.

3.4.2 Chemicals and Reagents

The work presented here utilized in 2,4-dichlorophenol (DCP) as a probe solute to evaluate the interaction of phenolic contaminants with soil modified to expose the humin type SOM. DCP was also selected because of its ability to participate in oxidative polymerization reactions. DCP is a derivative of widely used herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) and behaves like a weak acid in most water and soil environments. At neutral pHs, DCP exists in both neutral and ionic forms. At a pH of 7.0, approximately 83% of DCP exists in the neutral form while 17% exists in the ionized form. DCP can be detected in soil within 24 hours of 2,4-D application (Crespin et al., 2001). Exposure to high concentrations of DCP can cause damage to

the immune system and liver (EPA, 1980). Selected properties of DCP are shown in Table 3.2, while a more comprehensive listing is available elsewhere (Palomo, 2003).

Table 3.1. Selected properties of the alkali extracted soils.

Property	Agricultural		Woodland	
	Alkali Extracted	Original	Alkali Extracted	Original
pH:	6.80	6.10 [a]	6.80	6.90 [a]
Surface area (m^2/g)	16.9	9.0	15.6	6.9
Organic matter %:				
total	1.10	3.4[a]	1.80	6.20[a]
Texture (hydrometer):				
Sand %	12	32[a]	8	38[a]
Silt %	74	52[a]	72	52[a]
Clay %	14	16[a]	20	10[a]

[a] from Palomo and Bhandari, 2005-SOM determined by the Walkley-Black Method.

Table 3.2. Selected properties of DCP

Molecular weight (g/L)	163
Solubility g/L (20 ºC)	4.5
log K_{OW}	3.20
pKa (25 ºC)	7.69

Unlabeled and ^{14}C-labeled DCP (specific activity 20.9 mCi/mmol) were purchased from Sigma Aldrich, St. Louis, MO and used with no further purification. ^{14}C-labeled DCP was used as a tracer to track and quantify the distribution of DCP and DPP in the soils and aqueous phases. Five different working solutions (0.5, 5, 50, 100 and 500 μM DCP) were prepared in a pH 7 phosphate buffer (2.82 mM K_2HPO_4, 1.8 mM KH_2PO_4, and 500 mg/L of sodium azide as bacterial growth inhibitor) by dilution of accurate amounts of both unlabeled and ^{14}C-DCP. The

initial solutions were spiked with enough ^{14}C-DCP to have approximately 10,000 dpm (disintegrations per minute) of activity per milliliter of solution. Radioactivity in aqueous solutions was analyzed by transferring 250 μL of the solution into 7 mL scintillation vials that contained 5 mL of scintillation cocktail (Fisher Scientific, Scinti-Safe 50%). All aqueous samples were analyzed in a 6500 Beckman Liquid Scintillation Counter (LSC). Before analysis, the samples were allowed to sit overnight to minimize interference from chemiluminescence.

Horseradish peroxidase (HRP-type II, RZ2:2) and hydrogen peroxide (H_2O_2, 30% w/w, 8.2 M) were acquired from Sigma Aldrich and utilized without further purification. One activity unit (AU) of HRP was defined by Sigma Aldrich as the amount of HRP that formed 1.0 mg purpurogallin from pyrogallol in 20 seconds at pH 6.0 at 20 °C. A 0.612 mM H_2O_2 stock solution was prepared in distilled/de-ionized water one hour before the start of the experiment. Sufficient mass of HRP was added to the working solution to produce 2 AU of enzyme activity per milliliter of solution. The addition of H_2O_2 to the reactors initiated the oxidative polymerization reaction.

High performance liquid chromatography (HPLC) grade water and methanol (Fisher Scientific) were used for the HPLC analysis and extractions of DCP and DPP from soil. A 0.1 N sodium hydroxide solution (2 N NaOH, Fisher Scientific) was used to extract HA and FA from the surface soils. Glacial acetic acid purchased from Fisher Scientific was employed in the HPLC analysis of the samples.

3.4.3 Sorption Experiments

Sorption of DCP (no enzyme added) and DPP (generated in sity by addition of HRP to the reactors) was evaluated using completely mixed batch reactors (CMBRs) containing predetermined amounts of geosorbent. CMBRs consisted of 10 mL test tubes containing 1.5 gram of alkali extracted soil and capped with Teflon-lined screw closures. Three different sorption contact times (1, 7 and 28 days) and five different DCP concentrations were evaluated (0.5, 5, 50, 100 and 500 μM). Triplicate CMBRs and controls (no soil added) were prepared per concentration per contact time per solute studied. The tube + cap were weighed before and after soil addition and the weights were recorded to determine the exact mass of soil in each reactor. The sorption experiment was initiated by adding DCP working solutions to the test tubes. DPPs were produced in-situ by adding HRP and H_2O_2 to the solution in the CMBR. The volume of

solution transferred into the CMBRs was determined gravimetrically. Every sorption experiment consisted of 90 CMBRs (3 replicates + 3 controls, 5 concentrations and 3 contact times). Both DCP and DPP experiments were initiated on the same day. A touch mixer was used to homogenize the contents in the CMBR. All test tubes were placed in an end-over-end tumbler (20 rpm) until the desired contact time was reached.

The CMBRs were removed from the tumbler after predetermined contact periods and centrifuged for 45 minutes at 550 g to separate the solid and aqueous phases. Two aqueous samples were removed from each CMBR for LSC and HPLC analysis. HPLC was used to quantify the amount of DCP remaining in the solution after the sorption experiment, while LSC analysis accounted for the total radioactivity remaining in solution. From each CMBR, a sample of 250 μL was transferred to a 7 mL scintillation vial containing 5 mL of scintillation cocktail and analyzed in the LSC 24 hours later. One milliliter of the supernatant was removed with a gas-tight glass syringe and filtered using a 0.45 μm glass microfiber (Millipore filters and Millex-LCR syringe driven filter unit). Controls and the filtrate were analyzed using reverse phase high performance liquid-chromatography (RP-HPLC). HPLC samples were stored in the refrigerator when not analyzed the same day. Samples were analyzed within 72 hours. The remaining supernatant in the CMBRs was carefully removed with a disposable glass pipette connected to a peristaltic pump, and discarded. The radioactivity (dpm) measured by the LSC analysis was converted to its equivalent concentration (μM) of DCP. DPP concentration was expressed in DCP equivalents. A material balance on DCP or the radioactivity was conducted to estimate the solid phase concentration of DCP and DPP (q, μmol/kg). The q value was calculated from the difference between initial aqueous concentration of solute (Co , μM) and its concentration in solution at the end of the desired contact time, t (C_t , μM). These values were corrected to account for sorption to glassware, obtained by measuring the aqueous solute concentration in the control CMBRs.

3.4.4 Water and Methanol Extraction

The extractability of sorbed DCP or DPP was estimated using two types of extractions. The relative aqueous extractability of sorbed DCP and DPP was determined using a single, 24-h water extraction, while sequential methanol extractions were conducted to measure the total extractable solute. Methanol was selected as the solvent for extraction based on the work by Xu

(2002), who had demonstrated that methanol and five other organic solvents had the same efficiency to extract DCP from a surface soil. Once the supernatant in the CMBRs was removed after completion of sorption experiments, the reactors were weighed to determine the amount of residual solution in each tube. DCP-free phosphate buffer was added to each CMBR to perform the single-water extraction. After addition of the buffer to the CMBRs, the weight was recorded. The exact volume of the solution added to each tube was calculated gravimetrically. The contents of the CMBRs were mixed with a touch-mixer and placed in the end-over-end tumbler. Water extraction was allowed to proceed for 24 hours after which the CMBRs were removed and centrifuged (45 minutes at 550 g). The supernatant was sampled for HPLC and LSC analyses as described in section 3.4.3. Three sequential fill-and-withdraw methanol extractions were performed on the soil immediately after the single-water extraction. The procedure was identical to that used for the water extraction. Three methanol extractions were sufficient to reduce the measured radioactivity in the extract to below the detection limit (50 dpm). Once the methanol extractions were completed, the soil in the CMBRs was air dried inside the fume hood. The air-dried soil in each tube was combusted at 925 °C using an OX-500 Biological Material Oxidizer (BMO, R.J. Harvey Instrument) to determine the radioactivity associated with the soil.

Methanol extracted solute was calculated indirectly by subtracting the amount of solute remaining in soil after methanol extraction from the solute remaining in soil after water extraction. To verify the efficacy of the indirect calculation, a mass balance of solute was conducted for the methanol extraction procedure. The residual DCP/DPP concentration in supernatant (methanol) was measured, and the mass of methanol added to the test tube and that remaining in the reactors after each 24-h extraction cycle was determined gravimetrically. Density of methanol, 0.791 g/cm^3 at 20 C (Perry and Green, 1984), was utilized to calculate the volume of methanol added and remaining in each reactor. Measurement of the supernatant residual solute concentration was determined by means of HPLC and LSC. While, methanol losses in the test tubes were found to vary from 1.5 to 3.8 %, the solute mass balance results, for the majority of the reactors, were within 10 % of the indirectly calculated values.

3.4.5 Sample Analysis

HPLC Analysis- HPLC system equipped with a reverse phase column was utilized to determine the concentration of DCP in aqueous solutions and methanol. It consisted of a ProStar

230 tertiary solvent delivery module (Varian Associates, Inc.), a ProStar auto sampler (Varian Associates, Inc.), and a Varian Chromguard HPLC column (SS 50 mm x 3 mm). Identification of DCP concentration was done by a Varian ProStar 335 Photodiode Array Detector at an absorbance wavelength of 280 nm The mobile phase consisted of methanol: HPLC grade water: acetic acid mixture of 65:35:2 (v:v:v). The injection volume was 10μL and the flow rate was 1.0 mL/ min

LSC Analysis- A 6500 Beckman liquid scintillation counter was utilized to analyze the radioactivity in the aqueous solution after sorption experiments and in aqueous and methanol extracts. LSC results were determined in dpm but they were converted to their equivalent chemical concentration in μmoles or μM. The LSC analysis reported the total radioactivity in solution that included ^{14}C-DCP and ^{14}C-DPP. The concentration of aqueous (soluble) DPP was determined from the difference in concentrations of soluble (DCP + DPP) obtained from LSC measurements and the concentration of soluble DCP acquired from HPLC measurement.

Soil Combustion- The soil in each CMBR was combusted at 925 °C in an OX-500 biological material oxidizer (BMO, R.J. Harvey Instrument). The exact mass of soil combusted was determined by weighing a combustion glass boat before and after addition of the soil. The glass boat containing the soil was introduced into the combustion chamber and combusted for 4 minutes at 925 °C. Any ^{14}C-DCP or ^{14}C-DPP associated with the soil was converted to ^{14}C- CO_2 that was trapped in a 20 mL scintillation vial containing 10 mL Carbon-14 cocktail (R.J. Harvey Instrument). The scintillation vials were analyzed by LSC after 24 hours.

3.5 Results and Discussion

This section of Chapter 3 is divided in two main parts. The first part presents and disscuses the physicochemical properties and the ^{13}C NMR characterization of the two humin-mineral materials used in this research work. The ^{13}C NMR analysis was used to determine differences in organic matter composition of both geomaterials. The source of organic inputs and the management activities in both parent soils were different. Hence observed differences in sorption, extraction and binding of solutes in these geosorbents can be explained in the context of their organic and mineral compositions. The second part of section 3.5 presents the results for DCP and DPP sorption, binding and extractability in the context of the mode humin-mineral soils.

3.5.1 Physical Properties of the Humin-Mineral Geomaterials

Exhaustive alkali extraction was used to prepare the model humin-mineral soils since Wang and Xing (2005) had reported that alkali extraction of HA and FA from soil did not cause any significant alteration of the humin fraction. The SOM contents of the agricultural humin-mineral (AHM) and woodland humin-mineral (WHM) geomaterials were 1.1 and 1.8 % respectively. SOM content of the parent agricultural and woodland soils were previously reported as 3.4 and 6.2 %, respectively (Palomo and Bhandari, 2005). Texture analysis for both AHM and WHM fractions is presented in Table 3.4. Exhaustive NaOH extraction was observed to change the soil texture and impacted the goematerial's sand, silt and clay contents. This change was attributed to aggregate breakage in original soil caused by the solubilization and extraction of HA and FA.

A significant increase in surface area of the original soil was observed after alkali extractions. The increase of surface area was from 9 to 16.9 and 6.9 to 15.6 m^2/g, for the AHM and WHM, respectively. Chiou et al., (2000) reported increases in surface area when a humin sample was prepared by alkali extraction of peat. The surface area of peat was enhanced from 1.4 m^2/g to 4.5 m^2/g. Chiou et al. (2000) believed that enhancement in surface area was caused by the enrichment of high-surface-area-carbonaceous-material (HSACM) by sequential NaOH extractions. It is likely that the increase in surface area, of both soils was also impacted by disaggregation of the parent soils. The fraction of fine silt and clay particles was observed to increase while the fraction of the coarser sand decreased as result of alkali extraction.

3.5.2 ^{13}C NMR Characterization of Humin-Mineral Geomaterials

Solid-state ^{13}C NMR analysis was utilized to characterize the humin-mineral geomaterials. ^{13}C NMR analysis was conducted in this study to correlate the chemical composition of humin with results obtained from the sorption, and solute stabilization experiments. It was expected that the humin-mineral geomaterials would possess chemical characteristics similar to those attributed to the parent soils. The ^{13}C NMR spectra of AHM and WHM were acquired after 25,000 and 25,968 scans, respectively (Figure 31.). Both spectra showed two broad but clearly resolved peaks. Well resolved peaks were obtained in regions 0-100 and 100-200 ppm, indicating a similar chemical composition. The largest peaks in the spectra were observed in the aliphatic C region. The AHM and WHM aliphatic C comprised 80

and 76 % of the total C integrated by ^{13}C NMR, suggesting that the nature of both humin fractions was primarily aliphatic.

Table 3.3 summarizes the results obtained by solid-state ^{13}C NMR CP-MAS. The humin fractions in both sorbents were abundant in un-substituted or paraffinic C and substituted or *o*-alkyl C. While paraffinic C has been attributed to the selective preservation of cutin that is present in the cuticle covering the cellular wall of higher plants (Kang and Xing, 2005), the *o*-alkyl C (C-O, C-N bonds) could be attributed to carbohydrates, alcohols, ethers and amines (Chefetz et al., 2000). This result is consistent with the origin of humin since at the soil surface the input of fresh organic matter is continuous and the microbial activity is strong. Thus, the presence of wax-like material from plants, carbohydrates, and proteinaceous substances would be expected in a surface humin (Hatcher et al., 1985).

No individual resolved peaks were observed in the 30-50 ppm region. However, a broad peak with well defined shoulders around 30 and 33 ppm was noted. This can be attributed to highly cross-linked paraffinic macromolecules (Hatcher et al., 1985). The signals at 30 and 33 ppm are likely attributable to amorphous and crystalline poly(methylene) C, respectively (Hu et al., 2000). Such structures have been suggested to be responsible for the fast, nonlinear and irreversible interaction of HOCs with humin (Chefetz et al., 2000; Chiou et al., 2000). In the same region, AHM spectrum showed two other clear peaks at 25 and 40 ppm, while in the WHM two peaks were seen at 45 and 50 ppm. Dominant peaks around 70 and 105 ppm were observed in both spectra. These peaks can be attributed to the presence of polysaccharides (Hatcher et al., 1985). The signal in the 110-220 ppm range was broad with several peaks. However, none of the peaks were individually defined. The presence of some lignin might be possible since both spectra showed a peak at 150 ppm that is characteristic of methoxyl-substituted or phenolic C.

AHM appeared to have a slightly more aliphatic character than WHM, with the same proportion of paraffinic C but different proportion of *o*-alkyl groups. While 37% of total carbon was substituted C in AHM, WHM manifested 33% of substituted C in the region from 0 to 220 ppm. AHM appeared slightly less aromatic than the WHM although both showed the same composition of phenolic C (6%). WHM appeared to contain slightly more aromatic, carboxylic and carbonyl C.

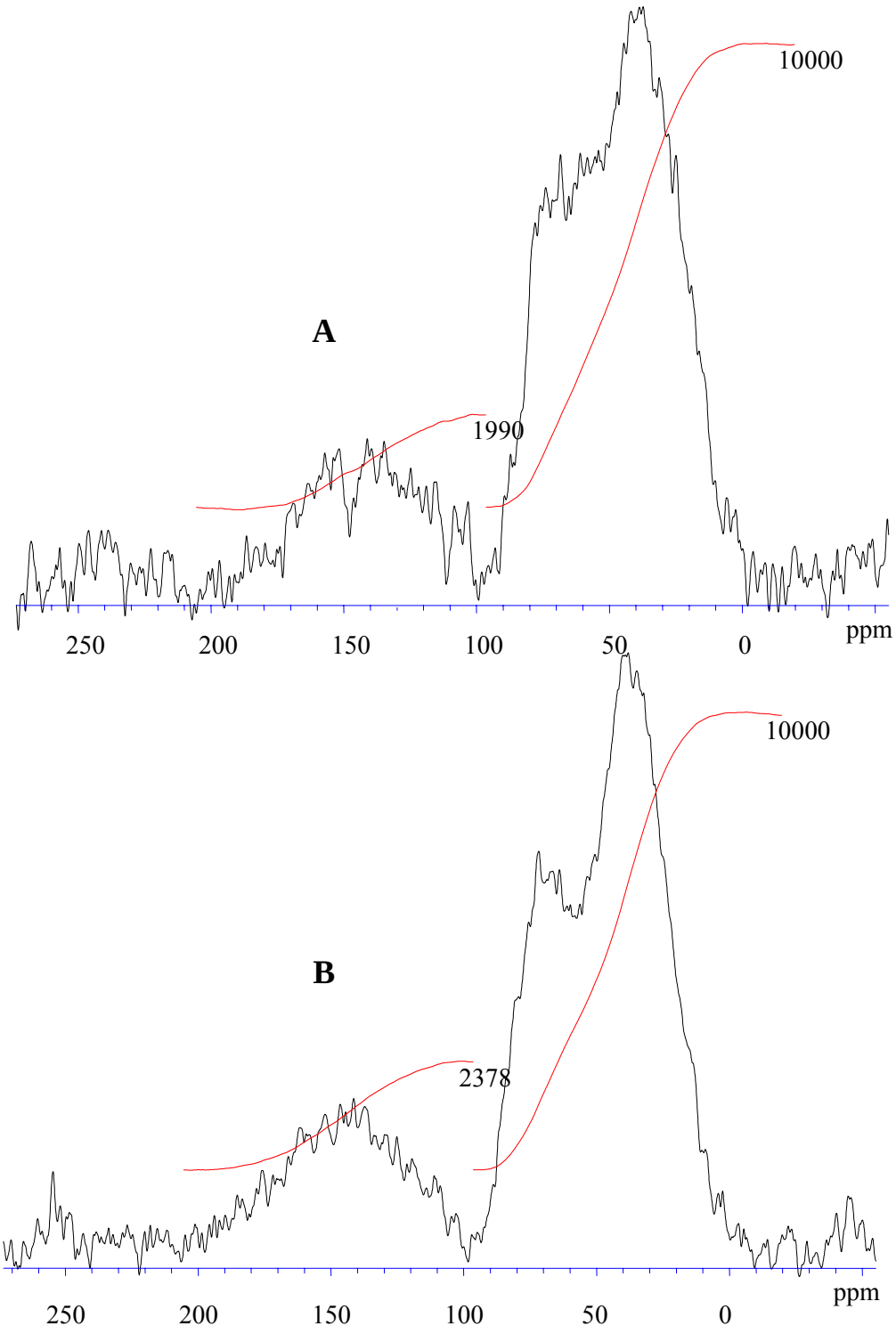

Figure 3.1. CP-MAS solid-state ^{13}C NMR spectra of humin-mineral fractions, extracted from an agricultural soil (A), and a woodland soil (B).

Table 3.3. Solid-state ^{13}C NMR integration results.

C-containing group	ppm range	Distribution of C chemical shift (%)			
		AHM		WHM	
		Including carbonyl	Carbonyl not included[a]	Including carbonyl	Carbonyl not included[a]
paraffinic	0-50	43	*43*	43	*44*
o-alkyl	50-110	37	*37*	33	*34*
aromatic	110-145	9	*10*	11	*11*
phenolic	145-163	6	*6*	6	*6*
carboxylic	163-190	4	*4*	5	*5*
carbonyl	190-220	1	---	2	---

[a] Distribution of C from ^{13}C NMR spectra without the carbonyl fractions was included to compare the values obtained in this study for the humin fractions with those reported for the original soils (Palomo and Bhandari, 2005)

The major chemical difference between the two humins was in the composition of the substituted C groups attributable to carbohydrates, amines, alcohols and ethers (Chefetz et al., 2000). The high content of *o*-alkyl groups in AHM and WHM suggested that they were preserved in the humin structure through interactions with the other C components of the fraction.

Comparing the ^{13}C NMR integration obtained for the original agricultural and woodland soils reported elsewhere (Palomo and Bhandari, 2005), it can be seen that the paraffinic C was slightly decreased after exhaustive alkali extraction (from 47 to 43% for agricultural soil and 45 to 44 % for woodland soil), while the *o*-alkyl C was enriched for the agricultural soil (from 32 to 37%) and reduced in the woodland soil (from 38 to 34%). Similar amounts of aromatic and phenolic C suggested that both humins were relatively young and in a similar stage of diagenesis (Huang and Weber, 1997).

The polarity index was calculated in accordance to Wang and Xing (2005), where the carbohydrate (50-110 ppm), phenolic (145-163 ppm) and carboxylic (163-190 ppm) carbon were

considered as polar carbons and polarity was defined as the ratio of polar C to total organic C (0-220 ppm). Aliphaticity was defined as the ratio of aliphatic C (0-110 ppm) to the sum of aliphatic and aromatic C (0-163 ppm). Similarly, aromaticity was defined as the ratio of aromatic C (110-163 ppm) to the sum of aliphatic and aromatic C (0-163 ppm) (Chefetz et al., 2000). The values obtained for polarity index, aliphaticity and aromaticity for the two alkali extracted soils are shown in Table 3.4. Polarity indexes for AHM and WHM were 0.47 and 0.46, respectively. These values are similar to those reported for humin fractions extracted from Amherst and Florida peat soils, which ranged from 0.35 to 0.54 (Wang and Xing, 2005). The AHM and WHM can therefore be considered as moderately polar. Low polarity of the SOM has been found to relate to an affinity for HOCs (Kang and Xing, 2005). Reduction in polarity has been reported to be caused by a reduction in the carbohydrate content (Chen et al., 2005). Kang and Xing (2005) reported that humin with low polarity (0.40) presented higher sorption capacity than HA's with high polarities, - ranging from 0.52 to 0.60.

Both humins associated with the humin-mineral geomaterials used in this study had similar aliphaticity, while less than 20 % of the total carbon was aromatic. Chen et al. (2005) suggested that abundant aliphatic components can cover the aromatic matter preventing its interactions with organic compounds.

Table 3.4. Selected properties of the humin-mineral sorbents

Property	AHM	WHM
Organic matter %:	1.10	1.80
Surface Area (m^2/g) BET N_2 P/P_o= 3	16.9	15.6
Aromaticity %	16	18
Aliphaticity %	84	82
Polarity Index	0.47	0.46

SOM % determined by the Walkley-Black Method

Chen et al. (2005) also studied the sorption of the polar phenol and 1-naphthol in differently treated aliphatic-rich cuticular fractions of green pepper. The cuticular fraction which

showed the highest polarity (0.69) was associated with the largest sorption capacity (*Koc*) values. Even though 100 % of both solutes were in non-ionized form at the neutral pH of the study, the results were attributed to the accessible aromatic and polar cores of the cuticle fraction that exhibited affinity for the polar compounds. These researchers also related the high paraffinic content and low polarity of the sorbent to high removal of nonionic organic contaminants (naphthalene and phenanthrene) from the aqueous phase. Similarly, Chefetz et al. (2000) observed a positive correlation between the sorption of the nonionic compound pyrene and the high aliphaticity (91.2%) of a humin material extracted from sapropel.

While Chiou et al. (2000) have attributed the specific interactions of reactive groups of SOM to the nonlinear sorption behavior of polar compounds; this study is the first to report the sorption affinity of DCP to aliphatic-rich and low polarity humin.

In summary, the NMR analysis demonstrated that the AHM and WHM organic matters have a predominantly aliphatic nature, and exhibit low polarity. Thus they can be expected to show a favorable interaction with nonpolar organic compounds. In this study the pH was fixed at 7, where about 83 % of DCP was in nonionic state while the remaining 17% was ionized. In such conditions, it is likely that the abundant nonpolar and less predominant polar components of the AHM and WHM humins will exhibit favorable sorption affinities to both forms of DCP.

3.5.3 Sorption of DCP to Humin-Mineral Soil

3.5.3.1. DCP Phase Distribution Relationships

The phase distribution relationships (PDRs) for DCP were constructed using five different initial aqueous concentrations and for three different contact times (1, 7 and 28 days). The Freundlich model was used in this study to obtain phase distribution parameters for the DCP and DPP. The Freundlich model is widely used to describe the sorption of organic contaminants to natural or model geosorbents. This model has been previously described in detail in section 1.1.2.2. The *n* parameter is a measure of sorption linearity and an *n*-value of unity means that the sorption process is dominated by partitioning. The K_F parameter is an indicator of the sorption capacity of the sorbent and depends on the chemical and physical properties of both sorbent and solute. The value of K_F is obtained at unit aqueous phase concentration and can be used to compare the sorption capacity of different geosorbents, at equal *n* values. However, when the *n* values obtained for different sorbents are dissimilar it is not advisable to compare the values of

K_F even for the same solute (Weber and DiGiano, 1996). In order to compare sorption capacities, the distribution coefficient (K_D) can be obtained from the linear model, where *n* equals one. Normalization of the solute distribution coefficient to the organic carbon (OC) or soil organic matter (SOM) content (K_{OC} or K_{OM} coefficients), is often performed to compare solute sorption on OC or SOM basis. This normalization acknowledges the sorption capacity of OC or SOM without taking into account the whole sample mass which includes the mineral component. Also SOM normalized sorption capacity coefficients were calculated for both humin-mineral soils and both solutes.

3.5.3.1.1 Woodland Humin-Mineral Soil

This section describes the interaction of DCP with the humin-mineral geomaterial obtained from the woodland parent soil. PDR's corresponding to three different contact times (1, 7 and 28 day) for DCP sorption to WHM are shown in Figure 3.2. DCP sorption on WHM appears to have reached equilibrium within 7 days. The Freundlich linearity parameter *n(t),*

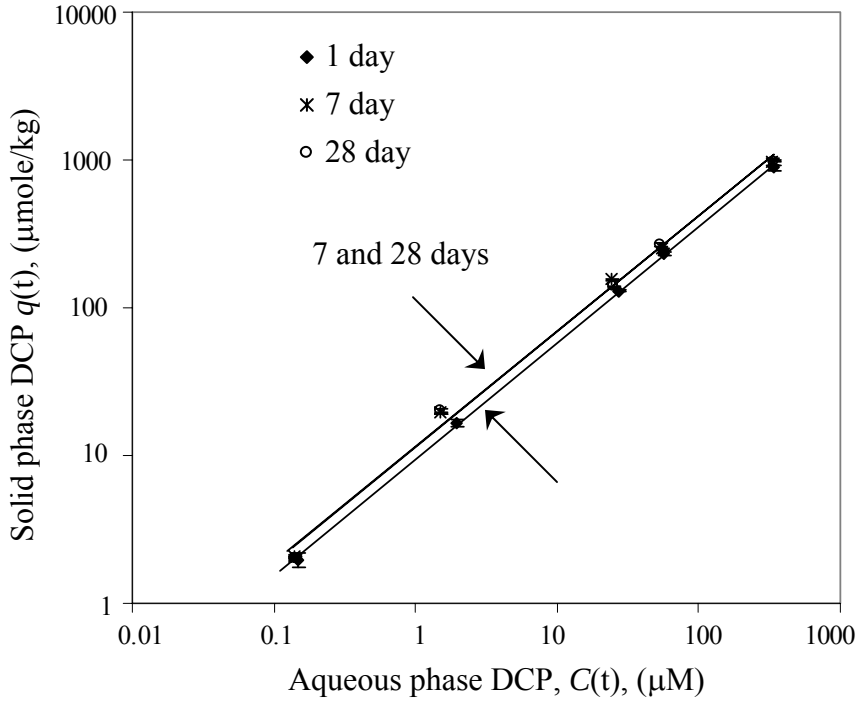

Figure 3.2. Phase distribution relationships for DCP sorption to WHM soil.

indicated a sorption behavior that was nonlinear (≈ 0.78) and remained constant through the experimental time. Since *n* values were not statistically different at the studied contact times, the values of $K_F(t)$ values have the same units and can be compared.

For PDRs shown in Figure 3.2, $K_F(t)$ at 1 day was statistically different from the values at 7 and 28 days. $K_F(t)$ varied from 9 μmole/(kg× μM^{-n}) at 1-day to approximately 11 μmole/(kg× μM^{-n}) at 7 and 28 days, indicating that DCP sorption to WHM was complete within 7 days. Values of the Freundlich parameters $n(t)$ and $K_F(t)$ and their 95% confidence intervals are summarized in Table 3.5. The nonlinear sorption of organic solutes to humin has been variously attributed to the abundance of low polarity aliphatic carbon (Kang and Xing, 2005), greater humin surface area exposure or exposure of higher energy sorption sites due to the removal of HA and FA. Chiou et al. (2000) attributed the nonlinear sorption of ethylene dibromide, diuron and 3,5-dichlorphenol to specific interaction between reactive sites of huimin instead of its surface area. However, surface area analysis of WHM showed a 2.3 fold increase in surface area compared to the parent woodland soil with clear implications for solute sorption. Since SOM content of WHM was 1.8%, sorption may be attributed to humin only without significant contribution of the mineral fraction. Pignatello (1989) suggested that SOM controls sorption of nonionic organic compounds for organic C levels > 0.1 %, while the interactions with mineral surfaces are significant below this SOM value.

Table 3.5. Freundlich parameters for DCP sorption to Woodland humin-mineral soil.

Contact Time (days)	Woodland Humin-Mineral Soil			
	n(t)	$K_F(t)$ [1]	$K_F(t)_{SOM}$ [2]	R^2
1	0.789 *(0.029)* [3]	9.33 *(1.38)*	518 *(76)*	0.996
7	0.781 *(0.031)*	11.52 *(1.80)*	640 *(100)*	0.995
28	0.783 *(0.031)*	11.30 *(1.82)*	628 *(101)*	0.995

[1] Units for $K_F(t)$ are $\frac{\mu mol}{kg}(\mu M)^{-0.78}$ [2] units for $K_F(t)_{SOM}$ are $\frac{\mu mol}{kg_{SOM}}(\mu M)^{-0.78}$

[3] 95% confidence intervals

3.5.3.1.2 Agricultural Humin-Mineral Soil

PDRs for the DCP sorption to AHM showed a somewhat different behavior than that observed for WHM (Figure 3.3). Although Freundlich constant *n(t)* was nonlinear and constant for all contact times, its value for AHM was lower (≈ 0.71) indicating a greater degree of nonlinear sorption behavior.

Although AHM had smaller SOM content than WHM, the aliphatic content and the surface area of AHM were higher and were the likely cause for the more pronounced nonlinear behavior. Palomo and Bhandari, (2005) had previously reported that the parent agricultural soil showed more pronounced nonlinear sorption behavior than the woodland soil. These researchers reported a greater *n* value of 0.81 for sorption of DCP to the bulk agricultural soil.

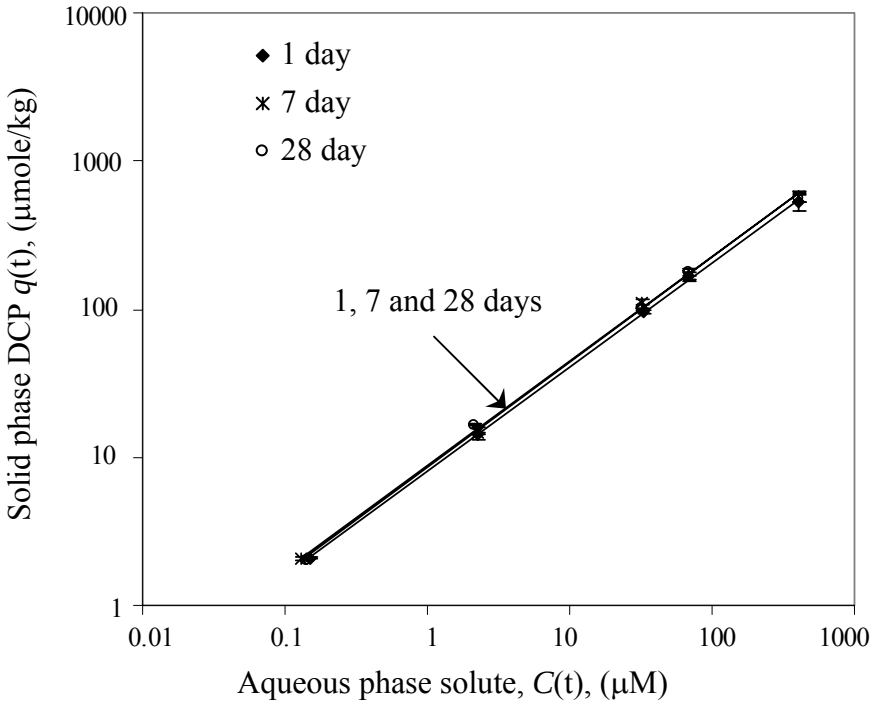

Figure 3.3. Phase distribution relationships for DCP sorption to AHM soil.

PDRs for the three contact times were found to overlap indicating that DCP sorption to AHM was complete within 1 day. The 95% confidence intervals indicated that the values of *n* and $K_F(t)$s at the three studied contact times, were statistically similar (Table 3.6). The SOM normalized $K_F(t)$ values are summarized in Table 3.6.

While the parent agricultural soil had a surface area of 9.0 m^2/g the AHM soil exhibited a greater surface area of 16.9 m^2/g. However, since AHM had a SOM content greater than 0.1%, the inorganic fraction of soil was considered to have minimal contribution to the overall sorption process (Pignatello, 1989).

Table 3.6. Freundlich parameters for DCP sorption to agricultural humin-mineral soil.

Contact Time (days)	Agricultural Humin-Mineral Soil			
	n(t)	$K_F(t)$ [1]	$K_F(t)_{SOM}$ [2]	R^2
1	0.704 *(0.015)* [3]	8.10 *(0.67)*	805 *(67)*	0.999
7	0.706 *(0.015)*	8.65 *(0.84)*	865 *(84)*	0.998
28	0.705 *(0.014)*	8.75 *(0.69)*	875 *(69)*	0.999

[1] Units for $K_F(t)$ are $\frac{\mu mol}{kg}(\mu M)^{-0.705}$, [2] units for $K_F(t)_{SOM}$ are $\frac{\mu mol}{kg_{SOM}}(\mu M)^{-0.705}$

[3] 95% confidence intervals

DCP sorption capacities of both humin-mineral geomaterials were also compared using single point, organic matter-normalized distribution coefficients ($K_{OM,Ct}$) obtained from the Freundlich *n* and K_F values as proposed by (Tang and Weber, 2006) using the following relationship:

$$K_{OM,Ct} = \frac{q_e}{c_e} = K_F C_e^{n-1} \qquad \text{(Eq. 3.5)}$$

where the units of $K_{OM,Ct}$ are L/kg $_{OM}$, and represent the volume of water that would contain the same quantity of DCP as one kilogram of soil after *t* days of contact. C_e and q_e represent the equilibrium concentration in the solid and aqueous phases, respectively. The $K_{OM,Ct}$ values were calculated once the sorption equilbrium was achived, i.e., at seven days of contact, and residual aqueous phase solute concentrations of 10, 100, 200 and 300 μM. Table 3.7 summarizes the results for both AHM and WHM. $K_{OM,Ct}$ is higher at lower concentrations for both sorbents. At most aqueous concentrations, $K_{OM,Ct}$ values for WHM were higher than those observed for AHM. The increased sorption capacity of WHM material can be attributed to its higher SOM

content than AHM. However, the difference in sorption capacity illustrated by the $K_{OM,C\,t}$ s values is not directly proportional to the SOM content of the sorbents. Thus, the $K_{OM,C\,t}$ values for AHM were consistently smaller, indicating a smaller sorption capacity compared to that observed for WHM

Table 3.7. Single point, organic matter-normalized sorption distribution coefficient, $K_{OM,C\,t}$ for WHM and AHM soils, after seven days of contact time.

Residual Aqueous Concentration (μM)	$K_{OM,\,Ct}$	
	WHM	AHM
10	387	439
100	234	223
200	201	182
300	184	162

*Units of $K_{OM,C\,t}$ are L/kg-OM

3.5.3.2 Readily Desorbing DCP

The relative water extractability of DCP from the parent woodland and agricultural soils was previously studied by Palomo and Bhandari (2005) as a means to compare the relative amounts of readily disorbing DCP from various sorbents. The relative ease of reverse mass-transfer of solute from the sorbent was studied using the empirically derived Extractability Index (*EI*) which utilized PDRs and the 24-hr water extraction data:

$$EI(t) = \log\left(\frac{q^t}{q^e - q^t}\right)_{|C_t} \qquad \text{(Eq. 3.6)}$$

where, q^t and q^e are the solid-phase solute concentrations obtained from the sorption and water extraction PDRs, C_t is the residual aqueous phase concentration and t is the contact time at which *EI* is calculated. Small values of *EI* indicate low aqueous extractability of solute, while values of *EI* approaching 1.0 indicate reversible sorption. *EI* values were calculated at three residual solute concentrations (1, 10 and 100 μM) and three contact times. To facilitate the discussion and comparison of *EI* values, a subscript will be used to correspond to the aqueous concentrations or contact time (x = 1, 10 or 100 μM; or 1, 7 or 28 days).

3.5.3.2.1 Woodland Humin-Mineral Soil

Sorption and extraction PDRs for WHM at three concentrations and three contact times are shown in Figure 3.4. *EI* values were observed to decrease in the order $EI_1 > EI_{10} > EI_{100}$ μM indicating that at low concentrations, a greater fraction of the sorbed DCP was extracted from humin, while at 10 and 100 μM, progressively less DCP was removed during 24-h water extraction. These results were in constrast to those reported by Palomo and Bhandari (2005) for parent soils. These researchers observed that the *EI* values for the original woodland soil increased at low initial solute concentration.

Although PDR's analysis does not provide information about the soil-solute interaction mechanisms, results from the *EI* analysis (higher extractability values at lower C_t's) suggest that besides surface adsorption, other mechanisms are operative and impact retention of DCP at higher solute concentrations. Since the overall number of adsorption sites on the humin surface is relatively constant for a sorbent, it is likely that at high C_t DCP molecules were able to penetrate deeper into the humin structure and access recessed sorption domains. These results can be attributed to diffusion of DCP molecules into the humin matter in addition to surface adsorption. The exposition and increased availability of the aliphatic rich WHM organic matter upon removal of HA and FA, may have resulted in enhanced solute penetration into the humin matter at higher solute concentrations. Huang and Weber (1997) reported that concentration-dependent hysteresis index (*HI*) values for sorption-desorption of phenanthrene increased or decreased depending on the chemical characteristics of the sorbent, such as the geological age and composition of SOM. In their study, solute extractability from most sorbents, was reduced at higher concentrations. Thus, the high sorption capacity of WHM for DCP, the nonlinear sorption behavior, and concentration-dependent solute extractability suggest not only surface adsorption of DCP but also diffusion of the solute molecules into the structure of the exposed and tightly mineral-bound humin.

It is also evident that the *EI* was impacted by time of contact with $EI_1 > EI_7 > EI_{28}$ days (Table 3.8). Thus, as time and solute concentration increased, sorption became less reversible. The *EI* value decreased from 0.55 to 0.40 from 1 to 28 days at 100 μM. These results indicate that reverse mass transfer of the sorbed DCP from humin was generally rapid and reversible.

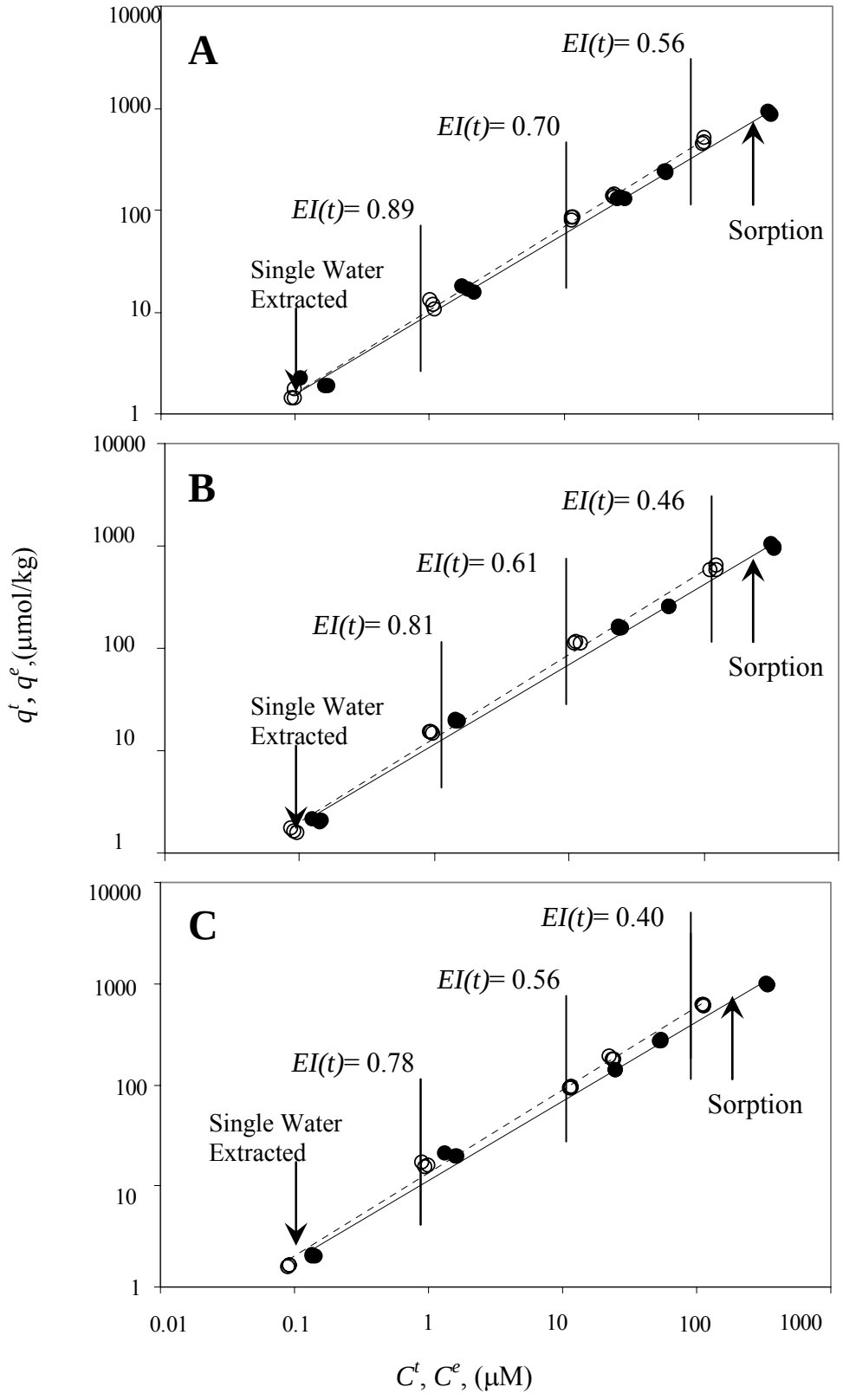

Figure 3.4. Extractability of DCP from WHM after sorption for A) 1, B) 7 and C) 28 days.

Table 3.8. Extractability index values for water extractable DCP from WHM.

Contact Time (days)	Woodland Humin-Mineral Soil		
	EI_1	EI_{10}	EI_{100}
1	0.894	0.697	0.555
7	0.814	0.610	0.463
28	0.781	0.560	0.403

3.5.3.2.2 Agricultural Humin-Mineral Soil

Sorption and water extraction PDRs for AHM are illustrated in Figure 3.5. In general, both contact time and concentration reduced DCP removal from AHM. *EI* decreased in the order of $EI_1 > EI_{10} > EI_{100}$ in terms of concentration and $EI_1 > EI_7 > EI_{28}$ in terms of time, except for the 1-day contact time where no concentration dependent trend was observed.

Extractability of DCP on day 1 was higher in the AHM soil than WHM. However, at larger contact times, DCP was less easily extractable from AHM soil. This implies that AHM was generally better in retaining DCP than WHM. The higher extractability, observed at 1 day for AHM may have been due to its lower SOM content. However, with increasing contact time and solute concentration, the SOM content appeared less influential than its aliphatic nature. The SOM associated with AHM was more aliphatic than in WHM. A graphical illustration of the effect of contact time on *EI* values for WHM and AHM is presented in Figure 3.6. It is also important to recall that the $K_{OM,\,C\,t}$ values had indicated that WHM consistently sorbed more DCP than AHM. Single point sorption data indicated greater fractional uptake of solute at smaller solute concentrations, and *EI* values showed higher reverse mass transfer from sorbent exposed to lower solute concentrations. At higher solute concentration the greater concentration gradient is likely to have driven the penetration of DCP molecules into WHM and AHM inter and intra particle space, while at lower solute concentration DCP molecules were associated with the more easily accessible and extractable near surface domains.

Results from this study indicate that the aliphatic-rich humin was able to interact strongly with DCP, consequently reducing its water extractability especially at higher concentrations and longer contact times. The *EI* values are summarized in Table 3.9.

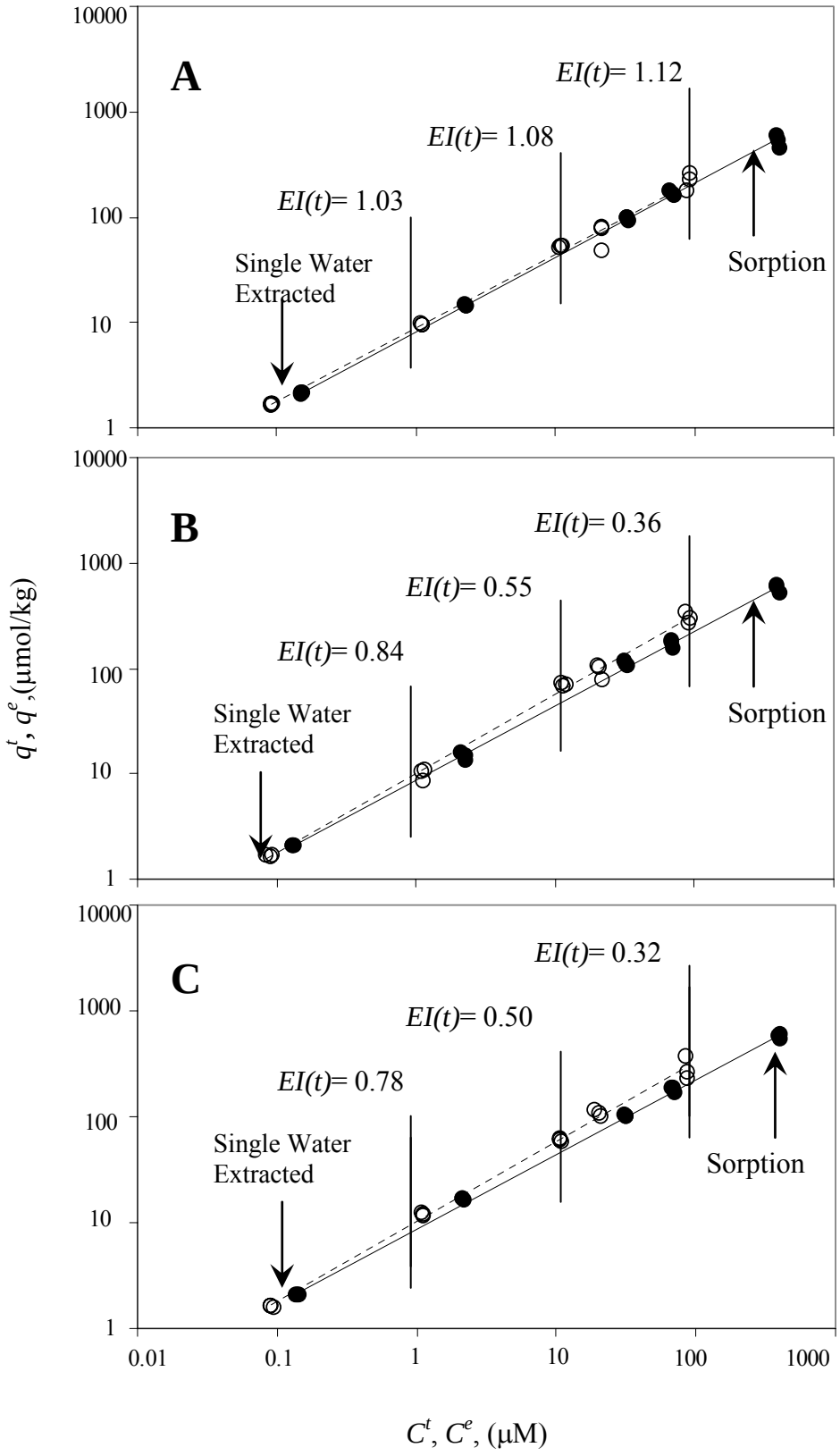

Figure 3.5. Extractability of DCP from AHM after sorption for A) 1, B) 7 and C) 28 days.

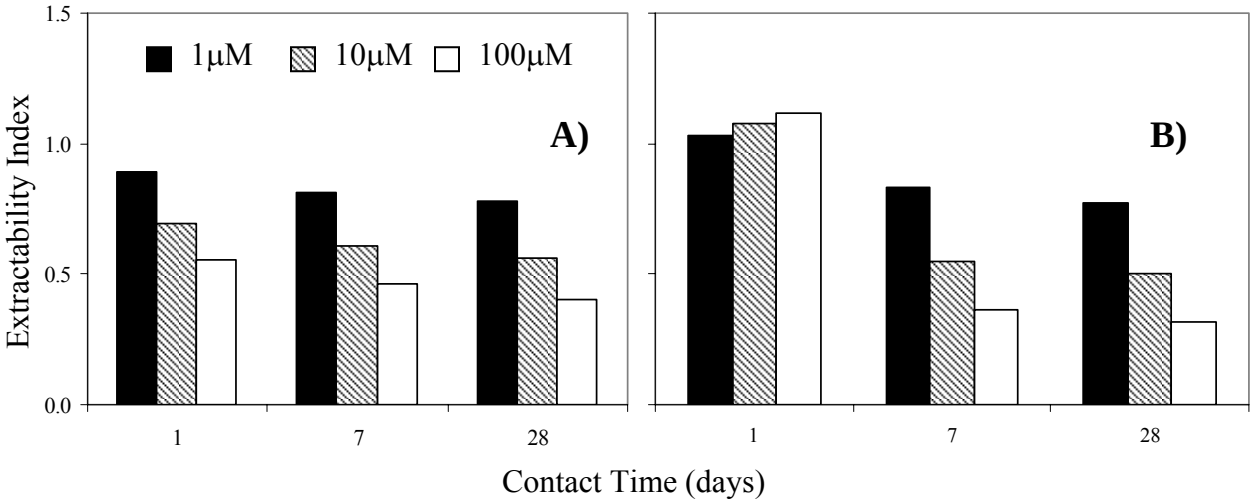

Figure 3.6. DCP extractability index as a function of sorption contact time. *EI* is shown for A) WHM and B) AHM.

Table 3.9. Extractability index values for water extractable DCP from AHM.

Contact Time (days)	Agricultural Humin-Mineral Soil		
	EI_1	EI_{10}	EI_{100}
1	1.034	1.075	1.119
7	0.836	0.549	0.362
28	0.776	0.501	0.316

3.5.3.3 Solvent Extractable and Stabilized DCP

A variety of solvents have been use by researchers to estimate the bioavilable fraction of sorbed organic solutes (Hatzinger and Alexander, 1995; Kelsey et al., 1997; Bhandari and Xu, 2001; Lesan and Bhandari, 2003). This study utilized sequential methanol extraction to estimate the removable and stabilized DCP from WHM and AHM soils. While section 3.5.3.2 discussed the relative ease of desorption of sorbed DCP using a 24-hr water extraction, in this section the extent of solute stabilization was evaluated by subjecting the water extracted sorbent to sequential extraction with methanol. The term "solvent extractable" represents the sum of DCP removed by water and consecutive methanol extractions, and can be considered a surrogate for the bioavailable fraction of the sorbed contaminant.

3.5.3.3.1 Woodland Humin-Mineral Soil

The effect of contact time on the mass of solvent extractable and stabilized DCP is shown in Figure 3.7. As discussed previously, for most of the DCP concentrations, sorption increased with contact time. Higher aqueous concentrations resulted in greater overall sorption of DCP, a significant fraction of which was removed during methanol extraction. Approximately 19 and 950 μmole/kg of DCP was observed to sorb to WHM within 28 days at initial aqueous concentrations of 5 and 500 μM, respectively. The amounts of stabilized DCP were 1.0 and 27 μmole/kg for these initial aqueous concentrations, respectively. Formation of bound residue was positively correlated to contact time at all initial DCP aqueous concentrations.

For the same 28-day period and the parent woodland soil, Palomo and Bhandari (2006) had previously reported that stabilized DCP amounted to 1.0 and 40 μmole DCP /kg at 5 and 500 μM, respectively. These researchers also reported that the DCP associated with the humin-mineral fraction obtained after extraction of HA and FA was approximately 0.26 and 11 μmole/kg at 5 and 500 μM, respectively. Thus, it appears that removing HA and FA and exposing the humin-mineral domain to DCP resulted in greater stabilization of the solute. Binding of DCP by the WHM was 2.5 to 3.8 times higher when the humin-mineral domain was directly exposed to DCP in the solution. The aliphatic and low polarity of the humin appeared to have played an important role during direct interaction with DCP molecules in solution.

3.5.3.3.2 Agricultural Humin-Mineral Soil

As described earlier, contact time had no impact on the total amount of DCP sorbed to the AHM (Figure 3.10). Most of the DCP sorbed to AHM was recovered by solvent extraction. However, the amount of stabilized DCP increased significantly with contact time at all initial concentrations. After 28 days of contact, the bound mass, at 5, 50,100 and 500 μM, was equal to 0.75, 4, 6 and 18 μmole/kg, respectively. These numbers were slightly lower than those for WHM (1.0, 5, 9 and 27 μmole/kg, respectively). This can be attributed to the higher organic matter content of WHM which resulted in greater sorption and retention of DCP.

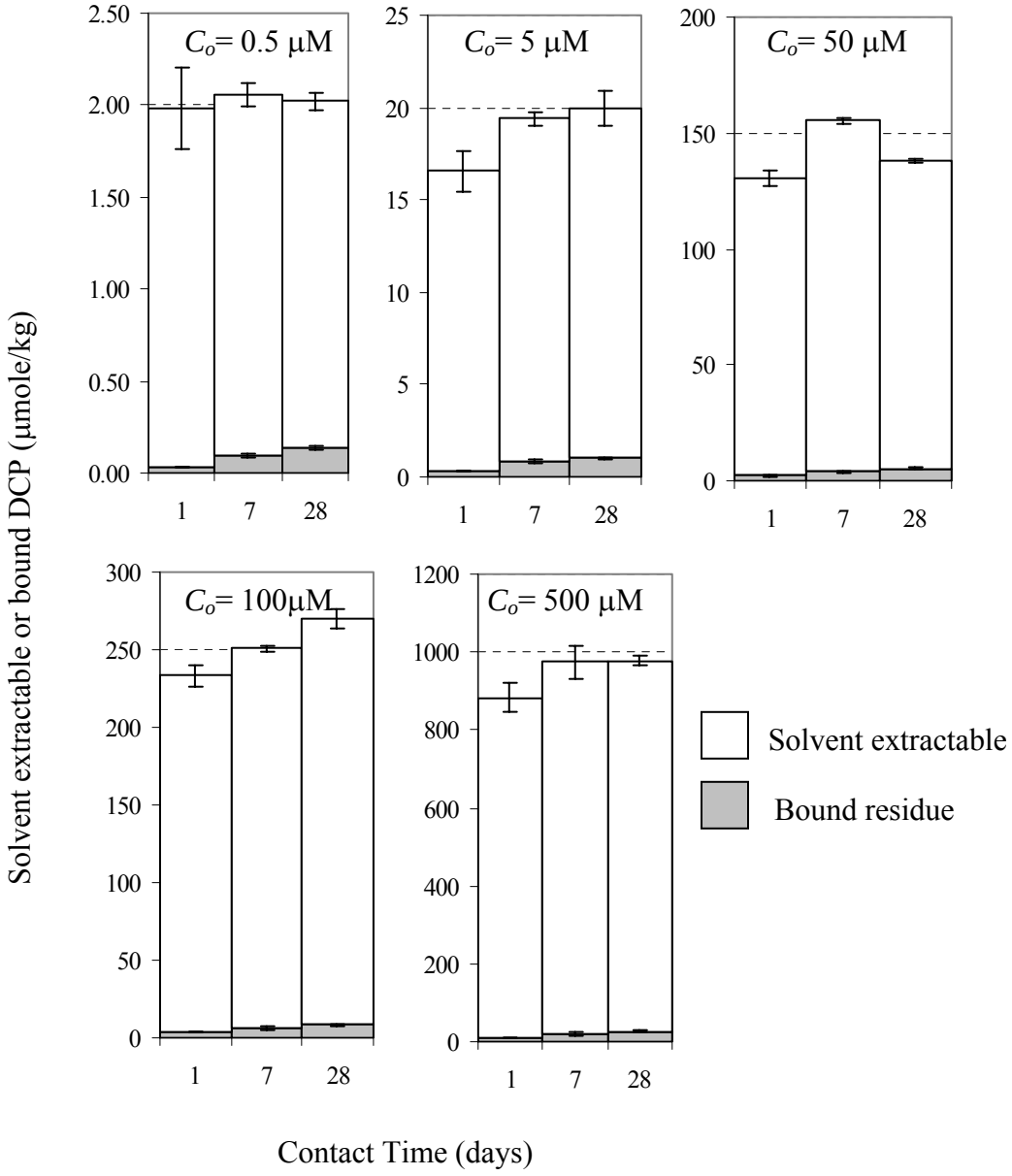

Figure 3.7 Solvent extractable and bound residue as a function of contact time. At different C_o's for WHM soil.

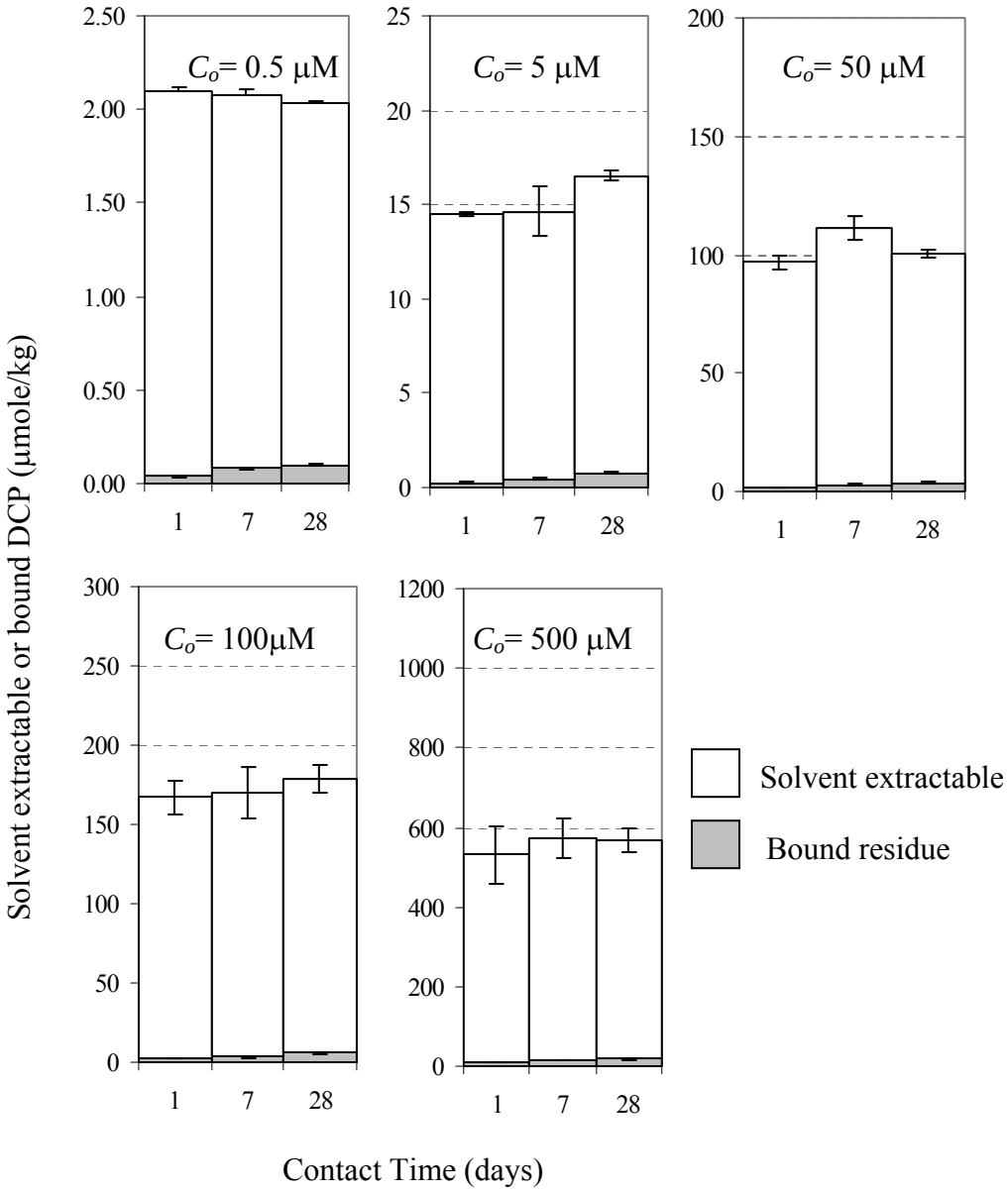

Figure 3.8. Solvent extractable and bound residue as a function of contact time. At different C_o's for AHM soil.

3.5.4 Solute Sorption inEnzyme-Amended Systems ContainingHumin-Mineral Geomaterials

The association of solute generated in HRP-amended systems was also evaluated using the phase distribution model described in section 3.5.3.1. In HRP-amended systems the term

solute refers to the sum of unreacted DCP + the generated dichlorophenol polymerization products (DPPs). Palomo and Bhandari (2005) previously defined DPPs as self-coupled, crossed coupled and sorbed unreacted DCP. In this study, the definition of DPP was expanded to the soluble polymers remaining in solution after the enzymatic polymerization and sorption processes were completed, including self-coupled, and crossed coupled polymers but excluding the unreacted DCP. However, phase distribution data is used to represent the sorption behavior of total solute present in the system rather than DPP sorption behavior alone.

3.5.4.1 Solute Phase Distribution Relationships

Phase distributions relationships have been used to study the interactions of phenolic polymerization products with surface soils (Bhandari and Xu, 2001; Palomo and Bhandari, 2005). Though these authors have utilized the term phenolic polymerization product, the sorption data they have presented represents total solute present in the systems (unreacted phenol+ phenolic polymers) rather than phenolic polymers sorption alone. Bhandari and Xu (2001) produced phase distribution relationships (PDRs) for HRP-mediated polymerization products of phenol, DCP, o-cresol and naphthol in contact with two surface soils that were different from those used in the current study. These researchers reported that phase distribution of the polymerization products was complete within seven days. The Freundlich linearity parameter n(t) was 0.89 while values of K_F(t) values ranged from 4.5 to 5.2 (μmole/kg)*(μM)$^{-n}$. Palomo and Bhandari (2005) used the parent woodland and agricultural soils of WHM and AHM to generate PDRs for DPP and reported values for n(t) near 1.0, while K_F(t) values were about 12.0 (μmole/kg)*(μM)$^{-n}$. These authors concluded that the enzymatically induced polymerization process and contact time had significant impact on the fate of the phenolic chemicals present in soils.

The remaining sections of this chapter discuss the association of total solute in HRP-amended systems in presence of humin-mineral soil. In the following discussion, the term sorption (combination of all processes responsible for removal of solute from solution) includes adsorption, absorption, precipitation of self-coupled oligomers, and cross-coupling of solute or oligomers with SOM.

3.5.4.1.1 Woodland Humin-Mineral Soil

Phase distribution of solute (DCP + DPP) in the presence of WHM is illustrated in Figure

3.9. Contact time appeared to have no impact on the phase distribution and the sorption capacity parameter $K_F(t)$ was constant at all contact times with a value of 32 (μmole/kg)*(μM)$^{-n}$. A similar behavior was observed by Palomo and Bhandari (2005) who noted that apparent PDR equilibrium was achieved within one day. In the current study, the linearity coefficient $n(t)$ also remained constant with an approximate value of 0.95. The Freundlich parameters and their standard deviations are summarized in Table 3.10.

Palomo and Bhandari (2005) had previously reported a constant $n(t) \approx 1.0$ and a constant $K_F(t) \approx 14.0$ (μmole/kg)*(μM)$^{-n}$ for the parent woodland soil. Solute association with WHM exhibited sliglhtly less linear sorption behavior than that observed for the parent soil. Nonlinear solute phase distribution suggests that mechanisms other than simple precipitation were responsible for the removal of total solute from solution. The nonlinearity can be attributed to the characteristics of humin that likely possessed a more condensed structure due to its association with the inorganic soil fraction (Kang and Xing, 2005). Nonlinearity of chlorophenols or polar compounds has been attributed to specific interactions with reactive sites of the humin fraction (Chiou, 2000).

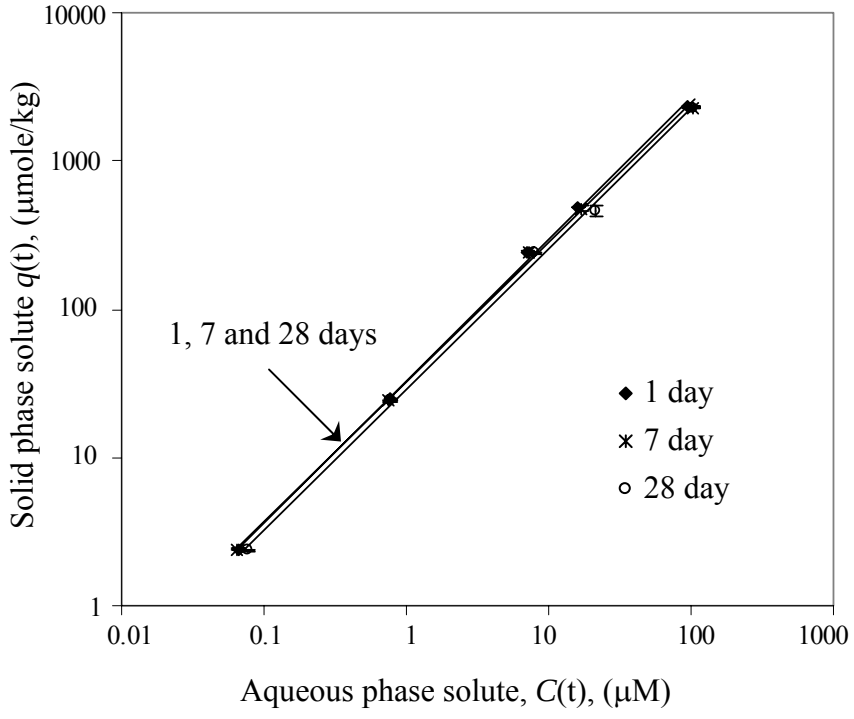

Figure 3.9. **Solute phase distribution (DCP+DPP) in HRP-amended systems at 1, 7 and 28 days, WHM**

Table 3.10. Freundlich parameters for solute phase distribution into WHM soil.

Contact Time (days)	Woodland Humin-Mineral Soil			
	n(t)	$K_F(t)$ [1]	$K_F(t)_{SOM}$ [2]	R^2
1	0.959 *(0.018)* [3]	32.9 *(2.42)*	1828 *(135)*	0.999
7	0.939 *(0.021)*	32.7 *(2.71)*	1816 *(151)*	0.999
28	0.946 *(0.037)*	29.0 *(4.40)*	1607 *(244)*	0.995

[1] Units for $K_F(t)$ are $\frac{\mu mol}{kg}(\mu M)^{-0.95}$ [2] units for $K_F(t)_{SOM}$ are $\frac{\mu mol}{kg_{SOM}}(\mu M)^{-0.95}$

[3] 95% confidence intervals

3.5.4.1.2 Agricultural Humin-Mineral Geomaterial

Contact time appeared to have no impact on the total solute phase distribution between water and AHM, suggesting that one day was sufficient to reach mass transfer equilibrium (Figure 3.10). Palomo and Bhandari (2005) had previously reported rapid phase distribution of total solute between water and the parent agricultural surface soil.

Freundlich parameters were constant with average values of 0.95 and 22.39 for $n(t)$ and $K_F(t)$, respectively, with K_F (t) having units of (μmole/kg)*(μM)$^{-0.95}$. The $n(t)$ values were higher for solute (unreacted DCP+ DPP) in HRP-amended systems than for DCP alone, indicating a more linear process and the possibility that precipitation might be an important mechanism in the removal of solute from the aqueous phase. However, it is also likely that other mechanisms like hydrophobic and electrostatic interactions were also taking place.

Since the linearity coefficient $n(t)$ averaged around of 0.95 for both AHM and WHM, a comparison between the sorption capacity of both sorbents can be done. The $K_F(t)$ value for WHM was 32, while that for AHM was 22, (μmole/kg)*(μM)$^{-0.95}$, indicating that while the alkali extracted woodland soil contained 39 % more SOM than AHM, its sorption capacity was only 31% greater than that of AHM. Table 3.11 summarizes the Freundlich parameters and the $K_F(t)_{SOM}$ values obtained for the total solute mass transfer on to AHM. Average $K_F(t)_{SOM}$ values for AHM varied from 2115 and 2331 (μmole/kg)*(μM)$^{-0.95}$. These values are summarized in Table 3.12. The values of $K_F(t)_{SOM}$ obtained for both soils, confirm that although AHM had a

lower SOM content, it exhibited a higher sorption capacity. This higher sorption capacity of AHM may be attributed to the more aliphatic nature and low polarity of the AHM material.

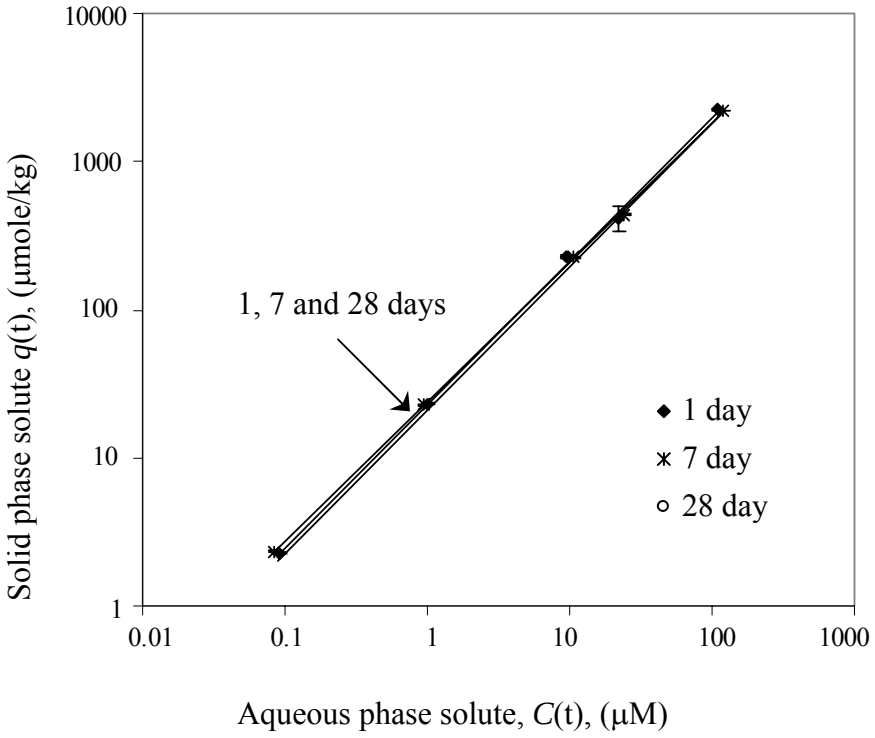

Figure 3.10. Solute distribution (DCP+DPP) in HRP-amended systems at 1, 7 and 28 days, AHM.

Table 3.11. Freundlich parameters for solute phase distribution into AHM soil.

Contact Time (days)	Agricultural Humin-Mineral Soil			
	n(t)	$K_F(t)$ [1]	$K_F(t)_{SOM}$ [2]	R^2
1	0.969 *(0.026)* [3]	23.3 *(2.47)*	2331 *(247)*	0.998
7	0.941 *(0.009)*	23.7 *(0.98)*	2374 *(98.0)*	0.999
28	0.963 *(0.016)*	21.2 *(1.48)*	2115 *(146)*	0.999

[1] Units for $K_F(t)$ are $\frac{\mu mol}{kg}(\mu M)^{-0.95}$ [2] units for $K_F(t)_{SOM}$ are $\frac{\mu mol}{kg_{SOM}}(\mu M)^{-0.95}$

[3] 95% confidence intervals

Table 3.12. SOM normalized sorption capacity parameters.

Contact Time (days)	WHM $K_F(t)_{SOM}^2$	AHM $K_F(t)_{SOM}^2$
1	1828	2331
7	1816	2374
28	1607	2115

Units for $K_F(t)_{SOM}$ are $\frac{\mu mol}{kg_{SOM}}(\mu M)^{-0.95}$

Single point OM-normalized distribution coefficients, calculated at 7 days of contact time, at four different residual aqueous concentrations, are illustrated in Table 3.13. In support to the results discussed previously, $K_{OM, Ct}$ values also showed that the sorption capacity of AHM was higher than of WHM at all the residual aqueous concentrations. These results suggested that total solute residues had higher affinity for the humin-type organic matter present in AHM. The greater affinity could be attributed to the slightly higher surface area, higher aliphaticity and polarity of AHM compared to WHM. Aliphatic SOM has been shown to produce favorable interaction with nonpolar organic compounds (Chefetz et al., 2000; Chiou et al., 2000).

Table 3.13 Single point OM-normalized distribution coefficients, $K_{OM, Ct}$, calculated at 7 days of contact time.

Residual Aqueous Concentration (μM)	$K_{OM, Ct}$	
	WHM	**AHM**
10	1578	2072
100	1371	1808
200	1314	1736
300	1282	1695

Units for $K_{OM, Ct}$ are $\frac{L}{kg_{OM}}$

3.5.4.2 Reverse Mass Transfer of Solute Sorbed and/or Precipitated

The relative ease of solute (sorbed and precipitated DPP+ sorbed unreacted DCP) reverse mass transfer was determined utilizing the same procedure described in section 3.5.3.2. The notation used to conduct the discussion of results for the EI_x, will be similar to that used previously, where the subscript could indicate total solute residual aqueous concentration (x= 1, 10 or 100 μM), time (x= 1, 7, or 28 days), or treatment (x= DCP or HRP).Where the HRP subscript will be used to described the total solute extractability in presence of HRP-amended systems. These subscript definitions are intended to facilitate the comparison of results obtained from using various conditions.

3.5.4.2.1 Woodland Humin-Mineral Geomaterial

HRP-mediated polymerization of DCP was successful in enhancing the removal of contaminant from solution in the form of DPP and some unreacted DCP (Figure 3.11). Total solute aqueous extractability decreased with increasing aqueous concentration of the solute and contact time. In the presence of HRP, *EI* values decreased in the order $EI_1 > EI_{10} > EI_{100}$ and $EI_1 > EI_7 > EI_{28}$. *EI* values obtained at all concentrations and contact times are summarized in Table 3.14.

At all concentrations and contact times, the values of EI_{HRP} were significantly smaller compared to the corresponding EI_{DCP} values. At 1 day the EI_{HRP} value decreased from EI_1= 0.350 to EI_{100}=0.139, while E_{DCP} decreased from EI_1= 0.894 to EI_{100}=0.555. In the same fashion, at 28 days, EI_{HRP} decreased from EI_1= 0.244 to EI_{100}= -0.145, while EI_{DCP} decreased from EI_1= 0.781 to EI_{100}=0.403.

Palomo and Bhandari, (2005) previously reported EI_{HRP} values for the parent woodland soil that decreased from EI_1=0.125 to EI_{28}= -0.033 at 10 μM, and from EI_1= -0.0414 to EI_{28}= -0.223 at 100 μM. In the current study, the EI_1 and EI_{28} at 10 μM were 0.236 and 0.027, respectively. While at 100 μM, EI_1 and EI_{28} values were 0.139 and -0.145, respectively. Extractability indexes for WHM were higher than for the parent soil, indicating that total solute reverse mass transfer was faster from humin-mineral geomaterial than from the parent woodland soil (6.4% SOM). Thus, the amoun or type of SOM content can impact the reverse mass transfer of solute during a 24-hr water extraction.

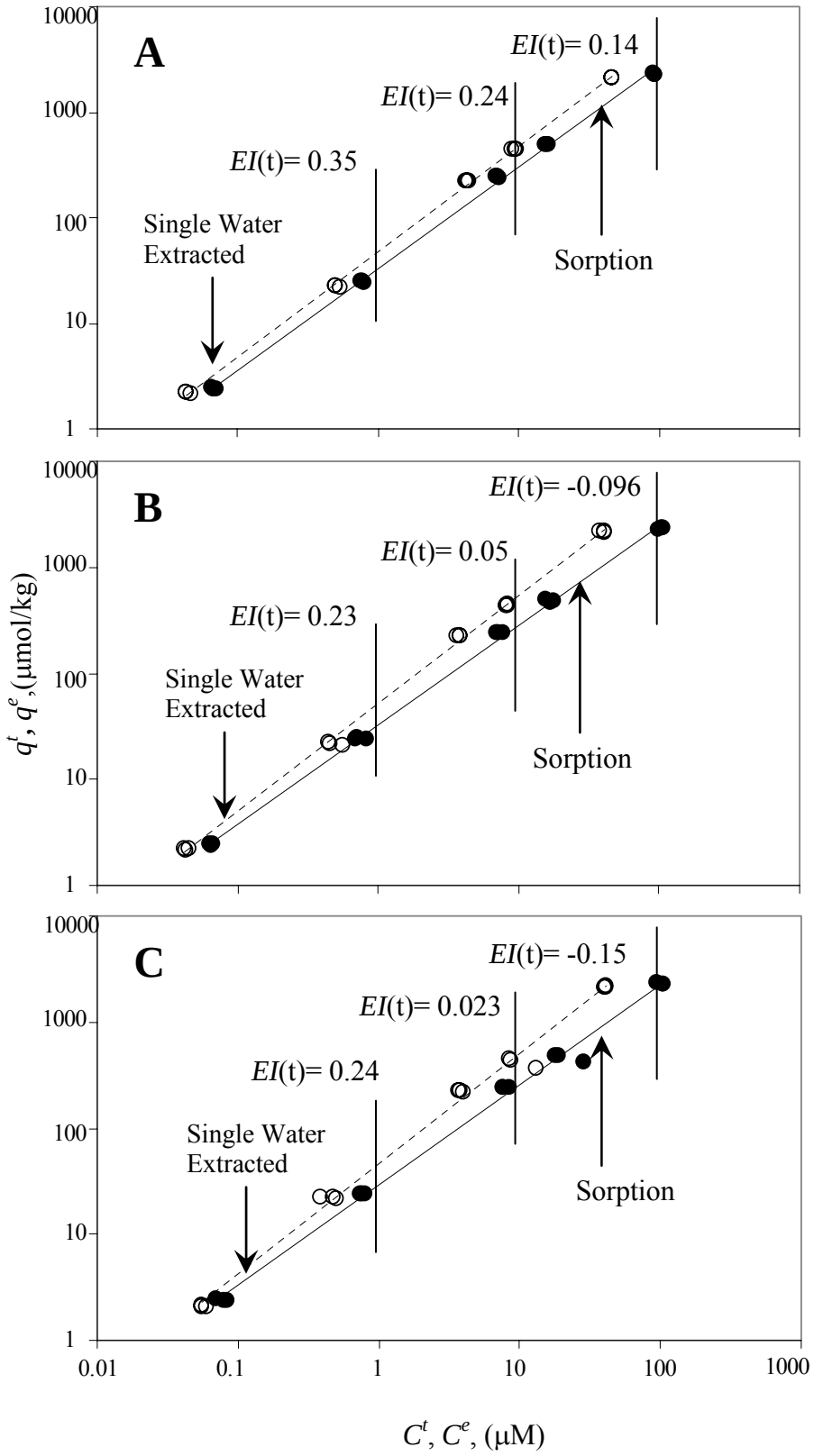

Figure 3.11. Solute (DCP +DDP) extractability at A) 1, B) 7 and C) 28 days, WHM

Table 3.14. Extractability index values for water extractable solute from WHM.

Contact Time (days)	Woodland Humin-Mineral Soil		
	EI_1	EI_{10}	EI_{100}
1	0.350	0.236	0.139
7	0.234	0.052	-0.096
28	0.244	0.027	-0.145

3.5.4.2.2 Agricultural Humin-Mineral Geomaterial

Phase distribution and extractability of solute in contact with AHM is illustrated in Figure 3.12. Both contact time and residual aqueous concentration were seen to impact the amount of total solute extracted from AHM soil. As time and concentration increased, the amount of total solute extracted from soil was reduced. This is clearly indicated by the *EI* values. The *EI* values at 1 μM of residual aqueous concentration decreased from 0.142 to 0.014, for 1 and 28 days, respectively; while at 100 μM, the values were -0.112 and -0.355 for 1 and 28 days, respectively. Negative values of *EI* indicated very high resistance to solute reverse mass transfer.

At 28 days EI_{DCP} decreased from 0.776 to 0.316 as concentration increased, while EI_{HRP} decreased from 0.014 to -0.355. EI_{HRP} values decreased more precipitously than EI_{DCP}, indicating that HRP-mediated polymerization enhanced DCP retention on soil through conversion to DPP and unreacted DCP sorption. It is important to note, however, that *EI* only represents the loosely associated total solute (DPP + unreacted DCP) that can be extracted with water in 24 hrs. The *EI* values are summarized in Table 3.15.

Table 3.15. Extractability index values for water extractable solute from AHM.

Contact Time (days)	Agricultural Humin-Mineral Soil		
	EI_1	EI_{10}	EI_{100}
1	0.142	0.006	-0.112
7	0.073	-0.115	-0.273
28	0.014	-0.186	-0.355

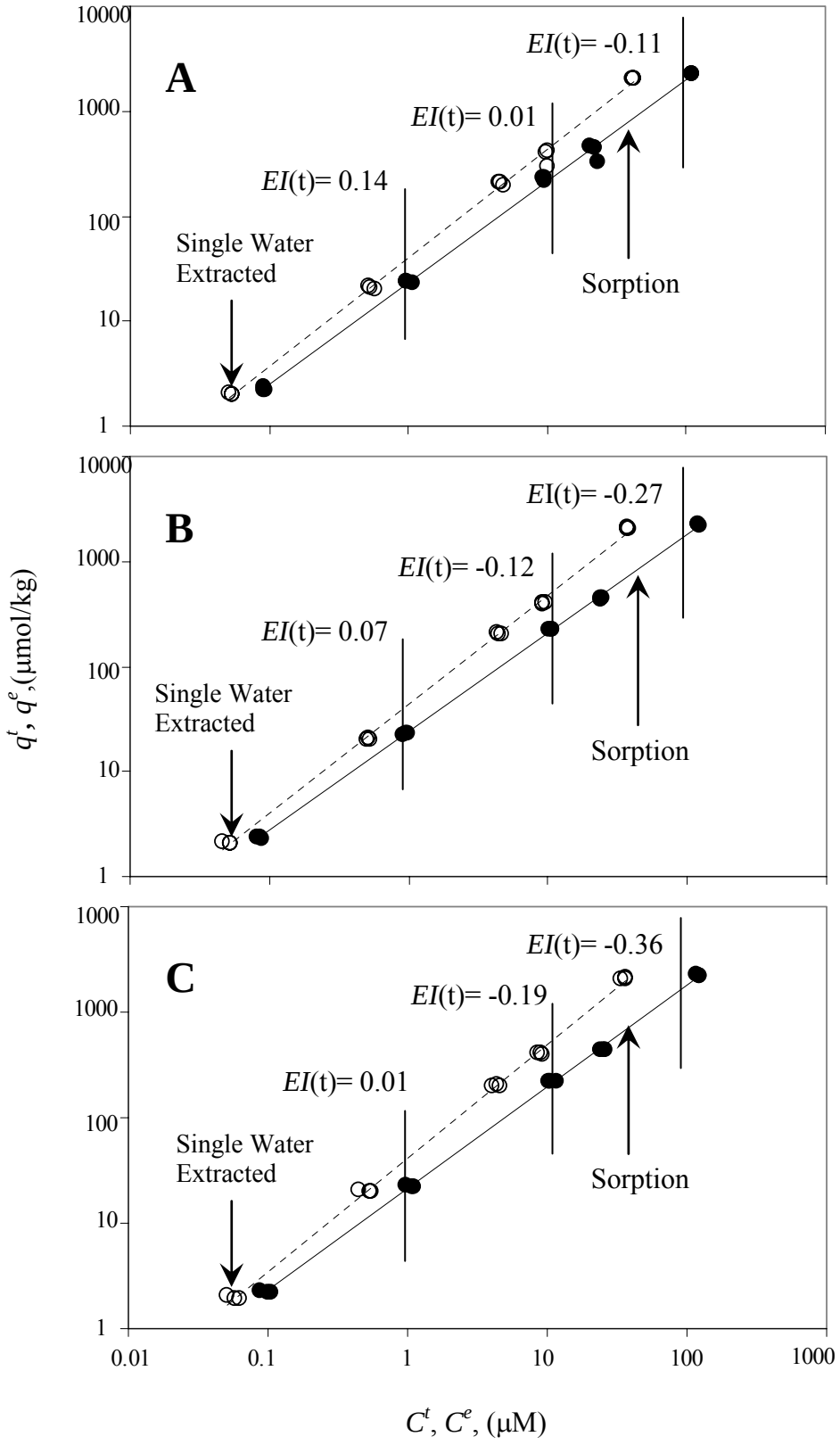

Figure 3.12. Solute (DCP +DDP) extractability at A) 1, B) 7 and C) 28 days, WHM

The greater the distance between the sorption and desorption phase distribution curves, the less mass of chemical is extracted from soil. This can be illustrated by comparing Figure 3.5 and Figure 3.12 at all contact-times. Palomo and Bhandari (2005) previously reported that the *EI* values for total solute associated with the parent agricultural soil decreased with contact time at all concentrations. Thus, at 100 μM the *EI* reduced from 0.171 to -0.122 at 1 and 28 days, respectively. While for the AHM soil, the values corresponding to the same concentration and time were -0.112 and -0.355. Thus, resistance to reverse mass transfer was significantly higher when only humin-type of organic matter was used, suggesting that perhaps the chemical nature of SOM, not just its quantity, also influences the interactions with organic compounds. The greater affinity of solute (DPP+ unreacted DCP) for humin in AHM may be attributed to the slightly more aliphatic and less polar nature of the humin.

The temporal changes in *EI* values for AHM and WHM soils are illustrated in Figure 3.13. AHM showed consistently higher resistance total solute extraction, than WHM at all concentrations and contact times. As mentioned previously, greater affinity of solute for AHM was attributed to the slightly more aliphatic and less polar nature of AHM than that of WHM. Reduction of solute extractability was observed as a consequence of contact time. Prolonged contact of humin with the generated DPP and unreacted DCP may have allowed the development of more complex interactions with humin matter.

Residual aqueous concentration also played an important role in hindering the removal of solute from soil. At the three studied contact times, for both, AHM and WHM, *EI* values were larger at low concentrations and decreased with increasing concentration, i.e., the solute extractability decreased with an increased solute concentration. Similar results were reported by Palomo and Bhandari (2005) and attributed to the formation of higher molecular-weight DPP at high solute concentrations. Macromolecules that partition less readily into the aqueous phase, due to their reduced solubility and more hydrophobic nature made reverse mass transfer more difficult, thus, reducing extractabilty.

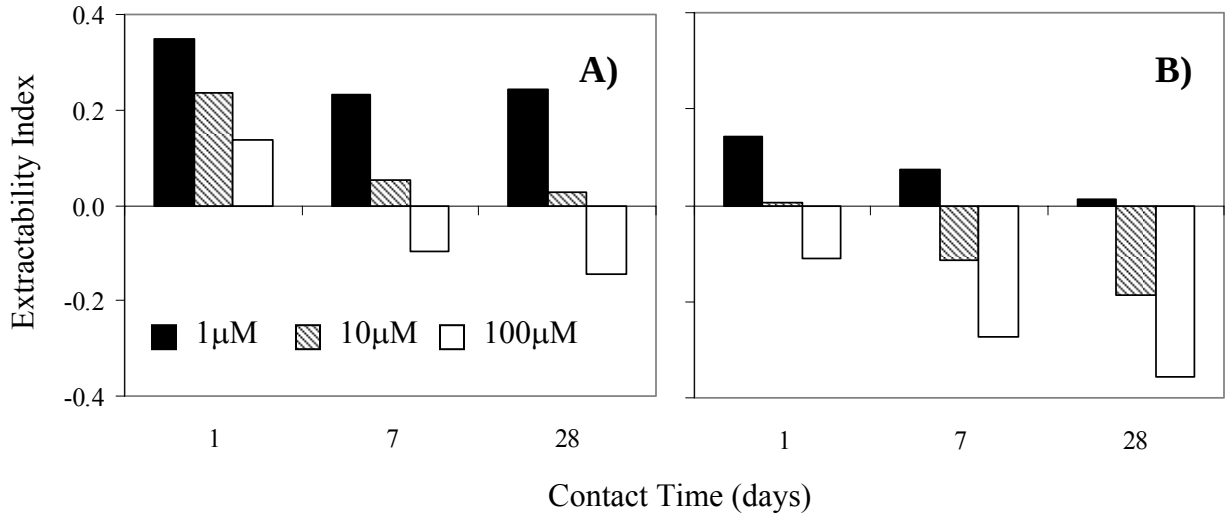

Figure 3.13. Solute extractability index as a function of sorption contact time. *EI* is shown for A) WHM and B) AHM.

3.5.4.3 Time-Dependent Mass-Transfer and Stabilization of Solute in Enzyme-Amended Systems

Time-dependent mass transfer and stabilization of solute (DPP+ unreacted DCP) from aqueous phase to WHM and AHM sorbents was studied and compared with time-dependent sorption of DCP to the same sorbents. The term solvent extractable was used to describe the sum of water extractable solute + methanol extractable solute. The amount of solute remaining associated with the geomaterial after sequential methanol extraction was described as stabilized.

3.5.4.3.1 Woodland Humin-Mineral Geomaterial

The distribution of solvent extractable and stabilized solute in WHM is illustrated in Figure 3.14. Overall, mass transfer of solute from the aqueous phase to WHM (bound + extractable solute) was not affected by contact time at all initial solute concentrations. At C_o = 0.5 μM, extractability and bound residue formation remained constant throughout the 28 days of study. However, for the remaining C_o s, total extractable solute decreased as a function of contact time, while the stabilized residue increased from 1 to 28 days.

After 28 day contact period, WHM sorbed 24 and 2,292 μmole/kg of solute expressed as equivalents of DCP, at 5 and 500 μM initial aqueous DCP concentrations, respectively. Whereas,

in the absence of HRP, WHM sorbed only 18, and 1,000 μmole/kg of DCP at the same initial

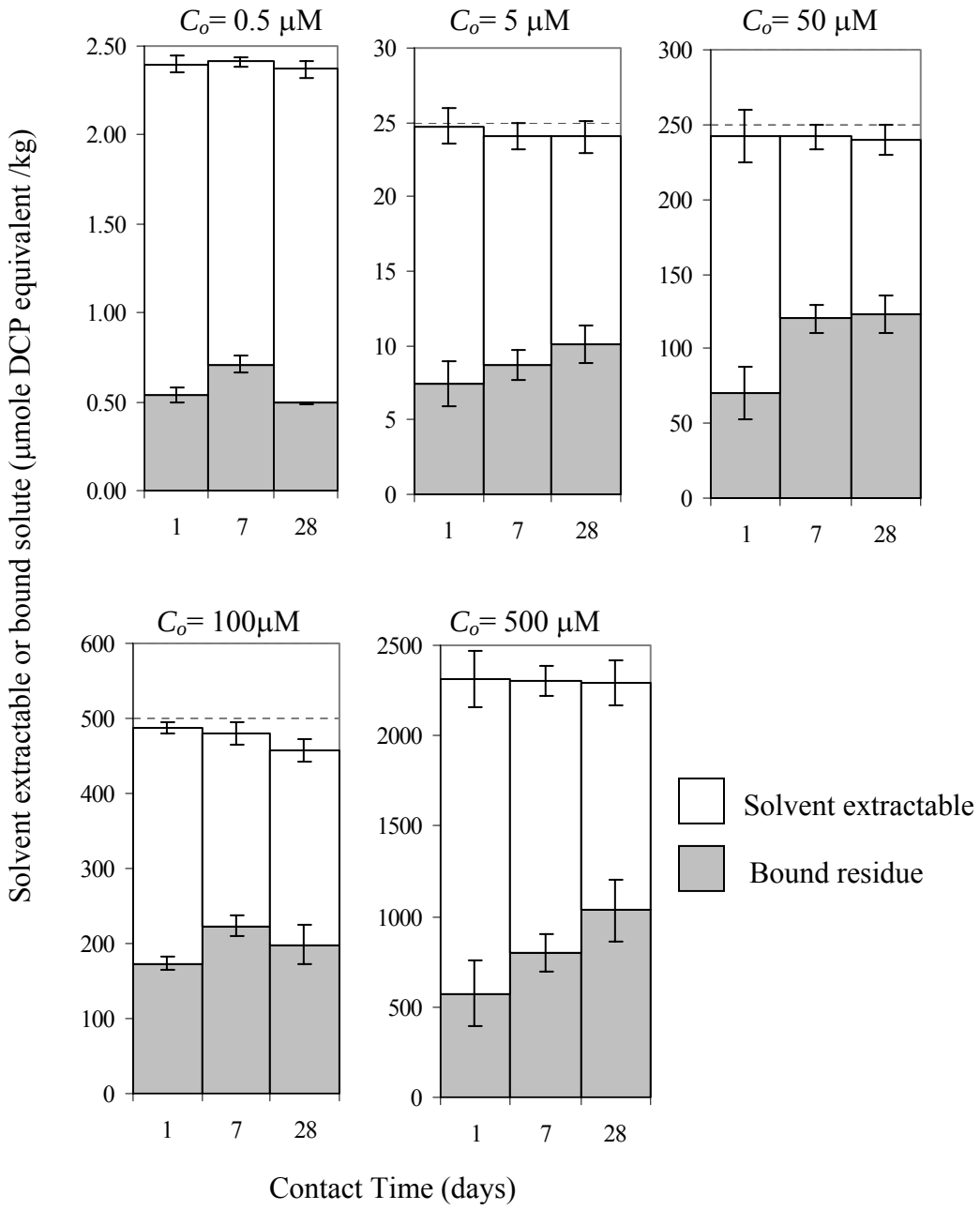

Figure 3.14. Solvent extractable and bound residue as a function of contact time. At different C_o's for WHM soil.

aqueous concentrations. The mass of solute sorbed was, therefore, significantly higher than that

of DCP for the same C_os. Furthermore, as the initial aqueous solute concentration of DCP increased, higher mass of solute was associated with the sorbent. Thus, at 500 μM, WHM sorbed 2.29 times more solute when enzyme was added. Palomo and Bhandari, (2005) previously reported that approximately 20 and 2,282μmole/kg of solute (DPP+ unreacted DCP) associated with the parent woodland soil at 5 and 500 μM, respectively. Their values were nearly identical to those obtained in the current study, indicating that the modified soil sorbed an equal amount of solute as the parent soil, which contained 3.5 times more organic matter. The results suggest that total solute phase distribution did not depend on the SOM type or content.

The reactive moieties of humic substances have been implicated in reducing the oxidative polymerization of phenols as a result of solute adsorption (Park et al., 2000). Sarkar and Bollag (1987) proposed that humic acids could complex with the metal in the enzyme active site, thus inhibiting the activity of the enzyme. Free radicals scavenging by humic acids has been reported (Vaughan and Ord, 1982) and proposed as a possible means of reducing the efficiency of the enzymatic-cross polymerization reaction (Park et al., 2000). It is also likely that removal of HA and FA reduced inhibition of enzyme activity by reduced adsorption to SOM thus, increasing the efficiency of DCP polymerization and solute retention by the sorbent.

The mass of total extractable solute decreased as contact time progressed at all concentrations. From 1 to 28 days, extractable solute decreased from 17 to 14 μmole/kg at 5 μM and from 1734 to 1259 μmole/kg at 500 μM. Conversely, bound solute increased with contact time at all the concentrations, except at the lowest one, where it remained constant. At the end of the experiment, for C_o = 5 and 500 μM, the bound solute residues were 10 and 1,033 μmole/kg respectively.

Although the sorbed mass of solute per kilogram of WHM appeared to be comparable in the presence or absence of HRP, assessment of the stabilized residues definitely demonstrated the advantage of enzymatic treatment. Recall that for DCP alone stabilized residues at 28 days were 1.0 and 27 μmole/kg at 5 and 500 μM, respectively, while, enzyme addition increased stabilized residue formation to 10 μmole/kg and 1033μmole/kg at 5 and 500 μM; thus, producing 10 to 38.2 times greater contaminant stabilization. The huge contrast suggests that polymerization products not only drop out of solution and precipitate, but have a significantly higher affinity for WHM than the parent DCP. Enzymatic polymerization is, therefore, able to attenuate transport of DCP by allowing the polymerization products to strongly associate with

the geosorbent.

Palomo and Bhandari (2006) had previously reported that the amounts of solute stabilized on the parent woodland soil were 3.1 and 1125.6 μmole/kg, at 5 and 500 μM, respectively, out of which the solute separated in the humin fraction accounted for 0.79 and 389 μmole/kg at 5 and 500 μM, respectively. The values reported for the separated humin fraction were smaller than those obtained in this study for the WHM soil, suggesting that removal of HA and FA and direct exposure of the humin surface to products of the enzymatic polymerization reactions enhanced contaminant stabilization in WHM soil.

3.5.4.3.2 Agricultural Humin-Mineral Geomaterial

The mass of solute sorbed to AHM soil did not change with contact time at all the initial solute concentrations studied (Figure 3.15). Extractable solute was found to decrease with contact time and the values were statistically different at the three studied contact times. For most C_o values, stabilized solute residue formation increased from 1 to 7 days and remained constant beyond that time.

The amounts of solute stabilized in AHM at C_o= 5 μM after 1 and 28 days of contact were 7 and 9 μmole/kg, respectively, while at C_o= 500 μM, the stabilized masses were 512 and 636 μmole/kg, respectively. Comparing solute stabilization resulting from a 28-day contact time with the stabilization of DCP within the same duration (at 5 and 500 μM ≈ 0.76 and 18 μmole/kg, respectively) illustrates that contaminant stabilization was 12 to 35 times greater in presence of HRP.

The mass of solute stabilized by AHM soil was smaller than that reported for WHM soil. At a 28-day contact time and C_o values of 5, 50, 100 and 500 μM, solute stabilized with WHM amounted to 10, 123, 199 and 1033 μmole/kg, respectively, while that stabilized with AHM was 9, 96, 185 and 636 μmole/kg. On the other hand, the mass of stabilized solute was larger for AHM at C_o= 0.5 μM. At C_o= 500 μM approximately 38.4% more contaminant stabilization was observed in WHM than in AHM. As mentioned earlier, WHM contains 38.8% more SOM than AHM. Thus, solute association with the sorbent and the SOM content of the materials may appear to be strongly correlated. However, since precipitation of the polymers occurs in the system this study does not provide direct evidence of the role of SOM in polymerization and precipitation of the products.

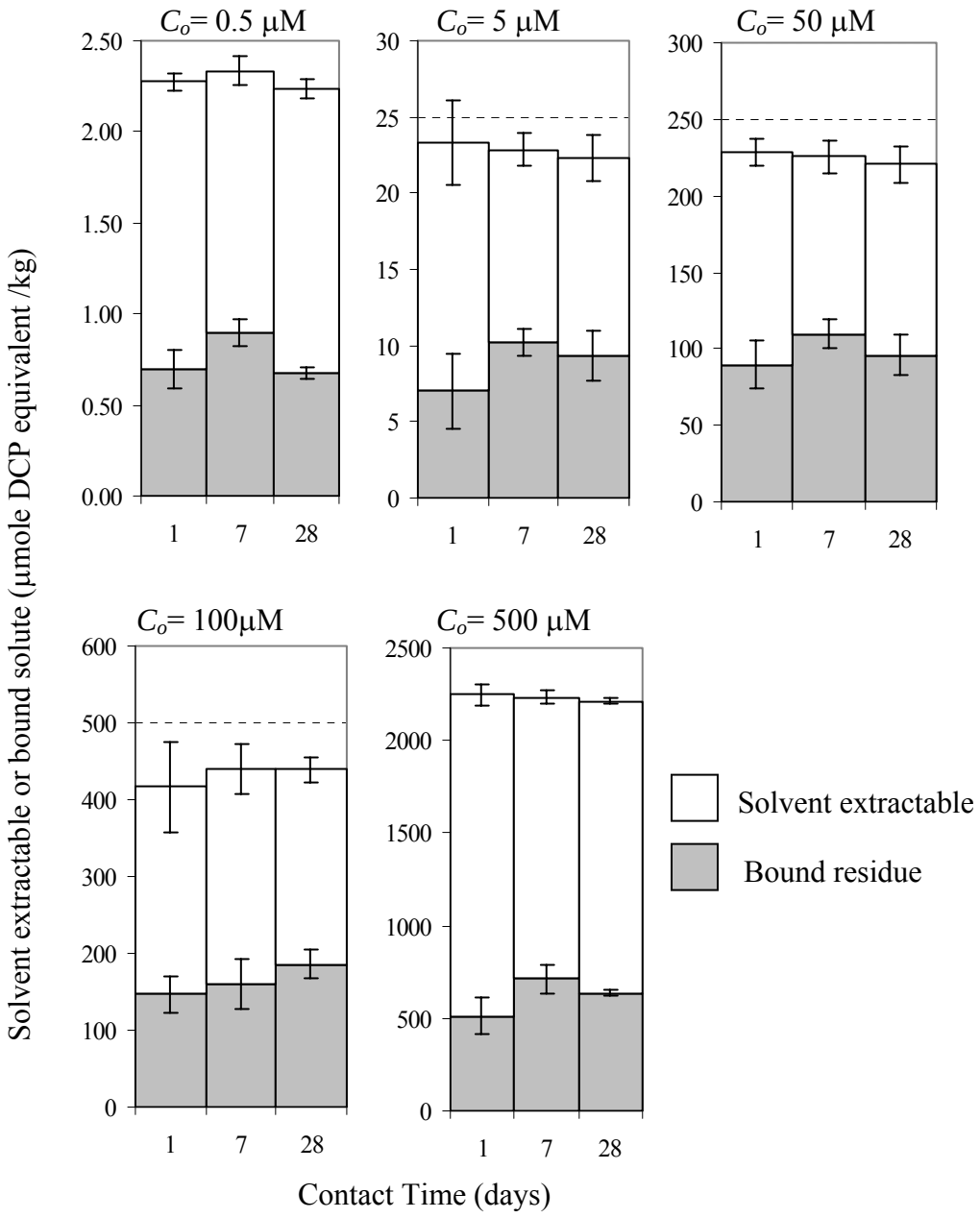

Figure 3.15. Solvent extractable and bound residue as a function of contact time. At different C_o's for AHM soil.

When the mass of contaminant stabilized, at 28 days and 500 μM, is normalized to the

SOM content, the data for AHM and WHM appeared to be similar: 57,810 and 57,389 μmole/kg $_{SOM}$, respectively. Thus, in terms of SOM content, AHM showed higher bound residue formation. When results are compared to those obtained for the parent agricultural soil at 28 days and 500 μM (Palomo and Bhandari, 2006) it can be noted that stabilized residue formation was significantly higher in AHM (1033 μmole/kg). Bound solute concentration in the parent bulk soil subjected to similar treatment was 795 μmole/kg out of which, the fractionated humin contained 182 μmole DPP/kg. Thus, alkali extraction appears to have resulted in the removal of HA and FA which exposed a humin-mineral domain that exhibited greater affinity for the solute after the HRP polymerization reaction.

3.5.5 Characterization and Quantification of Solute in Aqueous Phase

HRP-mediated polymerization of DCP has been shown to be effective in enhancing the removal of aqueous DCP through association with geosorbents matrices (Xu and Bhandari, 2003 b; Palomo and Bhandari, 2006). However, it has also been observed that in presence of geosorbents, the conversion of the phenolic compounds may not be complete (Kim, 2007), or that some of the polymeric products remain soluble in solution (Kim, 2007). Quantification of the amount of residual solute and polymerization products remaining in solution is important. In previous studies this has been accomplished by studying the distribution of the radioactivity associated with the solute between an aqueous and a solid phase. Identification, differentiation and quantification of the residual solute and soluble polymerization products have not been conducted in such experiments. These analyses are of significant importance since the presence of residuals in the aqueous phase makes them potential threats in the soil environment. Wagner and Nicell (2003) studied the impact of solids (silica gel, kaolin, bentonite, cellulose and peat moss) on the residual toxicity of supernatant after polymerization of phenol. These researchers concluded that the presence of solids (bentonite, kaolin and peat) decreased the toxicity of the supernatant. The reduction of toxicity was attributed to the capacity of the solids to interact and neutralize toxic reaction products. Since these authors created conditions to have nearly complete transformation of phenol, toxicity was attributed to transformation products only.

In a more recent study, Kong et al. (2007) reported that the toxicity in the supernatant was increased after polymerization and precipitation of DCP in the absence of solids. No information about the conversion of DCP was provided by the researchers. The residual toxicity

in the supernatant after DCP polymerization in the presence of solids such as soil particles is likely to be different. Wagner and Nicell's (2003) results suggest that in the presence of soil, the toxicity remaining after polymerization of DCP would be significantly lower than the toxicities observed by Kong et al. (2007).

The objective of this section of the chapter is to provide insight into the distribution of residual solute between the aqueous and solid phases. This study was conducted in the presence of AHM and WHM geomaterials. The aqueous conditions used in the study under solid-free conditions produced >95% DCP conversion. The significance of this study rests on results that show how soluble polymerization products and residual DCP are distributed in the system under study. Information on the residual toxicity is not within the scope of the study. This section presents, first a distribution of soluble polymerization products and residual DCP in the solution in contact with geomaterials. This is followed by characterization of the extractable polymerization products + unreacted DCP removed from soil during water and methanol extractions. While the supernatant after polymerization might contain residual DCP and soluble polymers, solute extracted from soil may consist of sorbed unreacted DCP, and sorbed, trapped, dissolved or disintegrated polymers.

3.5.5.1 Residual DCP and Soluble DPP Characterization in Supernatant after Sorption

Quantification of soluble residual solute, in the supernatant after polymerization of DCP, in the presence of WHM and AHM geomaterials is presented here. This section discusses results as fractions of the total mass that was initially added to the reactors. Thus, for 50, 100 and 500 μM of initial solute aqueous concentration and a solution volume of 8.5 mL, the initial mass added to the reactors (M) is equal to 0.43, 0.86 and 4.3 μmoles of DCP. The mass of DCP and soluble polymers measured in supernatant or remaining in soil is expressed as m.

3.5.5.1.1 Woodland Humin-Mineral Geomaterial

Figure 3.16 shows the impact of contact time and the amount of solute introduced to each reactor at the beginning of the experiment on the distribution of residual DCP and aqueous DPP. Results show that 95 to 97% removal of DCP occurred as a result of sorption or polymerization. The amount of soluble DPP in the supernatant increased with initial aqueous concentration of

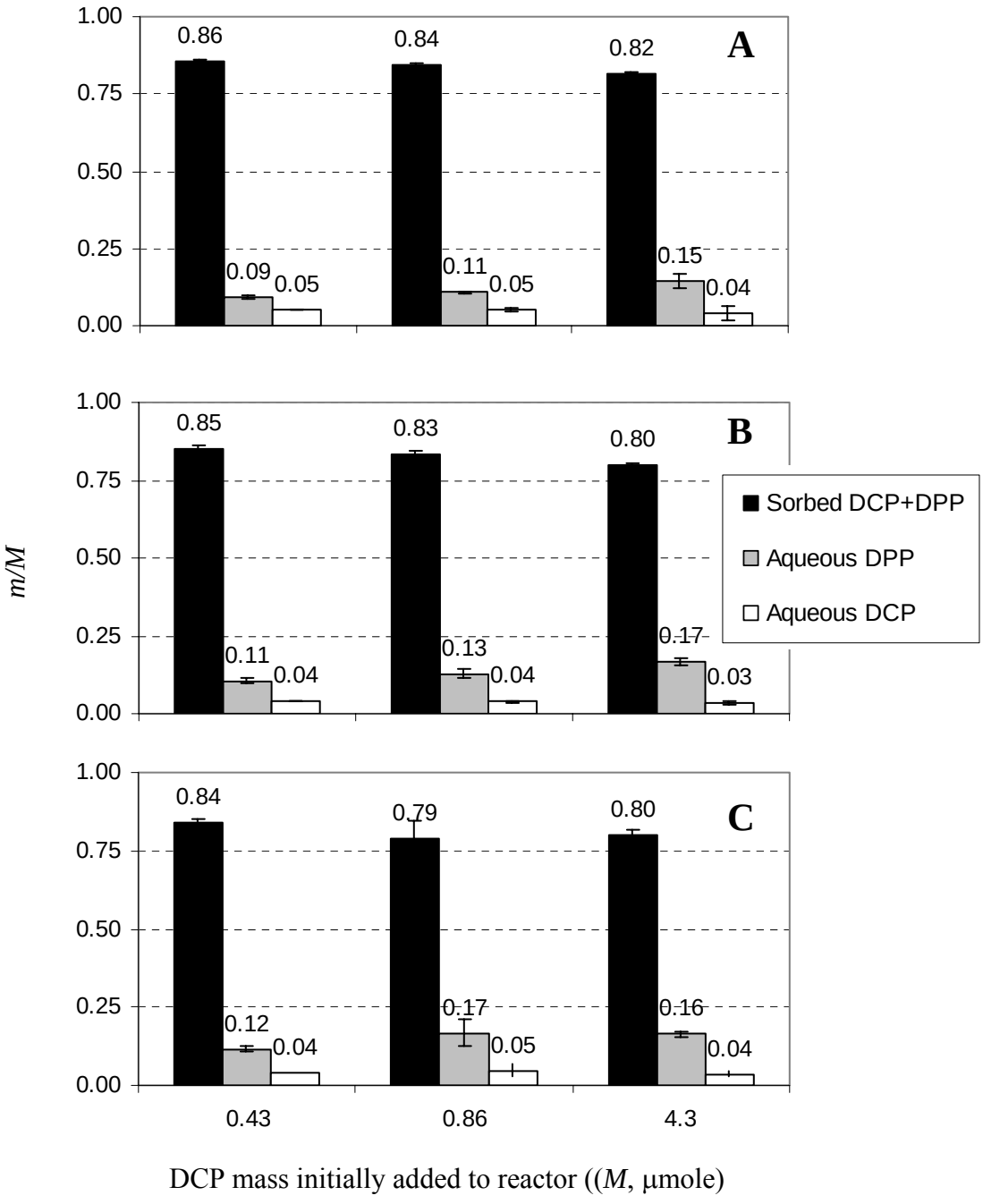

Figure 3.16. Distribution of residual DCP and soluble DPP between the aqueous and solid phases after (A) 1, (B) 7 and (C) 28 days of contact time, WHM.

DCP while the total sorbed mass of contaminant declined slightly. The sorbed solute fraction decreased and the aqueous solute increased from day 1 to 28, suggesting that equilibrium was not reached within one day and solute exchanges between water and the sorbent continued beyond one day. This result is in disagreement with the phase distribution relationship where equilibrium was reached after 1 day of contact.

In general, 9 to 17 % of the initially added mass of DCP was left in the supernatant as soluble polymers, and 3 to 5 % as residual unreacted DCP. Approximately 80 to 86 % of the total DCP introduced to the system was associated with WHM in the form of DCP or DPP. The near complete removal of DCP suggests that the overall toxicity due to DCP in the aqueous phase was likely reduced significantly. Kong et al. (2007) reported increase in the aqueous solution toxicity after DCP polymerization in soil-free systems. These researchers investigated the case where the entire mass of polymerized product remained in solution. In the current study, however, the toxicity of soluble polymers remaining in solution although unknown, it is likely to be significantly reduced due to sorption of residual DCP and soluble DPP to the geomaterials.

3.5.5.1.2 Agricultural Humin-Mineral Geomaterial

Distribution of the residual DCP and DPP between aqueous and solid phases in AHM soil is shown in Figure 3.17. Similar trends as those observed for WHM were seen for AHM. While only 3 to 5% of residual DCP remained in solution, its fractional removal slightly decreased with increasing C_o values and contact times. The fraction of soluble DPP increased as a function of time and initial aqueous concentration of solute. Soluble polymers represented 14 to 21% of the total mass initially added. On the other hand, the mass of sorbed DCP + DPP decreased as a function of C_o and contact time. Sorbed solute represented 75 to 81% of the total initial DCP added to the CMBRs.

The AHM system was observed to remain dynamic throughout the 28 days of study. It appears that true equilibrium was not reached in 28 days since exchange between sorbed polymers, sorbed DCP, residual DCP and soluble polymers was evident by the changing distribution.

The fraction of soluble polymers appeared to vary with time and initial mass of DCP added to the reactors. The fraction of soluble polymers was higher for AHM (14 to 21%) than WHM (9 to 17%); WHM sorbed more solute (80 to 86%) than AHM (75 to 81%), while both geomaterials exhibited similar residual DCP in aqueous phase (3 to 5%). It is likely that the

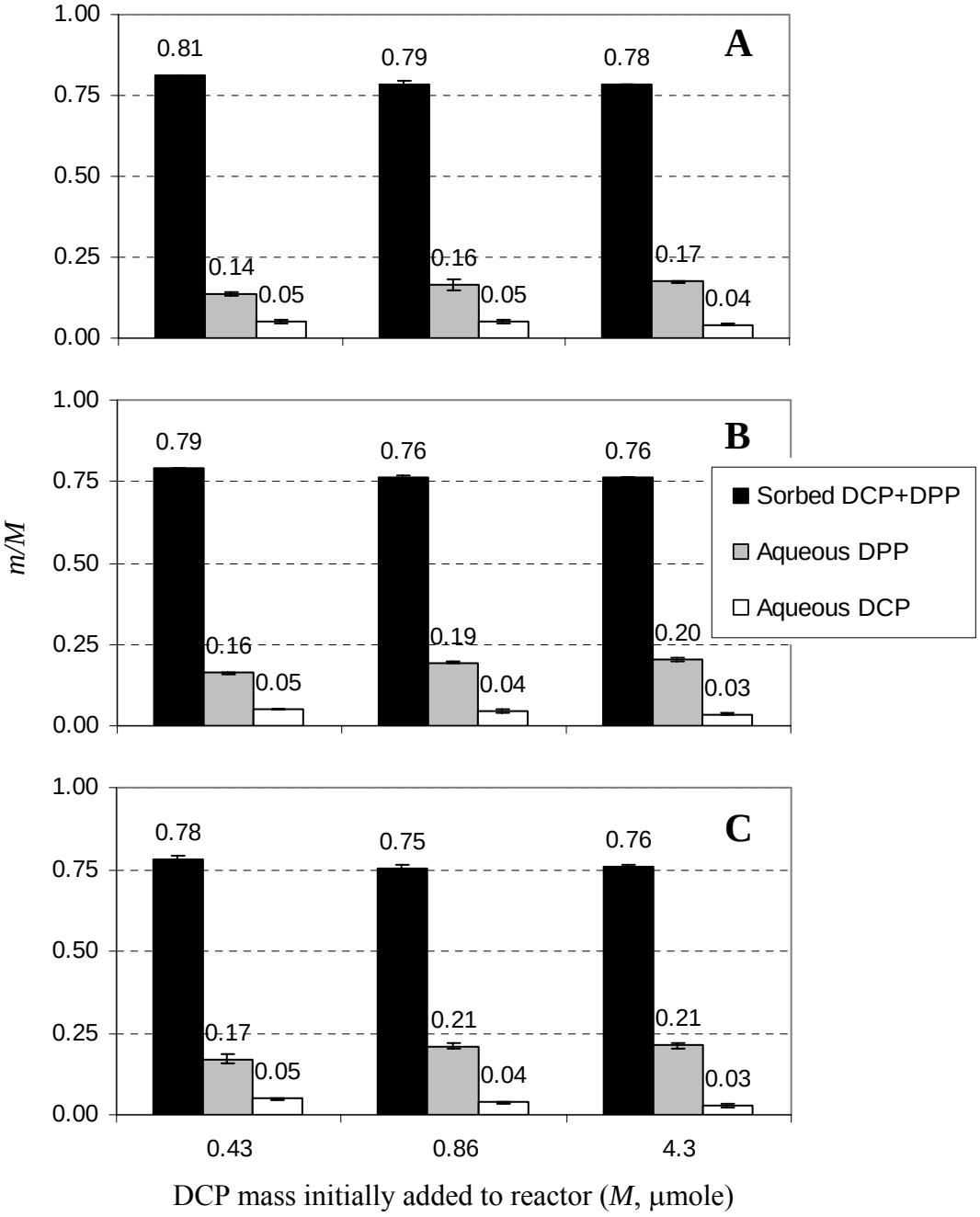

Figure 3.17. Distribution of residual DCP and soluble DPP between the aqueous and solid phases after (A) 1, (B) 7 and (C) 28 days of contact time, AHM.

difference in the fraction of sorbed solute was a function of the SOM content and the chemical nature of the humin matter in each material. Although AHM had 0.7% less SOM than WHM, the smaller amount of chemical sorbed by the AHM material was comparable to that sorbed by WHM.

3.5.5.2 Solvent Extractable unreacted DCP and DPP

This experiment characterized and quantified solute in the water and methanol extractable portions of contaminant that partitioned on to the geomaterials during enzymatic treatment. The total mass extracted is the sum of recovered DCP and extractable DPP. Data are presented as a fraction of the total mass that was added initially to the reactors per kilogram of soil. Thus, for 50, 100 and 500 μM of initial solute aqueous concentration, an average solution volume of 8.5 mL and a mass of 1.5 grams of sorbent, the initial mass added to the reactors (M) is equal to 283, 567 and 2,833 μmoles of DCP /kg of soil. The mass of DCP and DPP measured in the extract or left in soil is expressed as m.

3.5.5.2.1 Extractable Solutes from Woodland Humin-Mineral Geomaterial

The fate of recovered DCP and extractable DPP associated with the solid phase is presented in Figure 3.18. The solid phase contaminant was characterized as recovered DCP, extractable DPP and bound DPP. It is important to mention that the results in the graph represent fractions of the total solute initially added to the reactors so they are not expressed in mass basis. Results are illustrated in mass basis in Figure 3.14, for WHM and in Figure 3.15, for AHM.

Recovered DCP and bound DPP fractions were observed to decrease as a function of C_o, while the fraction of extractable DPP increased. Longer contact times increased the fraction of bound DPP, while the fraction of extractable DPP decreased. The recovered DCP fraction appeared to remain constant indicating that sorption of the unreacted DCP was controlled by its initial concentration in solution. These results indicate that longer contact times resulted in stronger association between bound DPP and the sorbent matrix.

3.5.5.2.2 Extractable Solutes from Agricultural Humin-Mineral Geomaterial

The distribution of recovered DCP, extractable DPP and stabilized DPP for AHM is illustrated in Figure 3.19. Similar to the trends observed for WHM, both C_o and contact time affected the distribution of the extractable and nonextractable fractions in AHM. Contact time

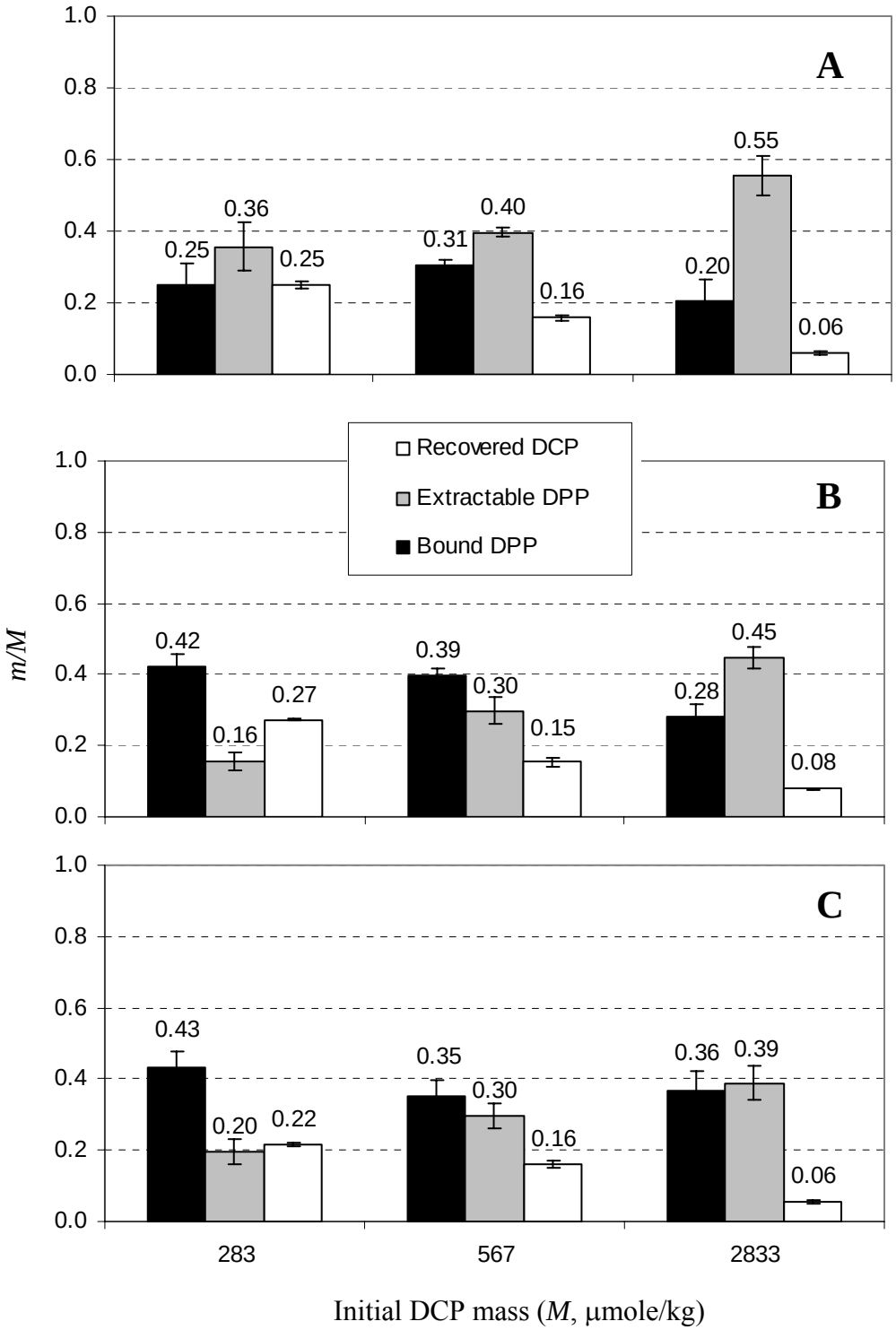

Figure 3.18. Fate of solid phase solute. A) 1, B) 7 and C) 28 days of contact time, WHM.

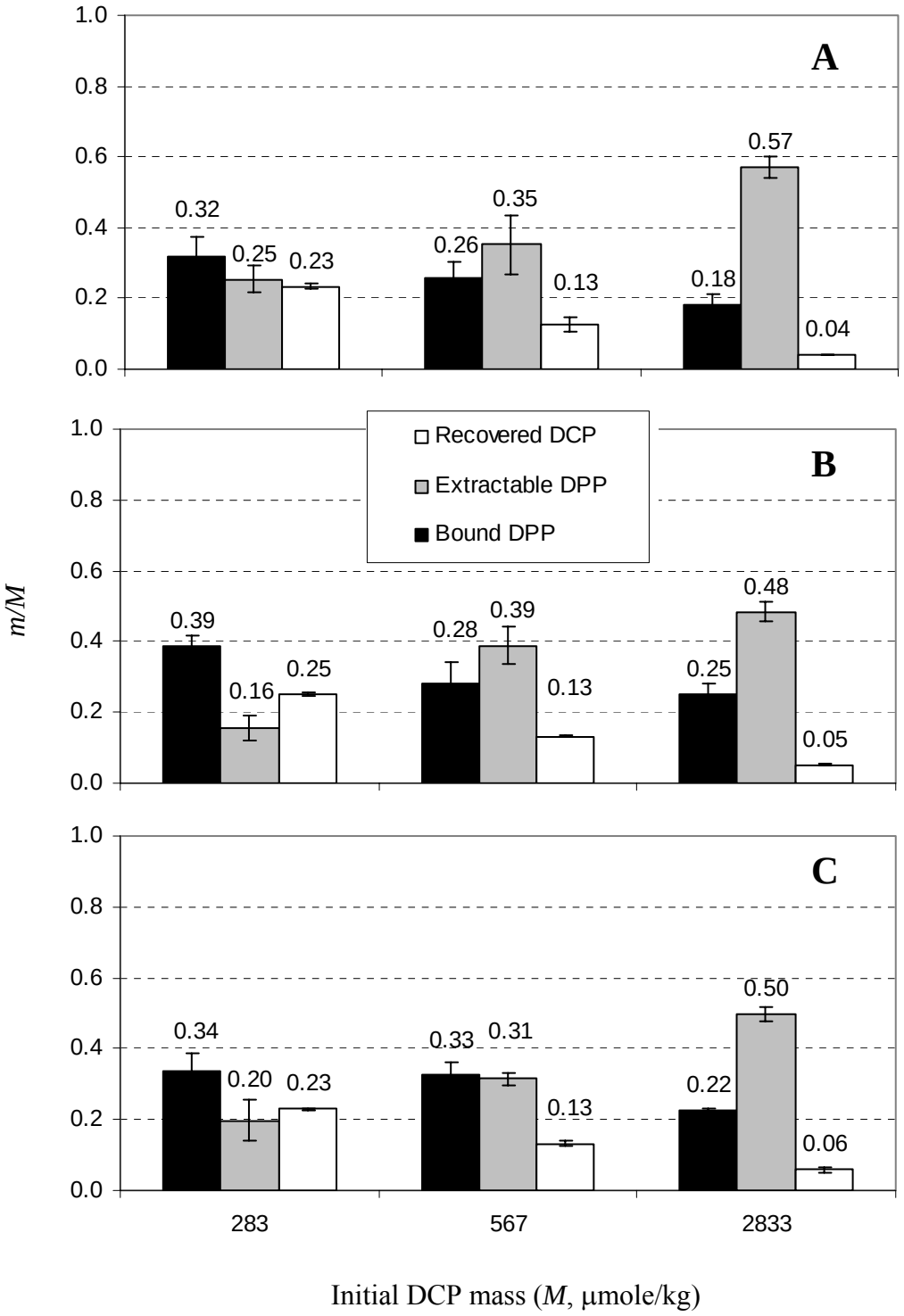

Figure 3.19. Fate of solid phase solute. A) 1, B) 7 and C) 28 days of contact time, AHM.

did not affect recovered DCP fraction at C_o = 50 μM and C_o = 100 μM, while at C_o = 500 μM, contact time caused a slight increase in the fractional DCP recovery (4 to 6%).

On the other hand, the fraction of extractable DPP increased with C_o. The fraction of extractable DPP was as high as 57 % of the total initial mass added at C_o = 500 μM. However, the aging effect caused a decrease in the fraction of extractable DPP. The decrease in the fractions of extractable DPP appeared to be greater in the presence of WHM than that observed for AHM. The fraction of bound solute increased as a consequence of contact time. However, the increase in the fraction of bound solute for AHM was not as high as that observed for WHM. Thus, in terms of fraction AHM soil seemed to have less solute bound.

3.6 Summary and Conclusions

The overall objective of this research was to assess the feasibility of utilizing horseradish peroxidase (HRP) in the chemical stabilization of phenolic contaminants in soils and aquifer materials. The realization of this goal was expected to result in an improved understanding of HRP-mediated polymerization of phenols in the presence of model matrices. The research utilized 2,4-dichlorophenol (DCP) as a model phenolic contaminant. Alkali-extracted humin-mineral geomaterials were used as model geosorbents

The following hypotheses were evaluated:

Hypothesis 1: Peroxidase-mediated oxidative coupling of DCP in the presence of humin-mineral geomaterials results in the formation of dichlorophenol polymerization products (DPPs);

Hypothesis 2: DPPs are removed from the aqueous phase as a result of sorption and retention of the oligomers on humin-mineral particles surfaces;

Hypothesis 3: DPPs associate strongly with the humin-mineral surfaces and are resistant to extraction by water and methanol; and

Hypothesis 4: Post-polymerization contact time, between DPP-containing aqueous phase and the humin-mineral geomaterial enhances formation of nonextractable DPP residues.

Hypothesis 1 was proven true by observing DCP removal and DPP generation during HRP-mediated oxidative coupling of DCP in the presence of two humin-mineral soils. Hypothesis 2 was proven true by observing soil-water phase distribution of solute in the HRP-

amended systems. Solute phase distribution was nonlinear for DCP and linear for total solute in systems where the enzymatic polymerization of DCP was induced. The amount of solute (DPP + unreacted DCP) associated with sorbents after 28 days of contact consisted of ~ 80% and 78% of the total mass of DCP initially added to the WHM and AHM slurries, respectively. The difference in sorption capacity was attributed to the difference in SOM content of both sorbents. The solute remaining in supernatant consisted of soluble DPP and unreacted DCP. Single-point, organic matter-normalized distribution coefficient, $K_{OM,Ct}$, showed that AHM sorbed more DCP than WHM at low solute concentrations. At higher solute concentrations, WHM sorbed more DCP than AHM. Results in HRP-amended systems suggested higher affinity of the solute for the organic matter in AHM than that in WHM. Higher affinity of solute (DPP + unreacted DCP) for the AHM geosorbent was attributed to its chemical characteristics, higher aliphaticity and lower polarity when compared to WHM.

Hypothesis 3 was proven true by observing low water and methanol extractability of sorbent-associated solute. The extractable solute consisted of sorbed DPP and sorbed, unreacted DCP. Aqueous extractability of the parent DCP, as measured by the extractability index (*EI*), decreased at high solute concentrations and contact times, suggesting that DPP precipitation and solute diffusion into the exposed humin matter were the predominant mechanisms for the removal of solute from the aqueous phase. Extractability of DCP from AHM was slightly higher than from WHM. In HRP-amended systems, extractability of solute decreased with contact time and aqueous solute concentration. Solute associated with WHM was more readily extracted with water than that associated with AHM. Enhancement in bound residue formation was significant in the presence of HRP. The extent of contaminant stabilization was generally similar in AHM and WHM at all initial aqueous concentrations except C_o= 500 μM where more DPP residue was observed in WHM soil.

Hypothesis 4 - was proven true for WHM but not for AHM soil. The amount of bound residue increased slightly with contact time for WHM, but remained steady for the AHM soil. Thus, the length of the contact between oligomers and geosorbent was observed to be important in determining the maginitude of the mass of solute sorbed and in the mass of solute stabilized. Results suggested that contact time allowed formation of stronger interactions between soil particles and oligomers, thus favoring their stabilization.

3.6.1 Real-World Implications

The stabilization of phenolic contaminants through enzymatic polymerization is a potential remediation technique for contaminated surface soil and subsurface material. Successful application of engineered humification requires a complete visualization of the fate of the target contaminants and their polymerization products. The significance of studying polymerization of DCP in the context of humin-enriched geosorbents is the fact that phenolic contaminants can be present in the subsurface where organic matter present is dominated by the humin type. Interactions between DCP polymerization products (DPP) with the humin fraction and their effect on the sorption, extraction and binding of DPP are, therefore, of interest in order to evaluate the effectiveness of this method under conditions different from that observed in a surface soil.

Results from this study suggest that, though the amount of organic matter present in soil is an important factor to determine the amount of organic chemicals sorbing and binding to the soil phase, the chemical characteristics of the SOM should also being taken into account to predict sorption behavior of the contaminant. This study showed that the mass of chemical associated with geosorbents with low SOM content and chemical character different from that predominant in surfaces soils, can be as high as that observed when high SOM contents are present in the geosorbent.

So, the application of the HRP-mediated stabilization of aqueous phase DCP was observed to be independent of the amount of SOM present in soil but favored by the chemical composition and physical arrangement of the humin fraction. The results from this work suggest that HRP-mediated stabilization would be successfully applied in a subsurface environment.

Lastly, phase distribution data implies that 24 hrs would be enough time to get significant mass of contaminant associated with the soil, since sorption equilibrium seem to be achieved after 1 day without further increase on the amount of mass sorbed to the geosorbent. Thus, field application of this remediation technology would not require prolonged amount of time.

3.7 Literature Cited

Almendros, G.; Guadalix, M. E.; Gonzalez-Vila, F. J. and Martin F. Preservation of aliphatic macromolecules in soil humins. *Org. Geochem*. 1996, 24(6/7): 651-659.

Anderson, D. W.; Paul, E. A. and Arnard, R. J. St. Extraction and characterization of humus with reference to clay-associated humus. *Can. J. Soil. Sci*. 1974, 54:317-323.

Bhandari, A.; Novak, J. T. and Berry, D. F. Binding of 4-monochlorohenol to soil. *Environ. Sci. Technol.* 1996, 30:2305-2311.

Bhandari, A.; Novak, J. T.; Burgos, W. D.; and Berry, D. F. Irreversible binding of chlorophenols to soils and its impact on bioavailability. *Journal of Environmental Engineering*. 1997, 506-513.

Bhandari, A. and Xu, F. Impact of peroxidase addition on the sorption-desorption behavior of phenolic contaminants in surfaces soils. *Environ. Sci. Technol.* 2001, 35:3163-3168.

Bollag, J. M. Enzyme catalyzing oxidative coupling reactions of pollutants. *Metal Ions in Biological Systems*. 1992-a,28:205-217

Bollag, J.-M. Decontaminating the soil with enzymes. *Environ. Sci. Technol.* 1992 b, 26:1876-1881.

Bonin, J; and Simpson, M. J. Variation in phenanthrene sorption coefficients with soil organic matter fractionation: the result of structure or conformation? *Environ. Sci. Technol.*2007, 41: 153-159.

Borisover, M.; and Graber, E. R. Hydration of natural organic matter: effect of sorption of organic compounds in humin and humic acids vs original peat material. *Environ. Sci. Technol.* 2004, 38: 4120-4129.

Capriel, P.; Haisch, A. and Khan, S. U. Distribution and nature of bound (nonextractable) residues of atrazine in a mineral soil nine years after the herbicide application. *J. Agric. Food Chem.* 1985, 33, 567-569.

Chefetz, B.; Deshmukh, A. P.; Hatcher, P. G.; and Guthrie, E. A. Pyrene sorption by natural organic matter. *Environ. Sci. Technol.* 2000, 34:2925-2930.

Chiou, C. T; Kile, D. E.; Rutherford D. W.; Sheng, G.; and Boyd, S. A. Sorption of selected organic compounds from water to a peat soil and its humic-acid and humin fractions: potential sources of the sorption nonlinearity. *Environ. Sci. Technol.* 2000, 34:1254-1258.

Crespin, M. A.; Gallego, M. and Valcarcel, M. Study of degradation of the herbicides 2,4-D and MCPA at different depths in contaminated agricultural soil. *Environ. Sci. Technol*. 2001, 35:4265-4270.

De Jonge, H and Mittelmeijer-Hazeleger, M. C. Adsorption of CO_2 and N_2 on soil organic matter: nature of porosity, surface area and diffusion mechanisms. *Environ. Sci. Technol.* 1996, 30: 408-413.

Dec, J.; Haider, K.; Benesi, A.; Rangaswamy, V.; Schäffer, A.; Plücken, U.; and Bollag, J.M. Analysis of Soil-Bound Residues of ^{13}C-Labeled Fungicide Cyprodinil by NMR Spectroscopy. . *Environ. Sci. Technol.*1997, 31, 1128-1135.

Dec, J. and Bollag, J. M. Phenoloxidase-mediated interactions of phenols and anilines with humic materials. *J. Environ. Qual.* 2000; 29(3): 665-676.

Dunford, H. B. Horseradish peroxidase: structure and kinetic properties. In: *Peroxidases 21in Chemistry and Biology*, Volume II, CRC Press, Inc. Editors: Everse, J.; Everse, K. E. and Grishman, M. B. 1991.

Duran, N. and Esposito, E.. Potential applications of oxidative enzymes and phenoloxidase-like compounds in wastewater and soil treatment: a review.*Applied Catalysis B. Enviromental*, 2000, 28:83-99.

Fabbri, D.; Chiavari, G.; and Galletti, G. C. Characterization of soil humin by pyrolysis(/methylation)-gas chromatography/mass spectrometry:structural relationships with humic acids. *J. Anal. App. Pyrol*. 1996, 37:161-172.

Goh, K. M.; Malloy, B. P. J. Radicarbon dating of paleosols using soil organic matter components. *J. Soil Sci*. 1978, 29, 567-573.

Gouveia, S.E M; Pessenda, L. C. R.; and Aravena, R.Dating of the humin fraction of soil organic matter and its comparison with ^{14}C ages of fossil charcoal. *Quimica Nova*. 1999, 22 (6):810-814.

Grasset, L.; and Ambles, A. Aliphatic lipids released from a soil humin after enzymatic degradation of cellulose. *Org. Goechem*. 1998 b, 4: 893-897.

Guetzloff, T. F.; and Rice, J. A. Does humic acid from a micelle? *Sci. Total Environ*. 1994, 152:31-35

Guthrie, E. A.; Bortiatynski, J. M. ; Van Heemst, J. D. H. ; Richman, J.E.; Hardy, K. S.; Kovach, E. M.; and Hatcher, P. G. Determination of [^{13}C]Pyrene sequestration in sediment microcosms using flash pyrolysis-GC-MS and ^{13}C NMR. *Environ. Sci. Technol.*1999, 33:119-125.

Hatcher, P. G.; Vanderhart, D. L. and Earl, W. L. Use of solid-state ^{13}C NMR in structural studies of humic acids and humin fromm Holocene sediments. *Org. Geochem*. 1980, 2: 87-92.

Hatcher, P. E. G.; Spiker, E. C.; Szeverenyi, N. M. and Maciel, G. E. Selective preservation and

origin of petroleum-forming aquatic kerogen. *Nature*. 1983, 305:498-501.

Hatcher, P; Breger, I.; Maciel, G.; and Szeverenyi, N. Geochemistry of humin. In *Humic Substances in Soils, Sediments, and Water: geochemistry, isolation, and characterization*. G. R. Aitken, D. M. MczKnight, R. L. Wershaw, and P. MacCarthy (eds) John Wiley, New York. 1985, 275-302.

Hayase, K. and Tsubota, H. Sedimentary humic acid and fulvic acid as surface active substances. *Geochimica et Cosmochimica Acta*. 1983, 47:947-952.

Hatzinger, P.B.; and Alexander, M. Effect of aging of chemicals in soil on their biodegradability and extractability. *Environ. Sci. Technol*. 1995, 29, 537–545.

Huang, W., and Weber, W. J. Jr. A distributed reactivity model for sorption by soils and sediments.10. Relationships between desorption, hysteresis, and the chemical characteristics of organic domains. *Environ. Sci. Technol*. 1997, 31: 2562-2569.

Hu, W-G.; Mao, J.D.; Xing, B.; Schmidt-Rohr, K. Poly(methylene) crystallites in humic substances detected by nuclear magnetic resonance. *Environ. Sci. Technol*. 2000, 34:530-534.

Huc, A.; and Duran, B. Etudes des acides humiques er de L'humine de sediments. Recents consideres comme precurseurs des kerogens. In *Advances in Organic Geochemistry*, 1973, Tissot and F. Bienner (eds). Editions Technip, Paris, 53-72.

Huc, A.; and Durand, B. Occurrence and significance of humics acids in ancient sediments. *Fuel*.1977, 56:73-80

Ibrahim, M.S.; Ali, H. I.; Taylor, K. E. Biswas, N.; and Bewtra, J. K. Enzyme-catalyzed removal of phenol from refinery wastewater: feasibility studies. *Water Environ. Res.* 2001, 73(2): 165-172.

Ishiwatari, R. Geochemistry of humic substances in lake water. *In* Humic Substances in Soils, Sediments, and Water: geochemistry, isolation, and characterization. G. R. Aitken, D. M. MczKnight, R. L. Wershaw, and P. MacCarthy (eds) John Wiley, New York. 1985, 147-180.

Kang, S.;and Xing, B. Phenanthrene sorption to sequentially extracted soil humic acids and humans. *Environ. Sci. Technol.* 2005, 39: 134-140.

Kelsey, J.W.; Kottler, B.D.; and Alexander, M. Selective chemical extractants to predict bioavailability of soil-aged organic chemicals. *Environ. Sci. Technol.* 1997, 31, 214–217.

Kohl, S. D.; and Rice, J.A. The binding of contaminants to humins: a mass balance. *Chemosphere*. 1998, 36(2):251-261.

Kong, I. C.; Kim, M. and Bhandari, A.Aqueous phase toxicity changes resulting from horseradish peroxidase-mediated polymerization of phenols and hydroxylated polynuclear aromatic contaminants. *Bull. Environ. Contam.* Toxicol. 2007, 79:104–108.

Kononova, M. *Soil organic matter*. Second Edition. Pergamon Press Oxford New York, 1966.

Lesan. H.M.; and Bhandari, A.Atrazine sorption on surface soils: Time-dependent phase distribution and apparent desorption hysteresis. *Water Res.* 2003, 37:1644-1654.

Lesan, H.M.; Bhandari, A. Contact-time dependent atrazine residue formation in surface soils. *Water Res*. 2004, 38:4435-4445.

Loiseau, L.; Barriuso, E.; Zegouagh, Y.; Largeau, C. And Mariott , A. Release of the atrazine non-extractable (bound) residues of two soils using degradative techniques. *Agronomie*. 2000, *20*, 513–524.

Liu, S-Y.; Minard, R. D. and Bollag J. M. Soil-catalyzed complexation of the pollutant 2,6-diethylaniline with syringic acid. *J. Environ. Quality*. 1987, 16(1): 48-53.

Malekani, K.; Rice, J. A.; and Lin, J-S. The effect of sequential removal of organic matter on the surface morphology of humin. *Soil Sci*. 1997, 162(5):333-342.

Mao, J.D.; Hundal, L. S.; Thompson, M. L. and Schmidt-Rohr, K. Correlation of poly(methylene)-rich amourphopus aliphatic domains in humic substances with sorption of a nonpolar organic contaminant, phenanthrene. *Environ. Sci. Technol.* 2002, 36,929-936.

Palomo, M. 2003. Effect of sorption contact time on the retention and binding of 2,4-dichlorophenol (DCP) and DCP polymerization products (DPP) in two surface soils. MS thesis, Kansas State University, Manhattan, KS.

Palomo, M.; and Bhandari, A. Time-dependent sorption-desorption behavior of 2,4-dichlorophenol and its polymerization products in surface soils. *Environ. Sci.Technol*. 2005, 39: 2143-2151.

Palomo, M.; and Bhandari, A.Impact of aging on the formation of bound residues after peroxidase-mediated treatment of 2,4-DCP contaminated soils. *Environ. Sci. Technol*. 2006, 40:3402-3408.

Park, J-W; Dec, J. Kim, J-E. and Bollag, J. M. Effect of humic constituents on the transformation of chlorinated phenols and anilines in the presence of oxidoreductive enzymes or birnessite. *Environ. Sci. Technol*. 1999, 33:2028-2034.

Park, J-W.; Dec, J.; Kim, J-E.; and Bollag, J. M. Transformation of chlorinated phenols and anilines in the presence of humic acid. *J. Environ. Qual*. 2000, 29:214-220.

Perry, R. H., and Green, D. W. Perry's Chemical engineers' handbook. McGraw-Hill, pg 3-88, 1984.

Pignatello, J. J. Sorption dynamics of organic compounds in soils and sediments. In *B.L. Sawhney and K. Brown (ed). Reactions and movement of organic chemicals in soil.* SSSA Spec. Publ. 22. SSSA and ASA, Madison, WI, pg. 45-80,1989.

Preston, C. M; and Newman, R. H. Demonstration f spatial heterogeneity in the organic matter of de-ashed humin samples by solid-state ^{13}C CP-MAS NMR. *Can. J. Soil Sci*. 1992, 72:13-19.

Rice, J. A.; and MacCarthy, P. Isolation of humin by liquid-liqud partitioning. *The Science of Total Environment*. 1989, 81/82: 61-69.

Rice, J. A.; and MacCarthy, P. Statistical evaluation of the elemental composition of humic substances. *Org. Geochem*. 1991, 17:635-648.

Rice, J.A. Humin. *Soil Sci*. 2001, 166 (11):848-857.

Russell, J.D.; Vaughan, D. Jones, D. and Fraser, A.R. An IR spectroscopic study of soil humin and its relationship to other humic substances and fungal pigments. *Goederma*, 1983, 29:1-12.

Salloum, M. J.; Chefetz, B; and Hatcher, P. G. Phenanthrene sorption by aliphatic-rich natural organic matter. *Environ. Sci. Technol*. 2002, 36:1953-1958.

Sarkar, J. M., and Bollag, J-M. Inhibitory effect of humic and fulvic acid on oxidoreductases as measured by the coupling of 2,4-dichlorophenol to humic substances. *Sci. Total Environ.* 1987, 62: 367-377.

Shah, R. K; Choski, M. R.; and Joshi, B. C. Development studies of soil organic matter: humin. *Chem. Era*. 1975, 6:1-3.

Shih, Y-H; and Wu, S-C.; Sorption kinetics in humin under two different levels of relative humidity. *J. Environ. Qual.* 2002, 31:970-978.

Simmos, K. A.; Minard R. D. and Bollag, J. M. "Oligomerization of 4-chloroaniline by oxidoreductases. *Eniron. Sci. Technol*. 1987, 21: 999-1003.

Simpson, M. and Johnson, P. C. E. Identification of mobile aliphatic sorptive domains in soil humin by solid-state ^{13}C nuclear magnetic resonance. *Environ. Toxicol. Chem*. 2006, 25 (1): 52-57.

Skujins, J. and; Klubek, B. Soil biological properties of a montane forest sere: corroboration of Odum's postulates. *Soil Biol. Biochem*. 1982, 14 (5): 505-513

Somani, L.L and Saxena, S. N. Distribution of humus fractions in some soil groups of Rajasthan.

Agrochim. 1982, 26: 95-103

Stevenson F.J. *Humus Chemistry: Genesis, Composition, Reactions*. New York: Wiley, 1982.

Tang, J.; and Weber, W. J. Jr. Development of engineered natural organic sorbents for environmental applications.2. Sorption characteristics and capacities with respect to phenanthrene. *Environ. Sci.Technol.* 2006, 40: 1657-1663.

Theng, B. K. G. *Formation and Properties of Clay-Polymers Complexes.* Elsevier, Amsterdam, 1979, page 362.

Thorn, K. A.; Pettigre, P.J.; Goldenberg, W. S. and Weber, E. J. Covalent binding of aniline to humic substances. 2. ^{15}N NMR studies of nucleophilic addition reactions. *Environ. Sci. Technol*. 1996, 30:2764-2775.

Vanderbrouke, M.; Pelet, R.; and Debyser, Y. Geochemistry of humic substances in marine environments. In *Humic Substances in Soils, Sediments, and Water: geochemistry, isolation, and characterization.* G. R. Aitken, D. M. MczKnight, R. L. Wershaw, and P. MacCarthy (eds) John Wiley, New York. 1985, 249-273.

Vaughan, D.; and Ord, B. G. An in vitro effect of soil organic matter and synthetic humic acids on the generation of free radicals. *Plant Soil.* 1982, 66: 113-116.

Wagner, M. and Nicell, J. Treatment of a foul condensate from kraft pulping with horseradish peroxidase and hydrogen peroxidase. *Water Res*. 2001, 35 (2):485-495.

Wang, K.; and Xing, B. Chemical extractions affect the structure and phenanthrene sorption of soil humin. *Environ. Sci.Technol.* 2005, 39: 8333-8340.

Weber, W. J.; and DiGiano, F. A. *Process Dynamics in Environmental Systems*; Wiley-Interscience: New York 1996

Welinder, K. G. Amino acid sequence studies of horseradish peroxidase. *Eur. J. Biochem.* 1979, 96: 483-502.

Xu, F. Horseradish peroxidase mediated sorption and binding of phenol, *o*-cresol, 2,4-dichlorophenol and 1-naphthol to two surfaces soils. PhD dissertation, Kansas State University, Manhattan, KS. 2002.

Xu, F.; and Bhandari, A. Retention and distribution of 1-naphthol and naphthol polymerization products on surface soils. *J. Environ .Eng*. 2003 a, *129,* 1041-4050.

Xu, F.; and Bhandari, A. Retention and extractability of phenol, cresol, and dichlorophenol exposed to two surfaces soils in the presence of horseradish peroxidase. *J. Agric. Food Chem.* 2003 b, 51, 183-188.

CHAPTER 4 - HORSERADISH PEROXIDASE MEDIATED STABILIZATION OF 2,4-DICHLOROPHENOL IN A MODEL SOM-FREE SOIL

4.1 Abstract

The fate and transport of phenolic agrichemical-derivatives such as 2,4-dichlorophenol (DCP), is of environmental concern since they are toxic at trace concentrations and represent a threat to surface water and groundwater resources. A variety of remediation methods, including contaminant transformation by peroxidase-mediated oxidative polymerization, have been proposed to manage soils and groundwater contaminated with chlorinated phenols. This study evaluated peroxidase-mediated transformation and removal of DCP from an aqueous phase in contact with an inorganic sorbent containing negligible (< 0.1%) soil organic matter (SOM). The sorbent was generated by oxidizing SOM associated with a surface soil with NaOCl. No DCP sorption was observed on the SOM-free soil. In enzyme-amended systems, the soil-water phase distribution of DCP polymerization products (DPP) was complete within 1 day. The aqueous extractability of DPP from SOM-free soil was lower at longer contact times and at smaller residual aqueous concentrations of DPP. Water- and methanol-extractable solute consisted entirely of DPP and no unreacted DCP. DCP stabilization was enhanced with contact time, and a greater fraction of the DCP was stabilized in soil at lower initial concentrations. Results from this study suggest that peroxidase can be utilized to stabilize DCP in contaminated mineral soils and aquifer media; thereby restricting its transport in the environment. DCP stabilization results from a combination of sorption and precipitation of the oligomeric products generated during oxidative coupling of the contaminant mediated by peroxidase enzyme and hydrogen peroxide.

4.2 Background

4.2.1 Oxidation of Soil Organic Matter

The removal of soil organic matter from soil and sediments is necessary to conduct mineralogical analyses like particle size-distribution and X-ray diffraction. One objective of the method used to remove SOM is to achieve minimum alteration of the remaining inorganic fraction. The use of hydrogen peroxide (H_2O_2) digestion is the most common and accepted

method to remove organic carbon from a soil sample. Soil samples to be treated with H_2O_2 are first acidified to improve the effectiveness of removal of SOM. Efficient removal of SOM allows complete dispersion of the inorganic aggregates of the soil material under study.

The H_2O_2 method, however, has been reported to significantly impact the integrity of the mineral soil fractions by causing dissolution of Si, Al, Fe (Lavkulich and Wiens, 1970), alkaline earth carbonates (Bourget and Tanner, 1953; Anderson 1963), and Mn oxides (Jackson, 1956; Lavklich and Wiens, 1970). Formation of calcium oxalate in soils treated by H_2O_2, has also been reported (Martin, 1954). Alteration of properties of the residues remaining after H_2O_2 digestion, include loss of phosphate sorption capacity (Williams et. al., 1958). Consequently, some researchers have utilized alternate SOM-oxidizing methods that allow better preservation of the inorganic fractions of the soil sample.

Bourget and Tanner (1953) studied the effectiveness of sodium hypobromite (NaOBr) in removing organic matter. These researchers compared the removal SOM from non-calcareous and calcareous soils. Their results suggested that for non-calcareous soils, the NaOBr showed a comparable SOM digestion capacity as H_2O_2. However, when calcareous soils were used, the H_2O_2 method removed calcium and magnesium carbonates, while the NaOBr technique did not.

Anderson (1963) proposed the utilization of a pH 9.5 sodium hypochlorite (NaOCl) solution to oxidize SOM. A pH of 9.5 was suggested for complete sodium saturation without causing dissolution of aluminum. The objective of the study was the determination of a method that could provide complete SOM removal without removal or complexation of sesquioxide coatings, silica and carbonates. Three consecutive treatments with NaOCl solution were sufficient to reduce the organic content to values as low as 0.03 %.

Similarly, Lavkulich and Wiens (1970) reported that NaOCl method was more effective in removing organic matter than H_2O_2 and with less alteration or destruction of the mineral oxides. NaOCl digestion method was modified from that proposed by Anderson (1963) by heating the soil slurry in boiling water for 15 minutes. The higher removal of organic matter was attributed to the higher oxidation potential of NaOCl at pH 9.5 when compared to the oxidation potential of H_2O_2. The amounts of SiO_2, Mn, Fe, and Al removed from the samples with NaOCl treatment were lower than the amounts removed when the H_2O_2 method was utilized. Five successive NaOCl treatments performed on 16 soil samples reduced the carbon by 98%. These authors also reported a pH of 9.5 as the optimum pH for the destruction of the organic matter

with minimum removal of oxides.

While the NaOCl utilized in the previously described studies was reagent grade, Omueti (1980) employed commercial bleach solution that was adjusted to a pH of 9.5 immediately before use. The oxidation with commercial bleach (NaOCl) was more effective in removing SOM than the H_2O_2 method. Other authors have also used the NaOCl method with other additives to extract organic matter while preserving the inorganic fractions of the soil sample (Shuman, 1983; Hettiarachchi, et al 2003).

4.2.2 Sorption of Phenolic Contaminants to Mineral Soils

Knowledge of the soil matrix composition is important for understanding sorption and transport of organic chemicals in the environment. In general, the components of the soil matrix are grouped into organic and inorganic domains. The inorganic or mineral components are clay, silt and sand. These particles are characterized on the basis of their size and mass, and they constitute the bulk of the soil. Soil inorganic components include crystalline, semicrystalline and amorphous oxyhydroxide minerals (Laird et al., 1994). In SOM-containing soils, the mineral particles are often covered by an organic matter layer. Even though the organic matter represents a small weight fraction of the bulk soil mass, the mineral surface that is covered is usually very large. SOM controls the sorption of nonionic organic compounds when organic C levels are higher that 0.1 %, while interactions with mineral surfaces become significant below this SOM value (Pignatello, 1989).

Soils with none or negligible SOM content can be classified as inorganic or mineral soils. The interactions of organic contaminants with mineral soils are mechanistically different than those with organic soils. Mineral soils play a significant role in the fate and transport of organic solute in the subsurface environment.

An extensive collection of studies addressing sorption of organic contaminants and pesticides on mineral surfaces is available in the literature (Sanchez-Martin and Sanchez-Camazano, 1984; Micera et al., 1988; Laird et al., 1992; Barriuso et al., 1994; Hatzinger and Alexander, 1995; Huang et al., 1996; Sannino et al., 1997; Bhandari and Weber, 1998; González–Pradas et al., 1999; Ran et al., 2005). Ionic and neutral pesticide species have also been reported to adsorb in clay particle interlayers (Sanchez-Martin and Sanchez-Camazano, 1984; Ainsworth et al., 1987; Micera et al., 1988; Huang et al., 1996; Sheng et al., 2001; Sheng

et al., 2002).

Protonated species of pesticides have been reported to sorb preferentially to smectite surfaces over nonprotonated species. Adsorption of neutral species is enhanced by increase of the organic surface coverage (surface density), solution concentration and higher solution pH (Ainsworth et al., 1987). Laird et al. (1992) suggested that the high adsorption potential of pesticides by smectites is likely due to the clay's soil abundance, high surface area and clay surface chemistry.

Bhandari et al. (1996) investigated the association of 4-chlorophenol (4-CP) with an oxidized soil containing 0.52 % SOM. Binding of 4-CP to the soil with such low SOM content was attributed to metal oxides present on the mineral surfaces, and to the availability of internal sorption surfaces in mineral micropores. The enhanced binding of 4-CP in the presence of H_2O_2 was attributed to the oxidation of the exposed surface transition metal oxides and their ability to catalyze oxidative polymerization of 4-CP.

Adsorption-desorption of the pesticide 2,4-dichlorphenoxiacetic acid (2,4-D) in the presence of a pure montmorillonite and chlorite-like complexes [$Al(OH)_x$- montmorillonite] was evaluated by Sannino et al. (1997). These researchers postulated two types of processes responsible for the adsorption: 1) physical adsorption through electrostatic interactions between COO^- moieties of the herbicide and the positive $Al(OH)^{2+}$ groups on the clay surfaces, and 2) chemisorption through ligand exchange of COO^- groups with surface –OH or water.

Celis et al. (1999) studied sorption of 2,4-D in binary and ternary model particles of montmorillonite, ferrihydrite and humic acid. These authors reported a reduction in the sorption of 2,4-D by Ca and K-saturated montmorillonites. At the end of the sorption process, the concentration of the pesticide in the aqueous phase increased and it was explained by the preferential sorption of clays for water, which resulted in an increased solute concentration. The anionic form of 2,4-D was predominant at the pH of experiment (pH > 7) and sorption was not likely to happen since the clay particles were negatively charged. The authors concluded that sorption behavior of soil aggregates is dependent on the physical-chemical characteristics of the aggregate surface that remains available for interaction with organic compounds.

Sorption isotherms for 2,4-D on ferrihydrate, were of the S-type, indicating that at low concentrations, the solute most likely competed for sorption sites with water and/or ionic species present in solution. Sorption of the pesticide by iron oxides was observed to be important at high

2,4-D concentrations. The authors confirmed, through IR spectroscopy, that the Fe oxide surfaces interacted with the $-COO^-$ groups of the pesticide, while the aromatic ring was oriented toward the solution. Thus, sorption occurred through Coulombic interactions between the anionic form of 2,4-D and the positively charged surface of the Fe oxides. The formation of van der Waals interactions between the aromatic rings of the sorbed molecules, and competition between the pesticide and Cl^-, OH^- water molecules for the iron oxide surface were also suggested as factors impacting 2,4-D sorption. Sorption and precipitation of Fe oxides on Ca-saturated Wyoming montmorillonite (Ca-SWy clay) significantly increased the sorption of the pesticide compared to sorption observed for clay alone. Higher charge density was noted to have reduced the sorption of the solute, while low surface charge density promoted the interactions of the anionic species of the solute with the clay surface.

Adsorption of pesticides in major components of aquifer material was investigated by Clausen et al. (2001). These authors used calcite and quartz, kaolinite and α-alumina, to investigate the sorption of ionic compounds (2,4-D, mecoprop and bentazone). Sorption of the pesticides on quartz occurred only when the solution pH was below or close to the point of zero charge. The adsorption of pesticides by calcite and α-alumina was attributed to the interaction between the COO- groups with positively charged surface groups. Adsorption decreased in the order mecoprop> 2,4-D> bentazon. At a pH of 2.4, the quartz $=SiOH_2^+$ sites were assigned as responsible for the sorption of the anionic pesticide. In calcite, the $=Ca(OH_2)^+$ sites, and in α-alumina the electrostatic interactions between the anionic molecules and the $=AlOH_2^+$ sites were suggested as responsible for the adsorption of the anionic pesticides.

Addition of $CaCl_2$ to the background solution enhanced adsorption of 2,4-D and mecoprop by kaolinite. Adsorption of anionic pesticides was found to be reduced with increasing ionic strength. Thus, through the variation of the electrolyte concentration in solution (ionic strength), these researchers demonstrated that phenoxy acids adsorbed to mineral surfaces through outer sphere interactions (nonspecific adsorption) by means of electrostatic bonding. These researchers concluded that anionic pesticides are weakly bonded to the mineral surfaces through electrostatic linkages.

The importance of the iron oxides present in aquifer sediments was evaluated by Clausen and Fabricius (2001) using same anionic pesticides as Clausen et al. (2001) and SOM-free iron oxides (ferrihydrite, goethite and lepidocrocite). Adsorption of anionic pesticides by the iron

oxides was reported to be considerable with the carboxyl-bearing pesticides exhibiting the highest adsorption. The adsorption behavior of the iron oxides minerals was different (ferryhydrate > goethite > lepidocrocite) and differences were attributed to the density of singly coordinated hydroxyl groups.

Sheng et al. (2001) studied the contribution of smectite clays (K-saturated clay SWy-2) on the retention of seven different pesticides, including 4,6-dinitro-o-cresol. These authors observed that basal spacing of K-saturated SWy-2 clay increased with increasing 4,6-dinitro-o-cresol adsorption. The high adsorption of 4,6-dinitro-o-cresol was attributed to the characteristics of the molecule like planarity, aromaticity and the strongly electron-withdrawing nitro groups, which favored the formation of electron-donor acceptor complexes, where the aromatic pesticide accepted the electrons donated by the negatively charged siloxanes surfaces. Sorption mechanisms occurring between the organic molecules and the clay were identified as electron donor-acceptor interactions, water bridges between polar substituents and hydrated exchangeable cations, interaction of negatively charged substituents with exchangeable cations and hydrophobic interactions between the pesticide molecules and the siloxane surfaces. These researchers also observed that low surface charge densities caused increase of the adsorption domains, thus enhancing adsorption of organic solutes.

The study of the sorption of 2,4-D to 10 different aquifer sediments was reported by Madsen et al. (2000). Sorption of 2,4-D was observed to decrease at higher pH. The investigators suggested that the process responsible for sorption was the electrostatic interactions between carboxylic anions and the positively charged sites like iron minerals and/or clay minerals. In general, the authors suggested that the specific surface area, pH and total carbon (TOC) determined the magnitude of the sorption affinity.

Sorption of phenols and nitrophenols onto aquifer material with an organic content of 0.038% was presented by Amiri et al. (2004). Five of the phenols studied (phenol, 4-nitrophenol, 2-chlorophenol, 3-chlorophenol, and 2,4-dimethylphenol) did not show significant sorption to the aquifer material; in contrast 2-methyl-4,6-dinitrophenol (MDNP), 2,4,6-trichlorophenol (TCP) and pentachlorophenol (PCP), exhibited significant affinity for the material. The study utilized flow-through column sorption experiments to evaluate the sorption of neutral or ionic species by varying the pH of the solution. In general, enhanced sorption at low pH indicated that neutral species of the solutes were responsible for the retardation rather than

the ionized species.

4.2.3 Sorption of Large Organic Macromolecules to Mineral Soils

Sorption of large organic macromolecules, like humic substances and polyacrylamides, to minerals has also been reported in the literature. The objective of some of these studies has been to investigate the effects of mineral-bound humic substances on the sorption of organic contaminants (Murphy et al., 1990; Murphy et al., 1994; Wang and Xing, 2005; Feng et al., 2006). Some other researchers have studied the sorption of humic substances to stabilize them on the mineral phase (Kalbitz et al., 2005), and to investigate the conformation of the HS-mineral complexes (Balcke et al., 2002, Juhna et al., 2003; Wang and Xing, 2005).

Murphy et al., (1990) were able to achieve carbon contents ranging between 0.01 and 0.5 % by allowing IHSS humic substances to sorb on kaolinite and hematite. Murphy et al. (1994) studied the sorption of humic acid to mineral phases as a function of pH, ionic strength and cation valance. These researchers reported a Langmuir-type isotherm (monolayer sorption) for HA sorption on kaolinite with greater amounts of HA sorbing at higher ionic strengths. However, the valence of the cation did not have any impact on sorption. Sorption of HA to hematite did not show significant differences with varying ionic strength until the HA reached a sorbed residue of 150 μmol C/m^2. Thereafter, higher HA sorption was reported to occur at lower ionic strength. The sorption behavior was explained by the formation of multiple adsorption layers and lateral interactions. HA sorbed more readily to hematite than kaolinite.

Juhna et al. (2003) reported that the amount of clay and low pH solutions enhanced the sorption of humic substances in aquifer material. Sorption to aquifer material was more pronounced for HA than for FA. The interaction between humic substances and the aquifer material was attributed to hydrophobic attractions. Wang and Xing (2005) reported the fractional sorption of HA to clay minerals. The aliphatic HA fraction adsorbed preferentially to the mineral surface while the aromatic fraction remained in solution. These researchers proposed that at low HA loads, the mineral-bound HA formed a more condensed structure on the mineral surfaces (due to closeness) while at higher HA loads the bound HA developed a more relaxed structure. Kalbitz et al. (2005) reported greater stabilization and sorption for aromatic, recalcitrant dissolved organic matter (DOM) than for labile DOM. While studying the conformation of HA-mineral complexes, Feng et al. (2006) observed that polymethylene groups were predominant at

the surface of the complex and that these groups enhanced sorptive interactions with hydrophobic organic chemicals. Formation of the HA-mineral complexes was not observed to be affected by the cation used in the sorption process, but it was affected by the solution chemistry (pH and ionic strength) and clay type.

The study of water soluble polymers like polyacrylamides has been of interest since they have been used in soil erosion and conditioning, water treatment and oil recovery. Sorption behavior of polyacrylamides and modification of mineral surface properties upon sorption of the polymers was reported by Deng et al. (2006). Anionic polymers were reported to increase the dispersion of clay particles, while the cationic polymer enhanced flocculation of the clays. The sorption of the polymers was reported to be irreversible independently of the type of polymer sorbed. Properties of the polymer-clay complexes (like hydrophobicity) did not differ significantly from those observed for clays alone. Cation exchange capacity was reported to be highest for the cationic polymer and lowest for the anionic one.

4.2.4 Phenol Stabilization in Mineral Soils via Oxidative Polymerization

The study of engineered humification to stabilize phenolic contaminants in mineral soils by the formation of nonextractable or bound residues has been sparsely reported in the literature (Shannon and Bartha, 1988; Kim, 2007). Some studies have described the immobilization of enzymes that catalyze the oxidative coupling of phenolic compounds like HRP and laccase, on mineral surfaces of clays and on soil, as a method to protect and preserve the activity of the enzyme and extend its catalytic life (Ruggiero et al., 1989; Gianfreda and Bollag, 1994; Pal et al., 1994; Park et al., 1999; Ahn et al., 2002). Other studies have addressed abiotic polymerization of phenols by means of minerals (birnessite), transition metal oxides (MnO_2, ZnO, CuO) and dissolved cations of Mn^{2+} and Fe^{3+} (Larson and Hufnal, 1980; Stone and Morgan, 1984; Kung and McBride, 1988; Colareti et al., 2002; Selig et al., 2003).

Impact of the presence of inorganic solids like silica gel, silica sand and clays on the peroxidase-catalyzed polymerization of phenol has also been discussed in the scientific literature (Wagner and Nicell, 2003; Huang and Weber, 2004). Wagner and Nicell, (2003) investigated the impact of the presence of silica gel, kaolin and bentonite on the HRP-mediated treatment of aqueous phenol. While silica gel had no effect on the conversion of phenol, other solids enhanced phenol transformation at pH of 5 and 7 at all solids concentration studied (100 to

10,000 mg/L).

To study the stability of HRP and H_2O_2 these researchers incubated both chemicals for 1 hr at pH 7 in presence of 10,000 mg/L of solids before initiating the reaction. When silica gel and kaolin were used, HRP and H_2O_2 were stable. Fresh suspensions of bentonite inactivated the HRP and reduced phenol polymerization. H_2O_2 pre-incubated with bentonite had no effect on phenol removal. However, exposure of bentonite to the aqueous phase prior to addition of HRP eliminated its ability to inactivate the HRP's activity.

Phenol solutions treated in presence of bentonite, and kaolin became less toxic than the controls with no solids added. However, kaolin and bentonite were unable to adsorb reaction products that were formed in solid-free aqueous solutions. These results suggested that these clays were able to interact with and partially deactivate precursors of toxic reaction products. Similar adsorption of phenol reaction products with talc, resulted in protection of HRP activity, higher phenol conversion, and improved removal of products from the aqueous phase (Arseguel and Baboulene, 1994)

Wagner and Nicell (2003) researched the oxidative polymerization of phenol in the context of water and wastewater treatment. Although no discussion of formation of nonextractable or solid bound residue was presented, their results provided important information that could also be applied to the soil environment. The presence of soil mineral phases could enhance the efficiency of the enzymatic polymerization process by reducing the amount of the residual soluble polymerization products and their toxic characteristics.

Huang and Weber (2004) studied the effect of silica sand on the formation of nonextractable polymerization products. Silica sand adsorbed the enzyme, forming enzyme-solid associations that reduced HRP inactivation. These researchers suggested that HRP associated preferentially with hydrophilic materials that contain abundant surface oxygen atoms. Formation of nonextractable products was slightly increased by raising silica concentration up to 200g/L.

Only two studies have reported data about enzyme mediated phenol stabilization in mineral soils (Shannon and Bartha, 1988; Kim, 2007). A study to determine the viability of employing chemical stabilization through enzymatic polymerization processes in a column aquifer system was performed by Shannon and Bartha (1988). Polymerization of 4-methylphenol (4-MP) and DCP induced in a sand-column system was found to enhance retention of both chemicals in Lakewood sand material with 90% sand and 1.3 % SOM content. Leaching of both

chemicals in a control (no enzyme) and a treated (with enzyme) column decreased with contact time. In the untreated column the amount of leached MP decreased from 98.1 to 70.7%, from zero to 21 days, respectively. Upon acidification of the leachate, humic precipitate was formed and ranged between 5.2 and 8.1% of the total ^{14}C activity added. Leachate was partitionated with CH_2Cl_2, and solvent extractable chemical from control column, ranged from 0.9 to 3.7 % and consisted of parent MP. Formation of bound residue was observed and it varied from 3.6 to 19.5 % within the 21 days of study. Minimal CO_2 evolution was measured throughout the experimental time.

The activity of MP measured in the leachate of the treated column was significantly less than in the control column (from 9.1 to 6.8%). Less humic precipitate was formed and it decreased with the progression of the experiment (from 4.1% at time zero to 2.3% at the conclusion of the experiment). However, most of the ^{14}C activity was solvent extractable (59 to 74 %), predominantly in the form of polymerization products with trace amounts of the unreacted MP. Bound residue formation was observed, and it decreased with increasing time from 17.7 to 10 %. The production of $^{14}CO_2$ was lower in the treated columns and it was attributed to the slower degradation rate of the polymerization products.

In the case of DCP, the leachate from the control column accounted for 91.6 and 63 % of total added chemical at zero and 21 days, respectively. After 21 days of experiment, half of the activity was associated with parent DCP while the other half was identified as water-soluble complexes of DCP. MP and DCP appeared to have experienced auto oxidation in controls columns; however, the authors did not provide further discussion. The material mass balance included some humic precipitate formed (0.7- 6.1%), the solvent extractable fraction (5.2 to 19.3 %), and a very small amount of bound residue (0.5 to 3.1 %).

In the case of the treated column, in the course of 28 days of study, the DCP leaching out decreased from 48.5 to 10 %. As time progressed, free-DCP activity in the leachate decreased to as low as 10 %, while the remaining activity corresponded to water-insoluble extractable polymers. Humic precipitate, resulting from acidification of the leachate, increased from 5.3 to 34.6 %, while the solvent extractable activity did not change significantly over time (26.9 to 23.1 %). Most of the activity detected in the solvent extractable fraction was associated with polymerization products and trace amounts to unreacted DCP. The soil bound fraction increased with time from 8.5 to 17.9 %. A further incubation study revealed that even after 4 more weeks

no remobilization of the bound products occurred.

The authors concluded that the enzymatic polymerization of organic compounds was a convenient method to prevent and control groundwater pollution, indicating that the efficiency of the process depended on the characteristics of the pollutant. No attempt to investigate the effect of sand on the polymerization reactions was made.

Kim (2007) evaluated the ability of the HRP mediated polymerization to remove phenol from aqueous solution under simulated aquifer conditions. The aquifer conditions were simulated by utilizing a saturated column, packed with Ottawa sand, and no organic carbon present in the porous media. The study included the effect of solution pH, ionic strength and enzyme dose on the polymerization efficiency and removal of phenol within the saturated porous media.

Removal efficiency at HRP doses of 0.5 and 1 AU/mL were similar and corresponded to a 70% reduction of the total phenol concentration. At a HRP dose of 2 AU/mL, the observed removal increased up to 90 %. The column effluent solution was observed to contain unreacted phenol and soluble polymers. Increasing the HRP dose resulted in higher production of soluble polymers, while lowering the concentration of unreacted phenol exiting the column.

Polymerization of phenol resulted in precipitation and accumulation of the insoluble polymers in the porous media. The accumulated mass of phenol polymers at 0.5, 1 and 2 AU/mL of HRP, was reported to be 51.1, 59.3 and 65.5 % of the total phenol entering the column system, respectively. Precipitation and accumulation of the phenolic polymers changed the pore volume of the media thus, changing the hydraulic properties of the system. The effect was observed to be more pronounced at higher enzyme concentrations. Thus, the higher the enzyme dose, the greater the reduction of the pore volume since greater deposition of polymers was observed. Pore volume was reduced by 5.8 and 7.8 % at HRP doses of 0.5 and 2 AU/mL, respectively.

The change of solution pH (5, 7 and 9) affected the amount of soluble products exiting the column although no consistent trend was reported. The highest concentration of soluble polymers exiting the system and insoluble polymers accumulated in the media was observed at pH 7, while the lowest concentrations were detected at pH 9. Kim (2007) suggested that at pH 9 there was minimal deposition of polymers on the media, thus, modification of its hydraulic properties was negligible.

The removal of phenol was observed to be higher at the ionic strength of 20 mM. The increase of solution ionic strength (5, 20 and 100 mM) was observed to decrease the amount of

soluble polymers produced and exiting the system. Deposition of the polymers also increased with increasing ionic strength. At 5 mM of ionic strength the system experienced 5% decrease of pore volume, while at 20 and 100 mM, the porosity decreased by 8%.

4.3 Research Goal and Hypotheses

The objective of the research reported in this chapter was to evaluate the sorption and extractability of DPP produced in the presence of model inorganic sorbent containing negligible SOM (0.1% SOM). The parent surface agricultural soil described in the previous chapter was treated with NaOCl to reduce its natural organic matter to < 0.1% SOM. The NaOCl oxidation technique ensured that the integrity of the original soil inorganic fractions was maintained. A comprehensive description of the NaOCl oxidation method is presented in the material and methods section of this chapter

The following research hypotheses were evaluated in this chapter:

Hypothesis 5

HRP-mediated oxidative coupling of DCP in the presence of SOM-free (mineral) geomaterial results in the formation of DPP.

Hypothesis 6

DPP macromolecules are removed from the aqueous phase as a result of sorption and deposition on mineral particle surfaces.

Hypothesis 7

DPP molecules associate strongly with mineral surfaces and are resistant to extraction by water and methanol.

Hypothesis 8

Post-polymerization contact time between the DPP-containing aqueous phase and mineral particles enhances the formation of nonextractable DPP residues.

Hypothesis 9

HRP-mediated removal of aqueous DCP is influenced by the presence/absence of SOM in the

geosorbent particles.

4.4 Material and Methods

4.4.1 Soil

4.4.1.1 Oxidation of SOM with Sodium Hypochlorite

The objective of this procedure was to reduce the content of SOM in the agricultural soil to a value of less than 0.1%, so that sorption of organic molecules would be minimally impacted by the sorbent organic matter (Pignatello, 1989). Regular household bleach (Sun Flo Bleach, product from Kansas Correctional Industries) containing 5.25 % by weight of sodium hypochlorite (NaOCl) was utilized as the oxidant (Hettiarachchi et al., 2003). The pH of the bleach was adjusted to 8.5 with hydrochloric acid. Forty grams of dried soil material were placed in 500 ml volumetric flasks. Two hundred milliliters of the pH 8.5 bleach solution were added to flasks containing dry soil material. The contents of the flasks were mixed and placed inside a water bath heated to 90 ± 5 °C. The flasks were mixed every 20 minutes for two hours. At the end of the two hours, the flasks were allowed to cool and the contents transferred into plastic bottles for centrifugation for 20 minutes at 1370 g. After centrifugation, the supernatant was discarded and another oxidation cycle initiated by addition of 200 mL of pH 8.5 bleach solution (Hettiarachchi et al., 2003). Six oxidation cycles were required to produce a SOM content lower than 0.1%

The oxidation cycles were followed by washing the solids with 0.01 M Ca $(NO_3)_2$ to remove the excess NaOCl left in the soil material. The contents of each flask, containing the original 40 grams of soil, were washed with a total 1-L of the $Ca(NO_3)_2$ solution. A total of 5 washing cycles per sample were performed. One washing cycle consisted of adding 200 mL of the $Ca(NO_3)_2$ solution to each bottle, mixing the contents thoroughly by means of a vortex mixer and continuing mixing by hand shaking for 2 minutes (Hettiarachchi et al., 2003). Washing was followed by centrifugation and disposal of supernatant as explained previously. After the contents of the flasks had been washed with 1 L of the $Ca(NO_3)_2$ solution, the bottles containing the sample were dried at 78 °C. Dried soil material was homogenized by means of mortar and pestle and stored in the refrigerator at 1 °C until further use. Selected properties of the original, alkali extracted and NaOCl oxidized soil are presented in Table 4.1.

Table 4.1. Selected properties of the original, alkali extracted and NaOCl oxidized soil

Property		Sorbent		
		Original	Alkali Extracted	Oxidized
Organic matter %:				
	total	3.40 [a]	1.10 [a]	0.05 [b]
Texture:				
	Sand %	32	12	8
	Silt %	52	74	64
	Clay %	16	14	28

[a] determined by the Walkley-Black Method

[b] determined by Leco procedure

4.4.1.2 Mineralogical Analysis of the Geosorbents

Mineralogical analysis of the parent agricultural soil, the SOM-free agricultural soil and the clays separated from both geomaterials were conducted by means of X-ray diffraction (XRD). The purpose of performing mineralogical analysis of the parent agricultural soil and SOM-free soil was to identify any difference in mineralogy between the two samples. Separation of the clay fraction < 0.2 μm was achieved by following the methods of Jackson (1975). Forty grams of parent soil (3.4 % SOM) were treated with NaOAc and 30 % H_2O_2 to remove carbonates and soil organic matter, respectively.

The sand fraction was collected in the 300-mesh sieve and the subsequent dispersion and fractionation of silt and clay minerals was done through eight sedimentation times. Stoke's law was utilized to calculate the length of the sedimentation step for a 10 cm vertical fall (Jackson, 1975). Flocculation of the clay minerals was achieved by addition of $MgCl_2$.

The samples were submerged in a bath of dry ice and acetone for quick freezing that was followed by freeze drying process. Mineralogical analyses of clay fractions from the parent agricultural soil and the SOM-free soil were also conducted to determine if NaOCl oxidation caused an alteration of the mineralogical properties of the particles compared to that observed after treatment with H_2O_2. Fractionation of sand, silt and clay was done similarly as for the parent agricultural soil.

XRD analysis was performed by Technology of Materials (Wildomar, CA) with a Phillips diffractometer at potential of 30 kV, and with a current of 20 mA, using Cu K-α radiation and a scintillation detector. Bulk soil sample was run after grinding it to pass through a 325 mesh (44 μm) sieve and scanned from 3°2θ to 50°2θ. The resulting patterns collected on a computer were matched with reference standards for various inorganic minerals stored in the database. Semi-quantitative estimation of mineral components was carried out from the peak intensities.

A clay suspension was used to make oriented clay mounts on a Millipore filter. The suspensions were filtered, through a 0.45 μm filter paper on a Millipore filter set-up, using vacuum. Particles were washed thoroughly with distilled water to remove excess salts. The clay cake on the filter paper was transferred, while still wet, onto a glass slide and kept in an ethylene glycol chamber for 24 hours. A drop of glycol was placed on the edge of each slide before placing them in the chamber.

4.4.2 Preliminary Experiments

This section describes three preliminary studies performed to improve experimental protocols. (1) The purity of bleach was evaluated to ensure that no phenolic compounds were present in the NaOCl solution; (2) The optimum solid/liquid ratio for sorption experiments was estimated by determininig the amount of sorbent that would cause 30 and 70% removal of solute; and (3) a DCP polymerization study was performed at two solute concentrations to compare the sorption behavior in the presence of oxidized agricultural and woodland soils.

4.4.2.1 Purity of NaOCl Solution

The purity of the commercial bleach solution was determined by HPLC. This test was performed to ensure that the bleach did not contain an impurity or inert substance that absorbed light at the same wavelength as DCP. Prior to HPLC analysis a sample of bleach was dechlorinated with sodium sulfite (1,775 parts of Na_2SO_3 per part of chlorine). Dechlorination was confirmed by testing for free chlorine with the Free and Total Chlorine Test kit (Mod. CN-66/66F-66T, HACH). The test was done by adding N,N-diethyl-p-phenylenediamine (DPD) indicator to the bleach sample. When free chlorine is present, it reacts with DPD indicator producing a pink color. The lack of color after addition of DPD indicated dechlorination of the sample. The purpose of the dechlorination was to prevent potential column damage by the highly

reactive free available chlorine (hypochlorite ion). The HPLC chromatograph revealed that there was no DCP in the bleach solution that could interfere with the experimental analysis. The HPLC analysis was performed using conditions as described previously in section 3.4.5.

4.4.2.2 Solid/Liquid Ratio Determination

A solid/liquid ratio determination study was conducted with the SOM-free soil material to estimate the amount of sorbent needed in each reactor to observe 30 to 70% sorption of DCP. A total of five different solid/liquid ratios were used: 1, 1.5, 2, 2.5 and 3 g of the sodium hypochlorite treated soil in a 10 mL test tube as CMBR. The experiment was performed according to the approach described in section 3.4.3.

4.4.2.3 DCP Polymerization

Once the solid/liquid ratio was determined, polymerization of DCP was induced in the presence of SOM-free agricultural and SOM-free woodland soils at two different aqueous DCP concentrations (50 and 500 μM). The objective of this study was to determine if there was a difference in solute sorption behaviors exhibited by the SOM-free agricultural soil and the SOM-free woodland soil sorption. The study was conducted according to the approach described in section 3.4.3.

4.4.3 Sorption Experiments

The procedure described in section 3.4.3 was utilized in this study to perform the sorption experiments.

4.4.4 Water and Methanol Extraction

The procedure described in section 3.4.4 was utilized in this study to perform the water and methanol extractions.

4.4.5 Sample Analysis

The procedure described in section 3.4.5 was utilized in this study to perform the analysis of all the samples.

4.5 Results and Discussion

4.5.1 Soil Analysis

4.5.1.1 Oxidation of SOM with Sodium Hypochlorite

The SOM content of a small portion of the oxidized sample was determined by the Walkley-Black method (Nelsom and Sommers, 1996) after the first and second NaOCl oxidation cycles, and by the Leco procedure (Nelsom and Sommers, 1996), after the third and through the ninth cycle. The Leco procedure was utilized in samples containing less than 0.2 % SOM since that value represented the detection limit for the Walkley-Black method. Results from the oxidation cycles with NaOCl are shown in Figure 4.1. Results indicated that six oxidation cycles were sufficient to lower the SOM below 0.1%.

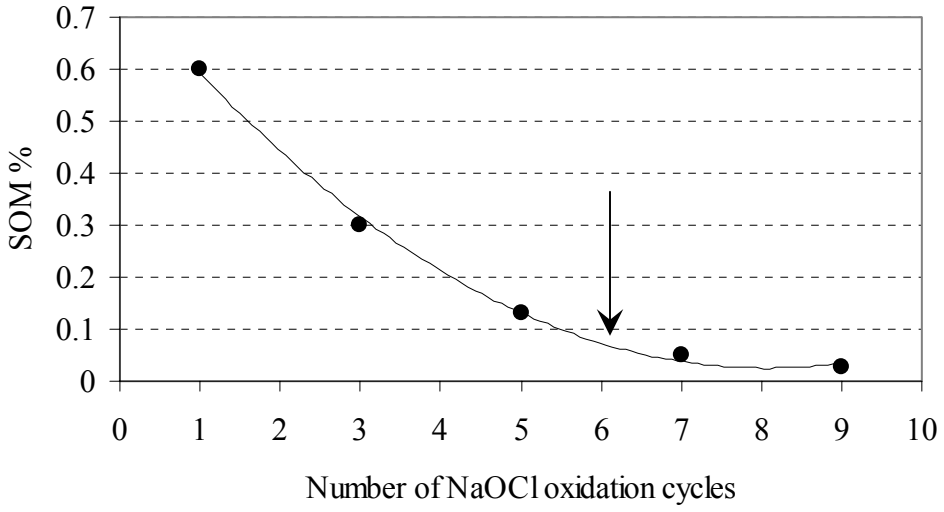

Figure 4.1. Oxidation of agricultural soil (3.4 % SOM) with NaOCl

4.5.1.2 X-Ray Diffraction Analysis of Geosorbents

The purposes of the mineralogical analysis were (1) to characterize the clay minerals present in the SOM-free soil, and (2) to evaluate the ability of the NaOCl oxidation to maintain integrity of the soil minerals as compared to the traditional H_2O_2 oxidation of SOM.

Mineralogical analysis revealed that the parent agricultural soil and SOM-free soil did not exhibit significant differences. The samples contained quartz (SiO_2) as their predominant mineral constituent, followed by feldspars (mainly Ca-Na feldspars), while clay minerals comprised a

small percentage of the geomaterials. The XRD spectrum of the parent agricultural soil is shown in Figure 4.2, while the spectrum for SOM-free soil is shown in Figure 4.3.

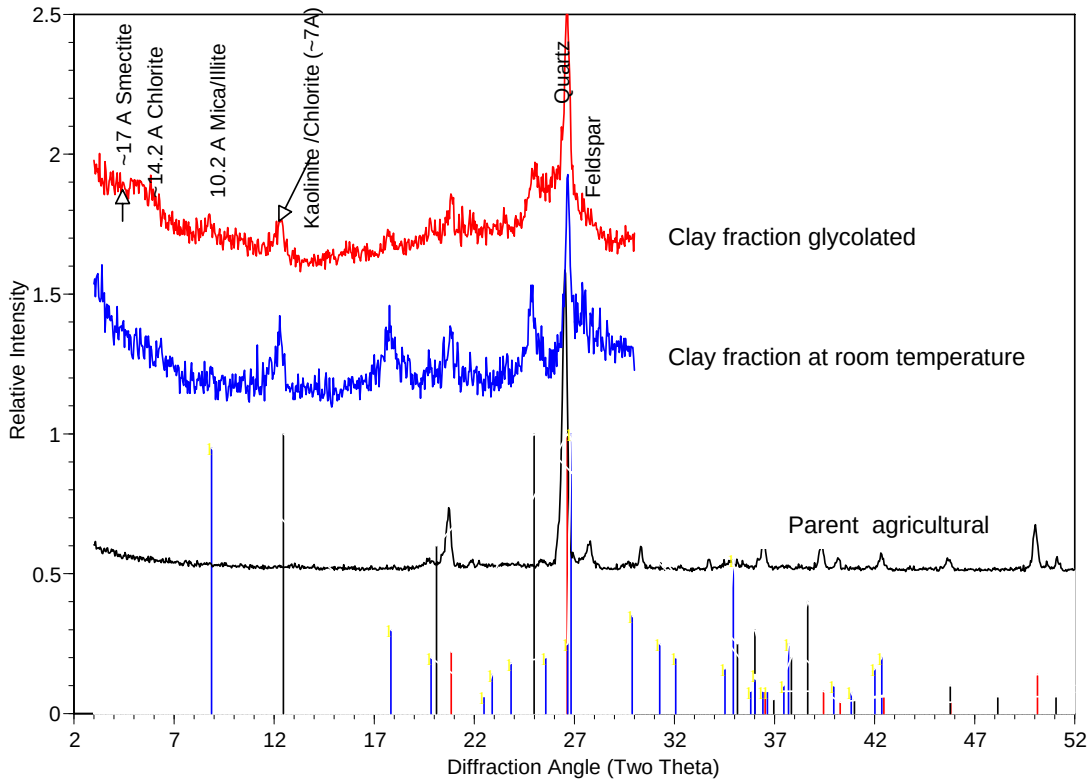

Figure 4.2. XRD patterns for bulk agricultural soil (black), glycolated clay fraction (red) and room temperature clay fraction (blue). Stick patterns for reference minerals are shown as vertical lines at the bottom on the figure, where quartz (red), mica (blue) and kaolinite (black) are shown.

The clay fractions associated with these geomaterials had similar mineralogy. For both clay minerals, the presence of smectite was confirmed by a small peak observed around 17.1 A. Chlorites were observed by the peak around 14 A. Mica/illite showed peaks around ~10 A and ~5 A, while kaolinite was identified by the peak around 7.2 A and 3.57 A. Clay-sized fractions of both samples are dominated by quartz and contain kaolinite (~10%), mica/illite (~5%), smectite (~2%), and vermiculite (~2%).

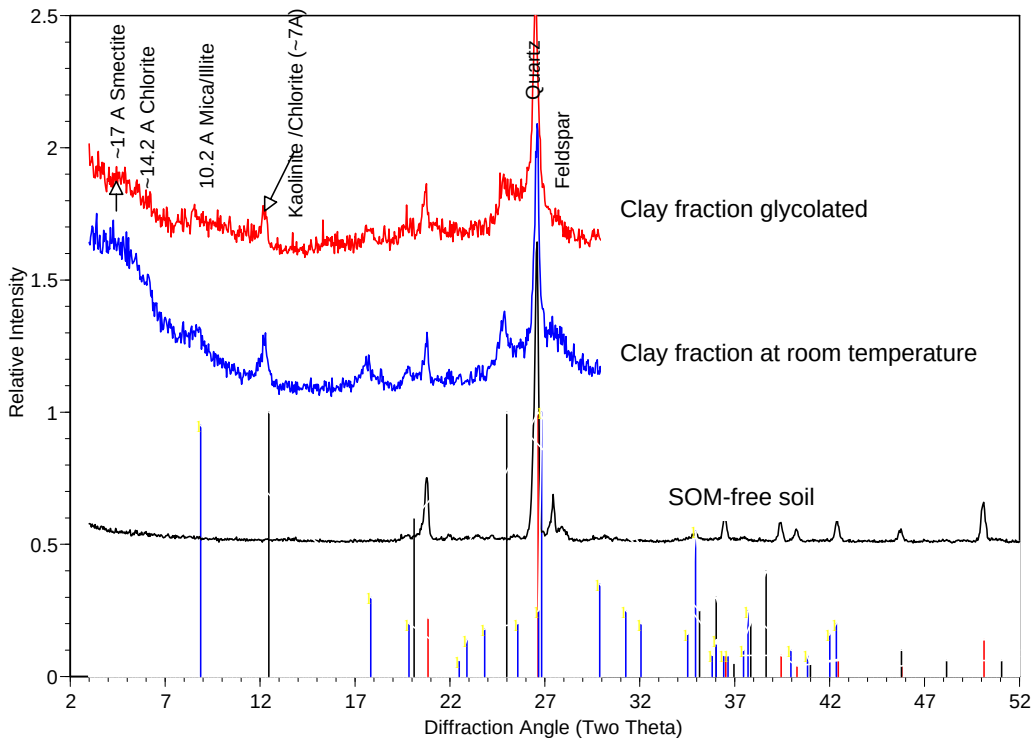

Figure 4.3 XRD patterns for SOM-free soil (black), glycolated clay fraction (red) and room temperature clay fraction (blue). Stick patterns for reference minerals are shown as vertical lines at the bottom on the figure, where quartz (red), mica (blue) and kaolinite (black) are shown.

The abundance of SiO_2 in the bulk and SOM-free soil suggested that quartz was the main mineral in the samples. While the presence of Ca-Na feldspars (with the general formula **X** $Al(Al,Si)Si_2O_8$, where **X** can be Na, K, Ca or Ba) suggested that plagioclase feldspars like albite, and anorthite (Na-Ca type of feldspars) were present in the samples. As reported by Technology of Materials, these types of feldspars are softer than quartz and slightly reactive. The presence of 1:1 and 2:1 mineral structures was confirmed by the presence of kaolinite and mica/illite, respectively.

4.5.2 Preliminary Experiments

4.5.2.1 Purity of Bleach

Standards of known DCP concentrations were analyzed along with bleach samples to help determine the concentration of any substance with similar elution time and UV absorption as DCP. The HPLC chromatographs indicated that such compounds were not present in the

bleach solution. These results ensured that pre-loading of a phenolic compound would not occur during oxidation of the soil with bleach.

4.5.2.2 Solid/Liquid Ratio Determination

Sorption of DCP was found to be similar in magnitude at all studied solid/liquid ratios. Figure 4.4 illustrates the trend between the aqueous phase concentration of DCP and its solid phase concentration for the two SOM-free soils. Less than 10% sorption of DCP was observed at all the solid/liquid ratios and for both soils. Higher solid/liquid ratios were considered impractical for the experimental procedure used. Due to very small observed sorption of DCP, further phase distribution experiments were not conducted for DCP in contact with SOM-free soils.

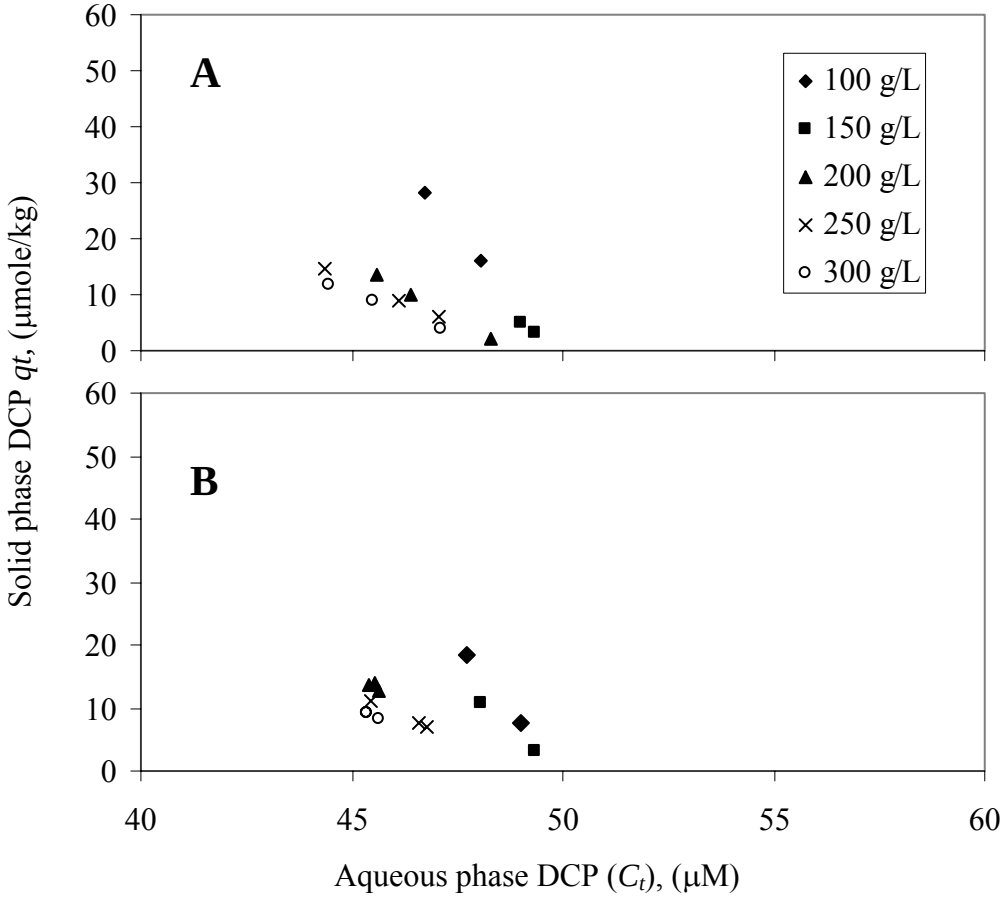

Figure 4.4. Relationship between DCP aqueous phase concentration and DCP solid phase concentration at different solid/liquid ratios. A) oxidized woodland soil, B) oxidized agricultural soil.

4.5.2.3 DCP Polymerization

The objective of this experiment was to investigate DCP polymerization in the presence of the SOM-free agricultural and woodland soils. Two different initial aqueous DCP concentrations were selected (50 and 500 μM). Figure 4.5 illustrates the relationship between the aqueous phase and solid phase DPP concentrations.

The results revealed that the amount of solute associated with both SOM-free soils was statistically similar. Thus, the differences in interactions between solute with SOM-containing materials in HRP-amended systems (seen in previous studies) were mainly due to the amount and type of organic matter present in each sorbent. These differences disappeared once most of the SOM was removed from the soils. Thus, it was decided that subsequent solute phase-distribution and extraction experiment would be conducted with one of the two SOM-free soils. The agricultural soil was selected for these experiments.

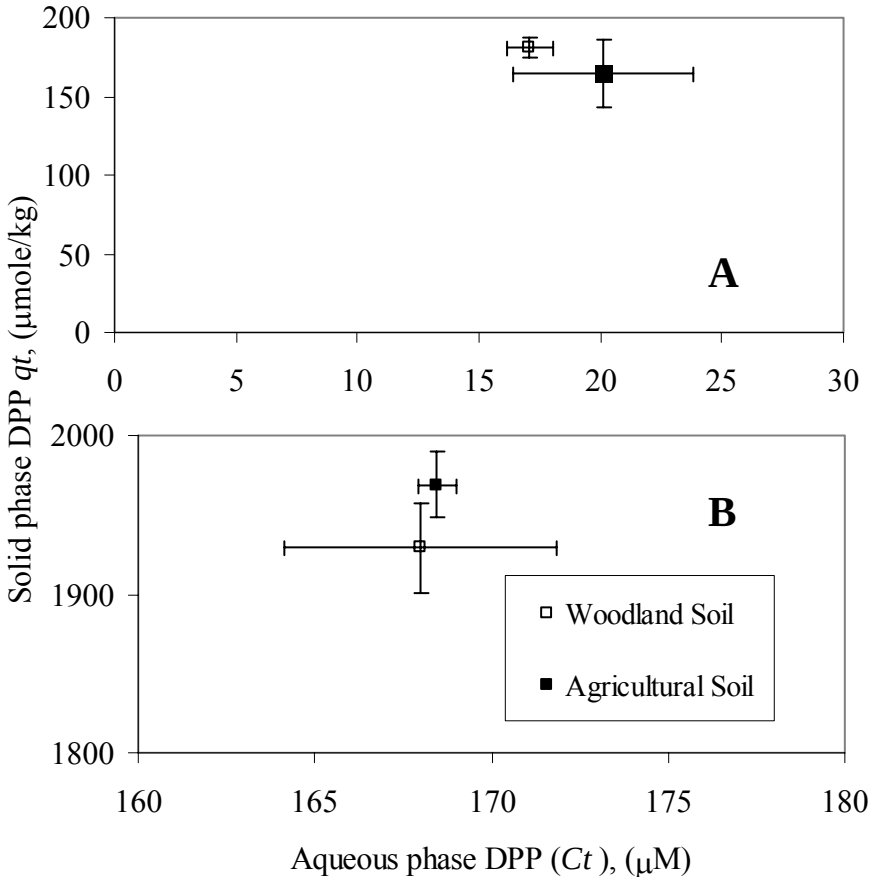

Figure 4.5. Relationship between aqueous and solid phase solute at a solid/liquid ratio of 250 g/L and at two different initial aqueous DCP concentrations in presence of enzyme. A) C_o= 50 μM and B) C_o=500 μM.

4.5.3 Solute Sorption in HRP-Amended Systems containing SOM-Free Geomaterial

Contamination of soil with phenolic chemicals is not exclusive to surface soils that contain significant amount of organic matter. Phenolic compounds may be present in predominantly mineral soils located at the soil surface or in the subsurface of the earth. Thus, the investigation of HRP-mediated oxidative polymerization of DCP in the context of predominantly mineral materials is of importance. It has been shown that in presence of organic matter when enzymatic polymerization of DCP is applied, a significant amount of solute can associate with soil. The extractability of solute (unreacted DCP +DPP) from solid phase was limited, and a large portion of solute is stabilized in the soil. The objective of the present study was to investigate how sorption, extractability and contaminant stabilization are impacted when oxidative polymerization of DCP is induced in solution in the presence of a predominantly mineral soil. As in previous chapters, the postpolymerization solute-solid contact time was also evaluated as an important variable that might affect the fate of dichlorophenol polymerization products.

4.5.3.1 Solute Phase Distribution

The distribution of solute between the aqueous phase and the mineral soil was studied by means of solid-liquid phase distribution. Figure 4.6 illustrates the phase distribution curves obtained at three different contact times. Analogous to what was seen for SOM-containing sorbents, phase distribution equilibrium of total solute in the system, was reached within 24 hours of contact with the mineral soil as noted by the three overlapping lines (Figure 4.6). When compared to solute phase distribution results obtained for the humin-mineral geomaterial in

Figure 3.10, phase distribution produced in the presence of SOM-free soil were shifted to lower q(t) and higher C(t) values at lower concentrations, while they remained similar at the highest residual aqueous concentration. These results indicate that at lower solute aqueous concentrations, less solute was associated with the SOM-free soil, compared to the humin-mineral soil, while at higher concentration the amount of solute sorbed were comparable. Although it is difficult to interpret precise mechanistic behavior from phase distribution results, it is possible that at low solute concentrations, adsorption dominated the interaction of solute with the sorbent (fewer polymers are produced and less sorbent's surface coverage occurs at lower

concentrations), while at higher solute concentrations, precipitation of DPP rather than solute sorption appeared to be responsible for the observed phase distribution. DPP precipitation at high solute concentrations was the likely cause for similar phase distribution behaviors at high concentrations observed for the SOM-free soil and the humin-mineral soils.

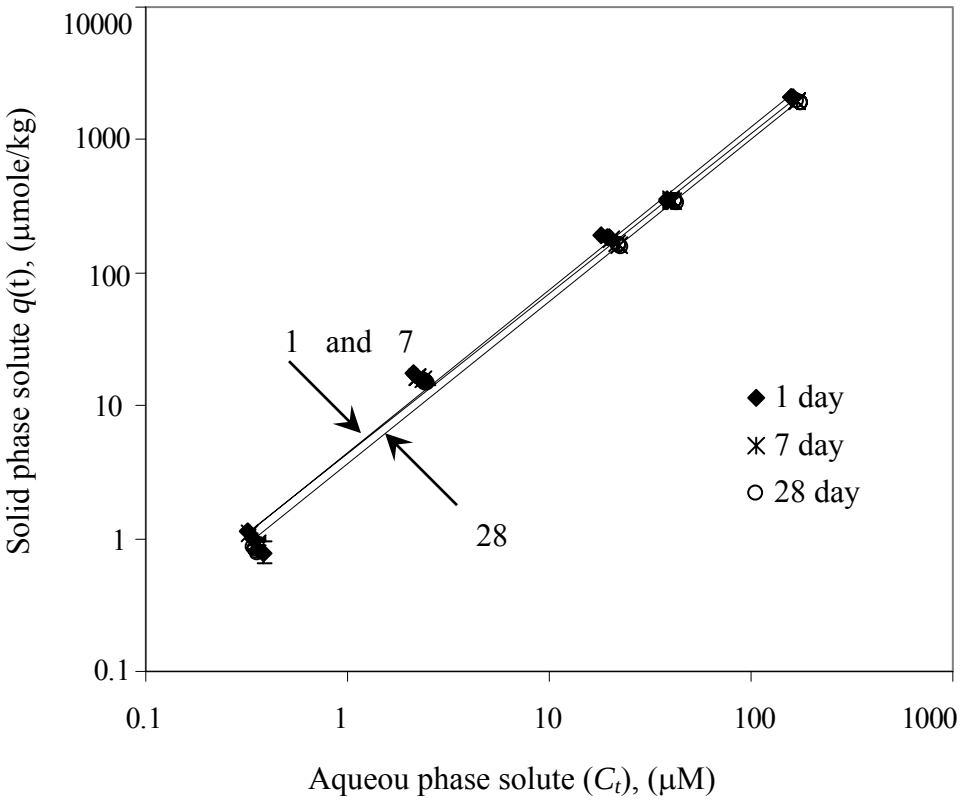

Figure 4.6. Solute phase distribution (DCP+DPP) in HRP-amended systems at 1, 7 and 28 days, SOM-free soil

The Freundlich parameters obtained fitting the Freundlich model to solute sorption data are listed in Table 4.2. As a result of the rapid equilibrium observed in the system, the n(t) and K_F(t) values did not change from 1 to 28 days of contact time. The average observed values for n(t) and K_F(t) were 1.2 and 4.1 (μmole/kg)*(μM)$^{-1.219}$.

The value of n(t) was higher than unity, indicating that the sorbent surface was modified by the solute in a way to facilitate further removal of solute (DCP+DPP) from the aqueous phase. Since the values of n(t) for solute distribution between water and SOM-free soil were different

from the ones for humin-mineral soil no appropriate comparison can be made between their respective Freundlich sorption capacity parameters. Wagner and Nicell (2003) reported that clays like bentonite and kaolin interacted with phenol polymerization products and enabled their removal from solution.

Table 4.2. Freundlich parameters for solute sorption on NaOCl treated soil. 95% Confidence limits are included in parenthesis.

Contact Time (days)	SOM-free soil		
	n(t)	***K_F (t)***	**R^2**
1	1.227 *(0.061)*	4.491 *(1.22)*	0.992
7	1.204 *(0.051)*	4.298 *(0.99)*	0.994
28	1.226 *(0.047)*	3.580 *(0.78)*	0.996

* Units for $K_F(t)$ are $\frac{\mu mol}{kg}(\mu M)^{-1.219}$

Single point sorption distribution coefficients, K_D were calculated as described in section 3.5.3.1.2. The units of K_D (L/kg), represent the volume of water that would contain the same quantity of DPP as one kilogram of soil after *t* days of contact. K_D values for SOM-free soil did not required normalization with respect to the organic matter content. These coefficients are summarized in Table 4.3.

The single point sorption distribution coefficient obtained for the SOM-free soil, increased at higher residual aqueous phase concentrations. At all residual solute concentrations, the K_D values were smaller than those observed for AHM and WHM. K_D values shown in Table 4.3 for WHM and AHM were not normalized to the SOM content. As the aqueous solute concentration increased, the mass of solute sorbed to WHM and AHM decreased, where WHM had the higher K_D at all concentrations. Additionally, at higher aqueous solute concentrations, the retention capacity of SOM-free soil increased and became similar to that of AHM. This was likely due to the formation of larger oligomeric products at higher DCP concentrations, which were more likely associated with the sorbent via precipitation rather than through sorption, therefore, solute removal via HRP-oxidative polymerization was not affected by the sorbent

SOM content.

Table 4.3. Single point sorption distribution coefficients, K_D from the 7 days of contact time data.

Residual Aqueous Concentration (μM)	Sorption Distribution Coefficients		
	WHM	AHM	SOM- free soil
10	28	21	7
100	25	18	11
200	24	17	13
300	23	17	14

* Units of K_D are L/kg

4.5.3.2 Reverse Mass Transfer of Solid-Associated Solute

The reverse mass transfer of solute associated with the SOM-free soil was studied and analyzed by means of the extractability index described in previous sections. It is important to recall that increased difficulty of mass transfer is indicated by small values of *EI.* While high positive values of *EI* indicate a reversible sorption behavior of the solute, negative *EI* values are indicative of extreme difficulty of solute extraction.

The sorption and desorption phase distribution curves are shown in Figure 4.7. It is apparent from the figure that *EI* values were affected by concentration and contact time. Solute extractability was lower (more negative *EI* values) at smaller residual aqueous concentrations. This behavior was opposite to what was observed for solute desorption from AHM soil. *EI* values obtained for SOM-free soil were always smaller than those observed for the AHM geomaterial (Figure 3.12) at all times and concentrations. The solute reverse mass transfer from the mineral soil into the aqueous phase was more difficult than from the aliphatic-rich, humin-mineral geomaterial. Furthermore, the values of the *EI* were observed to decrease with increasing postpolymerization contact time indicating greater difficulty of solute desorption from the SOM-free soil. The values of the *EI* are summarized in Table 4.4. The decline was larger at lower residual aqueous concentrations. These results are important because they support the idea that even in the absence of SOM, HRP-mediated oxidative polymerization of phenolic contaminants can be an effective process in stabilizing organic contaminants within soil matrices. The difficulty of solute removal from the solid was attributable to precipitation of oligomers with reduced solubility and to the strong interactions of the polymers with the mineral phase, and

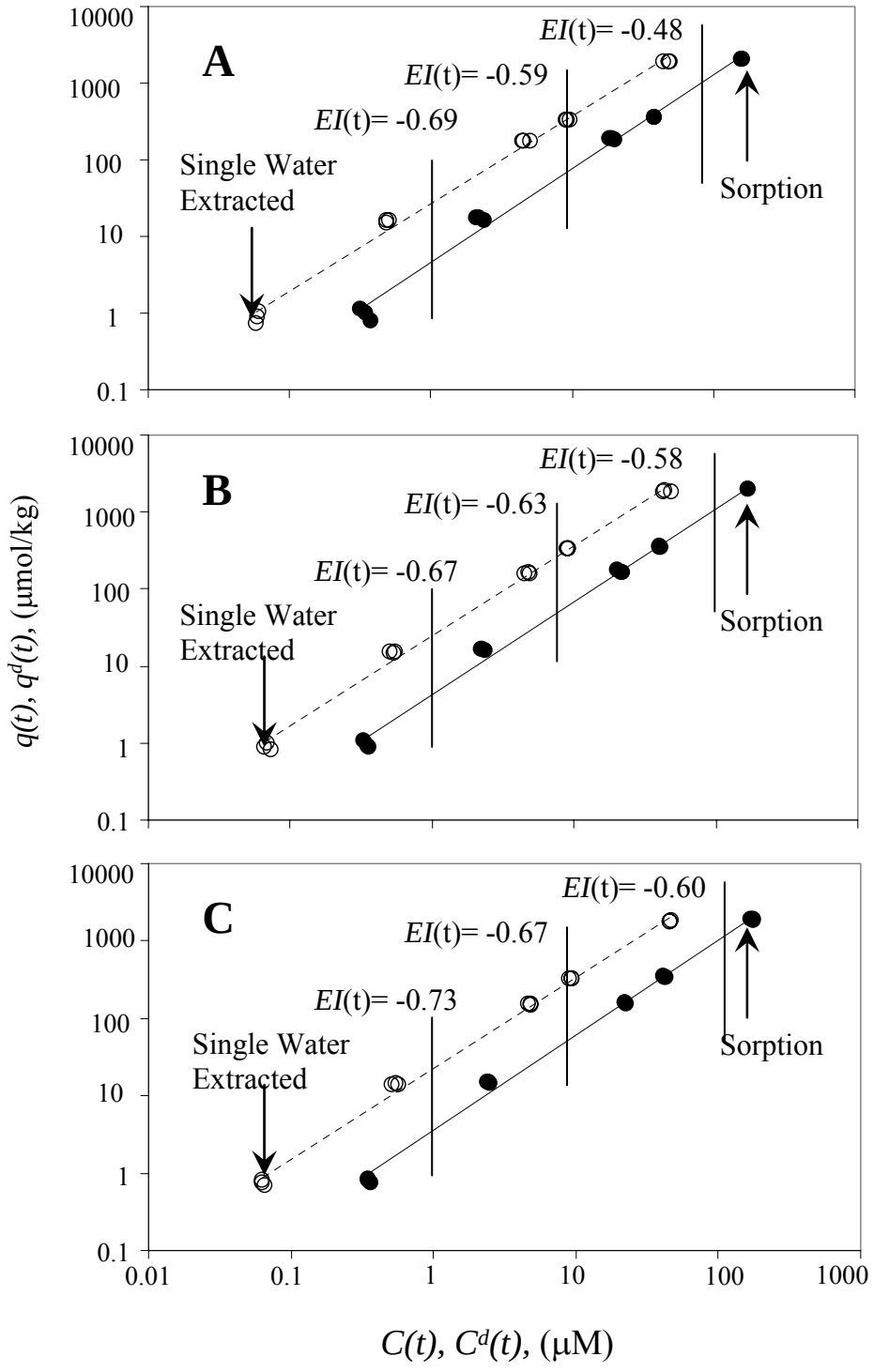

Figure 4.7. Solute desorption after at A) 1, B) 7 and C) 28 days, SOM-free soil

Table 4.4. Solute (DCP+ DPP) extractability index from SOM-free soil.

Contact Time (days)	SOM-free soil		
	EI_1	EI_{10}	EI_{100}
1	-0.69	-0.59	-0.47
7	-0.67	-0.63	-0.58
28	-0.73	-0.67	-0.60

possible coating of mineral particles. However, at low solute concentrations, the interaction between oligomers and minerals are likely to be stronger since fewer polymers are in solution, thus, reducing competition for the high site energy sites on the mineral domain. Hence, due to strong interaction between polymers and minerals at lower concentrations, the solute desorption is reduced. These results suggest that surface adsorption to minerals is the mechanisms controlling the retention of solute in the SOM-free soil.

It is evident that at 1 μM aqueous concentration, the temporal change in *EI* was very small, from -0.69 to -0.73. However, at 10 and 100 μM, longer contact periods made solute reverse mass transfer increasingly difficult. At 100 μM the *EI* varied from -0.48 to -0.60. Therefore, the lowest *EI* value was observed at the lowest solute concentration and at the longest contact time.

These results suggest that the reactive sites on the mineral surfaces of the sorbent, which have been exposed after removal of the SOM, interact strongly with the phenolic polymerization products. At the lowest concentrations, solute molecules (DCP +DPP) interact preferentially with high energy sorption sites, thus, stronger interactions are formed. These stronger interactions are proposed to be similar to those naturally occurring between humin and minerals (Kononova, 1966; Shah et al., 1975; Theng, 1979), or between minerals and aliphatic HA as proposed by Wang and Xing (2005). Interactions that have been attributed to stables links between the SOM molecules and the minerals, leading to condense configuration of SOM. However, at higher concentrations, a small fraction of the oligomer molecules form strong interactions with the minerals while the rest are associated less strongly. The increased ease of solute desorption at higher concentrations suggested that the abundant and less strongly associated oligomers may be

most likely adsorbed to or precipitated on the surface of the DPP-mineral complex.

4.5.3.3 Solute Stabilization in SOM-Free Soil

The effect of initial solute concentration (C_o) and contact time on the stabilization of solute was evaluated by means of a distribution analysis of the solute among sorbed, solvent extractable and soil stabilized fractions. The distribution data is shown in Figure 4.8. It is important to emphasize that the fractional distribution is not equivalent to mass of solute found in every phase. The objective of the fractional distribution is to characterize the total solute associated with the solid phase. Figure 4.8 shows the solvent extractable and stabilized solute. Solvent extractable represents the water extractable solute + solute removed after three consecutive methanol extractions.

The effect of initial solute concentration is evident at the three contact times. The lowest fractional extraction of sorbent associated solute was observed at lower initial concentrations. Thus, it looks like at lower solute concentration, solute molecules interacted more strongly with mineral surfaces that were exposed after oxidation of SOM.

Effect of contact time is also evident from Figure 4.8 at all initial solute concentrations. Although apparent sorption equilibrium was observed to have been reached within one day, the fractional distribution data suggest that equilibrium at the particle scale was not reached in 28 days. At 5 μM C_o, the solvent extractable fraction declined from approximately 0.57 to 0.32 for 1 to 28 days of contact, respectively. In other words, about 25% more solute was stabilized in the mineral matrix. At 50 μM C_o, the solvent extractable solute experienced a decrease of approximately 20% (from 0.65 to 0.45) in 28 days. While at the highest C_o, the extractable solute reduced from 0.75 to 0.60 or 15 % less solute was removed from soil. Thus, even in the absence of SOM, postpolymerization contact period appeared to exert significant influence on the retention of oligomeric products in soil. It is likely that besides precipitation, DPP are likely to be retained on mineral surfaces through hydrophobic interactions (Specht et al. 2000).

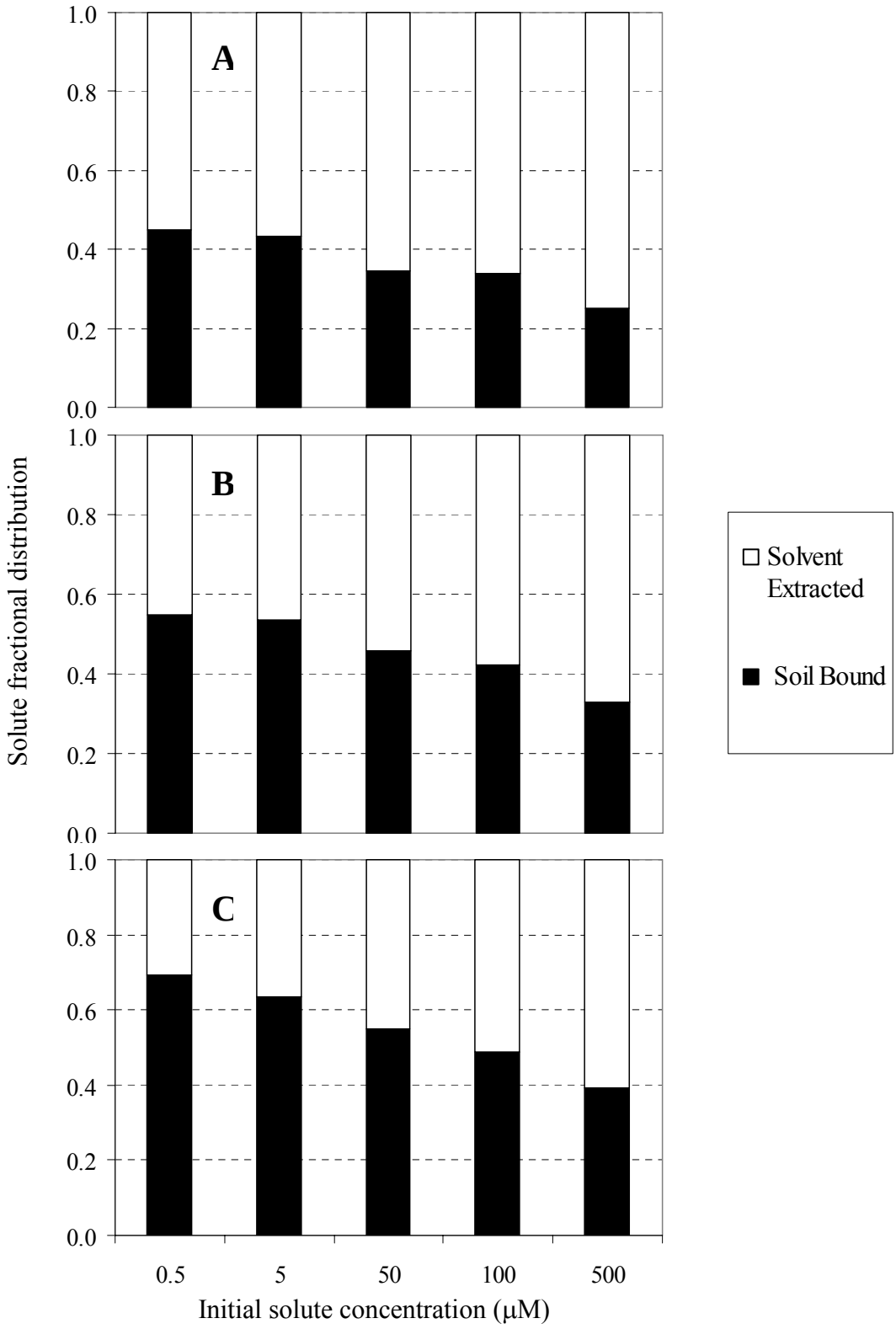

Figure 4.8. Effect of initial aqueous concentration on the fraction of extractable and soil-bound solute. A) 1, B) 7 and C) 28 days of contact time, SOM-free soil.

Dec and Bollag (1994) reported that the resulting dichlorophenol polymers beared hydroxyl moeties. Thus, besides hydrophobic interactions, the retention of DPP on the mineral surfaces may be caused by DPP hydroxyl moities that could undergo ligand exchange with the metal oxides of the mineral surfaces. Ligand exchange between humic substances (oxygen of hydroxyl or carboxylic groups of HA) and metal oxides, between humic substances and mineral surface hydroxyl groups, or between water of hydration and HS has been reported in the literature (Parfitt, 1977; Yost et al. 1990; Biber and Stumm, 1994).

The high retention of organic chemical in predominantly mineral soils was explained differently by Garbarini and Lion, (1986). These researchers suggest that H_2O_2 oxidation of SOM resulted in a type of residual SOM that was capable of binding organic solutes more effectively

4.5.3.4 Time-Dependant Distribution of Solute

Time-dependent distribution of mass of solute associated to the SOM-free soil, per kilogram of soil is presented in Figure 4.9. HRP-mediated DCP stabilization is evident since the mass of solute extracted decreased significantly from 1 to 28 days of contact, at all studied concentrations. After 28 days at C_o= 0.5 μM, the extractable solute decreased from 0.53 to 0.25 μmole/ kg of soil; at 50 μM from 122 to 71 μmole/ kg; and at 500 μM from 1552 to 1150 μmole/ kg. Therefore, contaminant stabilization was impacted positively by postpolymerization contact period.

When results of this study are compared with HRP-mediated DCP stabilization in the context of AHM soil, it is interesting to note that the mass of solute stabilized in SOM-free soil is comparable to that bound to AHM. Considering only the bound residues formed at the completion of the experiment, at 0.5 μM, the μmole of solute/ kg of soil are 0.68 and 0.56; at 5 μM, 9.3 and 9.3; at 50 μM , 96 and 87; at 100 μM, 185 and 167 and finally at 500 μM, 636 and 745, for AHM and SOM-free soil respectively. It is generally not expected to observe higher bound residue in predominantly mineral soil than in a soil with significant content of SOM, since SOM not only directly interacts with organics but through the HRP-mediated oxidative polymerization reaction also facilitates cross-coupling with phenolic chemicals (Hatcher, et al. 1993).

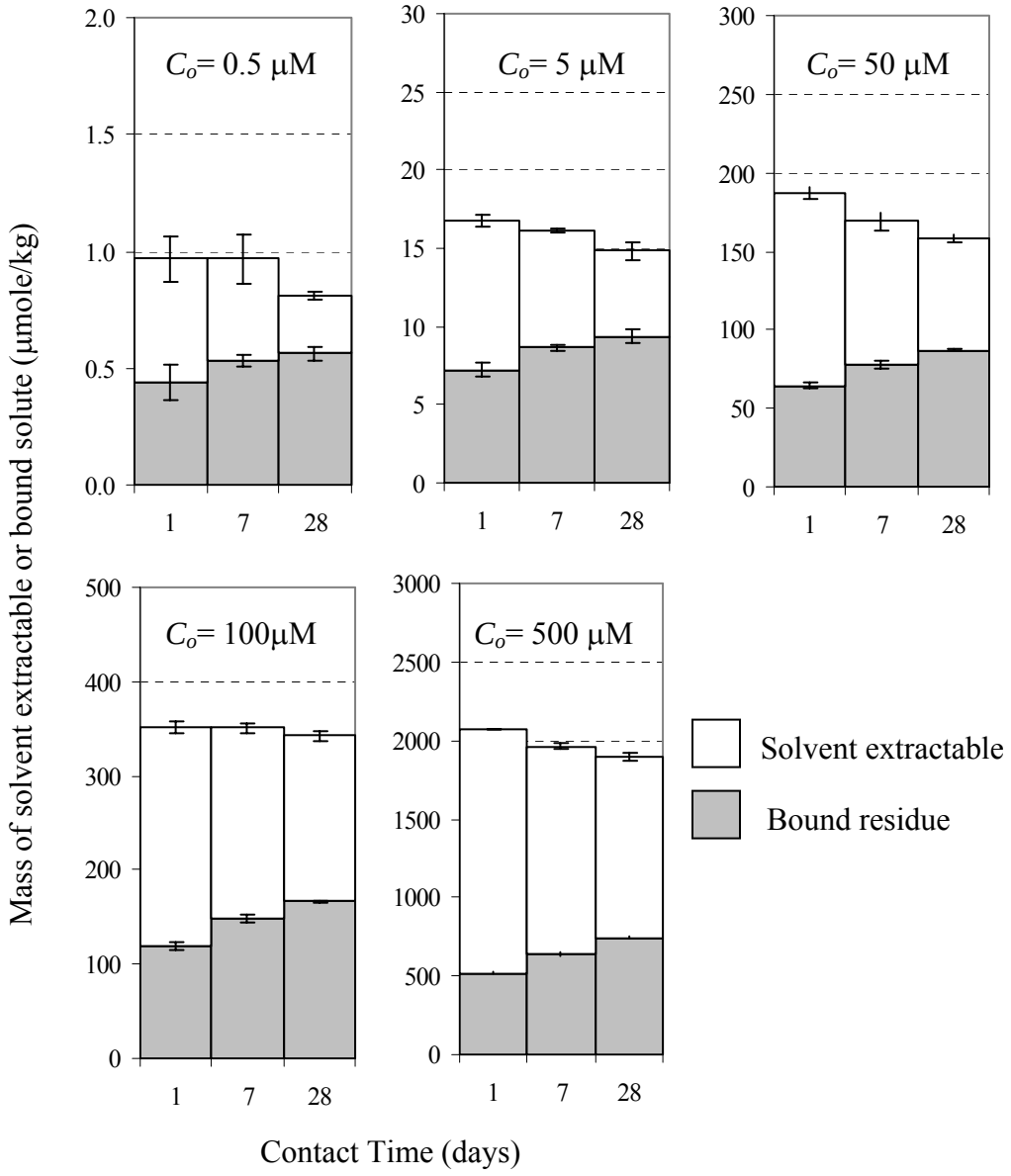

Figure 4.9. Solvent extractable and bound residue as a function of contact time. At different C_o's for SOM-free soil.

A possible explanation for the similarity observed in the extent of contaminant stabilization in the SOM-free and the humin-mineral soil may be the SOM associated with the latter. A lower efficiency of the oxidative polymerization process should be expected in a sorbent containing SOM due to sorption and reduced availability of DCP for the polymerization reaction. Additionally, the possible consumption of free radicals by SOM (Vaughan and Ord, 1982), and

the possible formation of HRP-SOM complex (Sarkar and Bollag, 1987) could significally decrease the conversion efficiency of the DCP polymerization process. The reduced conversion of DCP in presence of SOM was confirmed by the solute aqueous distribution study that was presented in Section 3.5.5. Thus, even though the transformation of DCP was less efficient in presence of the humin-mineral geomaterial, the SOM reactive and hydrophobic regions were important contributors in the retention of solute (DCP + DPP).

Although the amount of stabilized contaminants was similar in the two soils, the mass of solute sorbed was significantly lower in SOM-free soil at all concentrations. These results suggest that indeed, AHM sorbed more solute (DCP+ DPP) due to its higher SOM content. However, a large fraction of the oligomers associated with the mineral soil were resistant to solvent extraction. This is likely due to the stronger association between the oligomers and mineral surfaces in the SOM-free geomaterial.

Under SOM-free conditions, precipitation and sorption of the formed polymers are suggested to be the main mechanisms causing the removal of the solute from aqueous phase, while in SOM containing sorbents, a combination of DCP sorption, sorption, precipitation, and cross-coupling of DPP will cause solute retention in the solid phase. Results suggest that, in presence of HRP-oxidative polymerization reactions, the interactions between solute and minerals in the oxidized soil should be strong since solute resist extraction with solvent.

These results suggest the potential for successful application of engineered humification processes in contaminated soils that have negligible contents of SOM. Sorption of DCP to minerals was shown to be negligible in data produced from the preliminary study. Thus, DCP has a high potential to be transported across mineral soils near surface or in the subsurface. HRP-mediated stabilization of DCP results in the containment of the contaminant in the soil as a result of the precipitation and sorption of solute. Furthermore, postpolymerization contact period between the solution and SOM-free soil augmented solute stabilization.

This study also provides interesting information in the context of SOM formation in soil. When HRP-mediated polymerization is induced in the presence of mineral surfaces, the interactions between oligomers and minerals surfaces may be analogous to those between humin and minerals. Strong interactions were observed between solute and the exposed mineral surfaces of the SOM-free geomaterial. The resulting bound residues have the potential to mimic natural organic matter, affecting the fate of organic contaminants. Solute residues that could

behave as one of the natural SOM fractions (HA, FA or humin) found in natural soils.

4.5.4 Quantification of Solute in Aqueous Phase

The aqueous phase was characterized as unreacted DCP and soluble DPP at the end of HRP-mediated polymerization, in presence of soil particles. Experimental conditions in this study (solute concentration, enzyme and H_2O_2 dose) were similar to experiments described previously. Results are presented as fraction of the total mass added initially to the reactors. Thus, for 50, 100 and 500 μM of initial solute aqueous concentration and solution volume of 8.5 mL, the initial mass added to the reactors (*M*) is equal to 0.43, 0.86 and 4.3 μmoles of DCP. The mass of DCP and soluble polymers measured in supernatant or remaining in soil is expressed as *m*.

This section describes the distribution of residual DCP and soluble DPP left in the supernatant after completion of polymerization reaction. The distribution of extractable DCP and DPP, however, is not included as a subsection since no extractable DCP was detected and all the extractable solute was in the form of DPP. Further discussion is provided later in this chapter.

4.5.4.1 DCP and DPP in Supernatant after Sorption

The distribution of unreacted DCP and soluble DPP in the aqueous phase at the end of the requisite contact period is shown in Figure 4.10. Results confirm DCP removal of 95% or higher from the supernatant. Residual DCP was only detected in reactors with 500 μM initial aqueous concentration. The presence of inorganic chemicals like Al, Fe and Mn is less likely to be responsible for the apparent enhanced polymerization (Bollag, 1992) since preliminary experiments have shown that in the absence of HRP no significant sorption of DCP occurred. Removal of residual DCP from aqueous solutions can be attributed to clay minerals that have been reported to enhance the removal of phenolic polymerization products (Wagner and Nicell, 2003).

At all contact times, with increasing initial aqueous solute concentration, more solute was observed to be associated with the SOM-free soil. Thus, concentration had a positive effect on the sorption of DCP+ polymers. However, an opposite tendency was noted with respect to contact time. The sorbed fraction at 0.43 μmoles decreased from 0.62 to 0.55 as contact time increased from 1 to 28 days; at 4.3 μmoles the sorbed mass was reduced by 4%.

A corresponding increase in the amount of soluble polymerization products left in

supernatant was seen from day 1 to day 28. Soluble polymerization products increased by 7 %, 3 % and 3 % at 0.43, 0.86 and 4.3 μmoles, respectively. This may be due to soluble DPP that sorbed but later resolubilized. This can be attributed to the breakage of oligomers due to the shear forcers exerted by the continuous shaking of the CMBR.

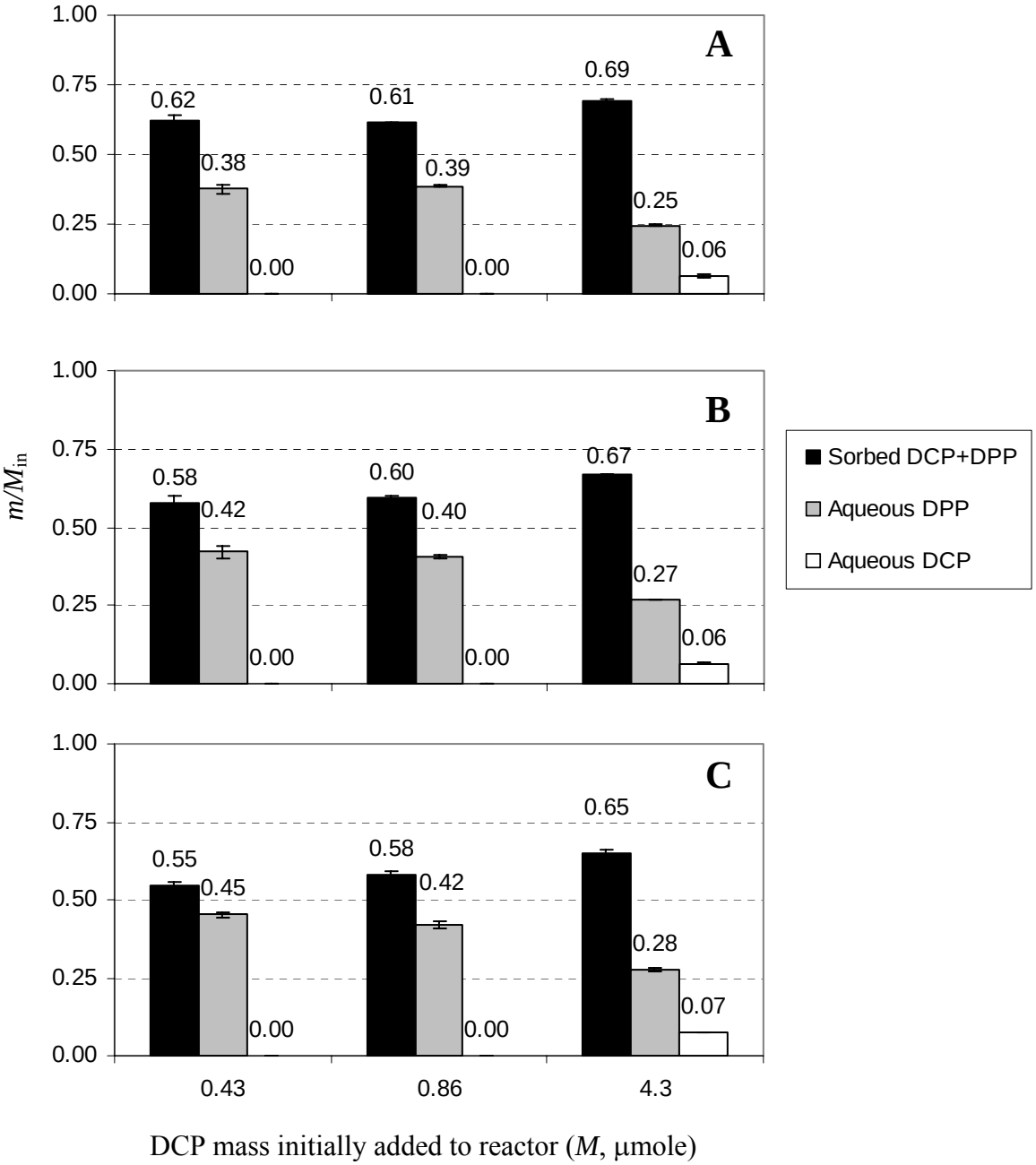

Figure 4.10. Distribution of DCP and DPP between the aqueous and solid phases after (A) 1, (B) 7 and (C) 28 days of contact time, SOM-free soil.

Juhna et al. (2003) observed release of organic matter from an aquifer material and attributed it to the physical interactions rather than chemical interactions between the organic and inorganic phases. Thus, sorption of HA and FA to an aquifer material (composed by sand, clay and small OC content) was mainly attributed to hydrophobic interactions. While acidic groups (carboxylic and phenolic) of HS were also considered to interact with the reactive oxide surfaces of the clays, but their contribution to the overall removal of HS was not considered as important as the hydrobophic interactions.

The overall concentration of aqueous DPP decreased with increasing initial aqueous phase DCP concentration. After 28 days, the total aqueous DPP accounted for 45, 42 and 28 % of the total mass initially added to achieve 50, 100, and 500 μM. This result is important in the context of the toxicity associated with the DPP in the aqueous phase (Kong et al. 2007). Wagner and Nicell (2003) found that for similar phenol aqueous phase concentrations (613 μM), the residual toxicity of the supernatant was reduced when the reaction was induced in presence of solids. Thus, reduced toxicity of residual supernatant would be expected for the range of solute aqueous concentrations used in this study, given that less soluble DPP are produced in presence of geosorbents.

The results from this study can be compared to those obtained for the AHM soil, Figure 3.17. As expected in presence of SOM, higher mass of solute was sorbed to the soil. The fraction of soluble DPP in supernatant for the AHM geomaterial was significantly lower than that observed for the SOM-free soil. The smaller amount of aqueous DPP in AHM supernatant can be attributed to the interaction of DPP with the SOM, consequently increasing their retention. Although higher solute sorption was reported for the AHM soil, the fraction of solute associated with the SOM-free soil was significant to conclude that this process could be equally effective when used in the presence of SOM.

An important difference between the results observed for AHM and the SOM-free geomaterials was the characteristics of the extractable solute. The solute extracted from AHM geomaterial consisted of unreacted DCP and DPP, while the extracted solute from SOM-free soil consisted only of extractable polymers. These observations appear to support our early contention that removal of SOM enhanced solute transformation.

The total solvent extractable fraction for the SOM-free soil is shown in Figure 4.8, where total solvent extractable is composed of DPP with no parent DCP. It can be observed that at the

end of 28 days, the fractions of extractable solute were 0.45, 0.50 and 0.60 at 50, 100 and 500 μM of initial solute concentrations, respectively. While for the AHM soil in Figure 3.19, the fractions of extractable solute at the same initial DCP concentrations were 0.44, 0.44 and 0.56. Even though AHM sorbed more solute, the bound residues in both sorbents had comparable magnitudes, meaning that more solute was extracted from the humin-mineral material, see Figure 3.15 and Figure 4.9.

4.6 Summary and Conclusions

The objective of the research reported in this chapter was to evaluate the sorption and extractability of DPP produced in the presence of an inorganic sorbent containing negligible SOM (0.1% SOM). The parent surface agricultural soil described in the previous chapter was treated with NaOCl to reduce its natural organic matter to < 0.1% SOM. The NaOCl oxidation technique ensured that the integrity of the original soil inorganic fractions was maintained.

The following hypotheses were evaluated:
Hypothesis 5: HRP-mediated oxidative coupling of DCP in the presence of a SOM-free geomaterial results in the formation of DPP;
Hypothesis 6: DPP macromolecules are removed from the aqueous phase as a result of sorption and deposition on mineral particle surfaces;
Hypothesis 7: DPP molecules associate strongly with mineral surfaces and are resistant to extraction by water and methanol;
Hypothesis 8: Post-polymerization contact time, between the DPP-containing aqueous phase and mineral particles, enhances the formation of nonextractable DPP residues; and
Hypothesis 9: HRP-mediated removal of aqueous DCP is influenced by the presence/absence of SOM in the geosorbent particles.

Hypothesis 5 was proven true by observing DCP removal and DPP formation when peroxidase and H_2O_2 were added to DCP solution in contact with SOM-free soil. DPP formation appeared to be as high in SOM-free geomaterials as in natural soils with SOM.

Hypothesis 6 was proven true by observing removal of DPP macromolecules from the aqueous phase as a result of sorption and/or precipitation on sorbent surfaces. The SOM-free soil

surface appeared to have been modified upon coverage with polymers, thus enhancing the association of DPP at higher initial solute concentrations.

Hypothesis 7 was proven true by observing low water and methanol extractabilities of the sorbent-associated DPP. The extractable solute was made up entirely of DPP; no unreacted DCP was detected. Solute extractability was lower and a greater fraction of the solute was converted to bound residues at lower initial concentrations.

Hypothesis 8 was proven true since post-polymerization contact time decreased the extractability of DPP from the sorbent. Although contact time appeared to have no impact on the association of DPP with sorbent surfaces resulting from sorption/precipitation after polymer formation; water and methanol extractabilities of sorbent-associated DPP were reduced at longer contact periods. The interactions between DPP and the SOM-free soil were strong, indicating significant contaminant stabilization. Greater contaminant stabilization as a function of contact time was observed at all initial DCP concentrations evaluated.

Hypothesis 9 was proven true by observing that the association of DPP was significant even in the absence of SOM. In presence of the SOM-free soil higher efficiency of DCP conversion was observed. Precipitation of DPP appeared to be the main mechanism responsible for the removal of DCP from the aqueous phase.

Thus, HRP mediated-oxidative polymerization of DCP is an effective process that is capable of stabilizing DCP in the presence of predominantly mineral materials.

4.6.1 Real-World Implications

Mineral soils are abundant in the earth. Inorganic soils might be present in the surface or in the subsurface. Contamination of soils is not exclusive of those containing SOM. Understanding of the interaction of DPP with mineral soils is important to extend the application of HRP mediated-oxidative polymerization of phenolic contaminants as a remediation technology for soil containing none or negligible SOM.

Results from this study are important because they support the idea that even in the absence of SOM, enzyme-mediated oxidative polymerization of phenolic contaminants can be an effective process to stabilize the organic contaminants in soil. It was reported that the HRP-stabilization of DCP was not dependent of the amount of SOM present in the sorbent. Thus, this remediation treatment could be effectively used to remediate predominantly mineral

contaminated soils.

Similarly to what was observed in the case of the humin-mineral geosorbent, about 24 hrs were required to achieve significant solute sorption with the mineral phase, suggesting that in a real situation the amount of time required to conduct the treatment would be relatively short.

Contact time appeared to be important in the bound residue formation of DPP in mineral soils. In a real situation these results would imply that, time will allow the increase of the amount of stabilized contaminant within the mineral soil. The HRP-stabilization of phenolic chemicals in a mineral soil can be related to the formation of synthetic SOM coverage that can provide potential sorption capacity to the geosorbent.

4.7 Literature Cited

Ainsworth, C. C., Zachara, J. M.; and Schmidt, R. L. Quinoline sorption on Na-montmorillonite: contributions of the protonated and neutral species. *Clays & Clay Miner*. 1987, 35: 121-1128.

Amiri, F.; Rahman, M. M.; Bornick, H.; and Worch, E. Sorption behaviour of phenols on natural sandy aquifer material during flow-through column experiments: the effect of pH. Acta Hydrochim. Hydrobiol. 2004, 32(3): 214-224.

Anderson, J. U. An improved pretreatment for mineralogical analysis of samples containing organic matter. *Clays & Clay Miner*. 1963, 10: 380-388.

Ahn, M.-Y.; Dec, J.; Kim, J.-E.; and Bollag, J. M. Treatment of 2,4-dichlorophenol polluted soil with free and immobilized laccase. *J.Environ.Qual.* 2002, 31: 1509-1515.

Arseguel, D.; and Baboulene, M. Removal of phenol from coupling of talc and peroxidases. Application for depollution of waste water containing phenolic compounds. *J. Chem. Technol. Biotechnol.* 1994, 61: 331-335.

Balcke, G.; Kulikova, N. A.; Hesse, S.; Kopinke, F.-D.; Perminova, I. V. and Frimmel, F. H. Adsorption of humic substances onto kaolin clay related to their structural features. *Soil Sci. Soc. Am. J.* 2002, 66:1805-1812.

Barriuso, E.; Laird, D. A.; Koskinen, W. C.; and Dowdy, R. H. Atrazine desorption from smectites. *Soil Sci. Soc Am. J*. 1994, 58: 1632-1638.

Bhandari, A.; Novak, J. T.; and Berry, D. F. Binding of 4-monochlorophenol to soil. *Environ. Sci. Technol.* 1996, 30: 2305- 2311.

Bhandari, A. and Weber, W. J. Sorption of 1,2,4-trichlorobenzene to natural geosorbents. In: *Proceedings Of The Thirieth Mid –Atlantic Industrial And Hazardous Waste Conference*. July 12-15, 1998 Villanova University, Villanova, Pennsylvania. 1998.

Biber, M. V., and Stumm, W. An in-situ ATR-FTIR study: the surface coordination of salicyclic acid on aluminum and iron (III) oxides. *Environ. Sci. Technol*. 1994. 28: 763-768.

Bollag, J.-M. Decontaminating the soil with enzymes. *Environ. Sci. Technol*. 1992, 26:1876-1881.

Bourget, S. J.; and Tanner, C. B. Removal of organic matter with sodium hypobromite for particle-size analysis of soils. *Can. J. Agr. Sci*. 1953, 33: 579- 585.

Celis, R.; Hermosín, M. C.; Cox, L.; and Cornejo J. Sorption of 2,4-dichlorophenoxyacetic acid by model particles simulating naturally occurring soil colloids. *Environ. Sci. Technol.* 1999, 33: 1200-1206.

Clausen, L.; Fabricius, I.; and Madsen, L. Adsorption of pesticides onto quartz, calcite, kaolinite

and α-alumina. *J. Environ. Qual.* 2001, 30: 846-857.

Clause, L; and Fabricius, I. Atrazine, isoproturon, mecoprop, 2,4-D, and bentazone adsorption onto iron oxides. *J. Environ. Qual.* 2001, 30: 858-869.

Colareti, M. L.; Toscano, G.; and Greco, G. Jr. Soil-catalyzed polymerization of phenolics in polluted waters. *Water Res*. 2992, 36: 3015-3022.

Dec, J. and Bollag, J-M. Dehalogenation of chlorinated phenols during oxidative coupling. *Environ. Sci. Technol*. 1994, 28: 484-490.

Deng, Y.; Dixon, J. B.; and White, G. N. Adsorption of polyacrylamide on smectite, illite, and kaolinite. Soil Sci. Soc. Am. J. 2006, 70: 297-304.

Feng, X.; Simpson, A. J.; and Simpson, M. J. Investigating the role of mineral-bound humic acid in phenanthrene sorption. *Environ. Sci. Technol*. 2006. 40: 3250-3266.

Garbarini, D. R.; and Lion, L. W. Influence of the nature of soil organics on the sorption of toluene and trichloroethylene. *Environ. Sci. Technol.* 1986, 20:1263-1269.

Giafreda, L. and Bollag, J-M. Effect of soils on the behavior of immobilized enzymes. *Soil Sci. Soc.Am. .J*. 1994, 58: 1672-1681.

Gonzáles-Pradas, E.; Villafranca-Sánzchez, M.; Socías-Viciana, M.; Fernández-Pérez, M.; and Urena-Amate M.D. Preliminary studies in removing atrazine, isoproturon and imidacloprid from water by natural sepiolite. *J. Chem. Technol. Biotechnol*. 1999, 74: 417-422.

Hatcher, P. G.; Bortiatynski, J. M.; Minard, R. D.; Dec, J.; Bollag, J. M. Use of high-resolution ^{13}C NMR to examine the enzymatic covalent binding of ^{13}C-labeled 2,4-dichlorophenol to humic substances. *Environ. Sci. Technol*. 1993, *27*, 2098-2103.

Hatzinger, P. B. and Alexander, M. Effect of aging of chemicals in soil on their biodegradability and extractability. *Environ. Sci. Technol*. 1995, 29:537-545.

Hettiarachchi, G. M.; Ryan, J. A.; Chaney, R. L.; and La Fleur, C. M. Sorption and desorption of cadmium by different fractions of biosolids-amended soils. *J. Environ. Qual*. 2003, 32: 1684-1693.

Huang, Q. and Weber, W. J. Jr. Peroxidase-catalyzed coupling of phenol in the presence of model inorganic and organic solid phases. *Environ. Sci. Technol.* 2004, 38: 5238-5245.

Huang, W., Schlautman, M. A. and Weber, W. J. Jr. A distributed reactivity model for sorption by soils and sediments. 5. The influence of near-sruface characteristics in mineral domains. *Environ. Sci. Technol.* 1996, 30: 2993-3000.

Jackson, M. L. *Soil Chemical Analysis*. Advance course. 2nd edition Published by author,

Madison, WI.

Juhna, T.; Klavis, M.; and Eglite, L. Sorption of humic substances on aquifer material at artifitial recharge of groundwater. *Chemosphere*. 2003, 51: 861-868.

Kalbitz, K.; Schwesig, D.; Rethemeyer, J. and Matzner, E. Stabilization of dissolved organic matter by sorption to the mineral soil. *Soil Biol. Biochem.* 2005. 37: 1319-1331.

Kim, W. Phenol removal from saturated porous media using horseradish peroxidase mediated oxidative polymerization process. Ph.D. dissertation, Department of Civil Engineering, Kansas State University, 2007.

Kononova, M. *Soil organic matter*. Second Edition. Pergamon Press Oxford New York, 1966.

Kung, K-H; and McBride, M. B. Electron transfer processes between hydroquinone and hausmannite (Mn_3O_4). *Clays & Clay Miner*.1988, 36(4): 297-302.

Laird, D. A.; Barriuso, E.; Dowdy, R. H and Koskinen, W. C. Adsorption of atrazine on smectites. *Soil Sci. Soc. Am. J*. 1992, 56 62-67.
Laird, D. A.; William, P. Y. Y.; Koskinen, C.; Steinhelmer, T. R.; and Dowdy, R. H. Sorption of atrazine on soil clay components. *Environ. Sci. Technol*. 1994, 28: 1054-1061.

Larson, R. A. and Hufnal J. M. Jr. Oxidative polymerization of dissolved phenols by soluble and insoluble inorganic species. *Limnol. Oceanogr*. 1980, 25(3): 505-512.

Lavkulich, L. M.; and Wiens, J. H. Comparison of organic matter destruction by hydrogen peroxide and sodium hypochlorite and its effects on selected mineral constituents. *Soil Sci. Soc. Am. Proc*. 1970, 34, 755-758.

Li, H.; Sheng, G.; Johnston C. T.; Teppen, B. J.; Johnston, C. T.; and Boyd, S. A.Sorption and desorption of pesticides by clay minerals and humic acid-clay complexes. *Soil Sci. Soc. Am. J.* 2003, 67: 122-131.

Madsen, L.; Lindhardt, B., Rosenberg, P.; Clausen, L.; and Fabrocous, I. Pesticide sorption by low organic carbon sediments: a screening for seven herbicides. *J. Environ. Qual.* 2000, 29: 1488-1500.

Martin, R. T. Calcium oxalate formation in soil from hydrogen peroxide treatment. *Soil Sci.* 1954, 77: 143-145.

Micera, G.; Pusino, A.; Gessa, C. and Petretto, S. Interaction of fluazifop with Al-,Fe^{3+}, and Cu^{2+}- saturated montmorillonite. *Clays & Clay Miner*. 1988, 36 :354-358.

Murphy, E. M.; Zachara, J. M.; and Smith S. C. Influence of mineral-bound humic substances on the sorption of hydrophobic organic compounds. *Environ. Sci. Technol.* 1990, 24: 1507-1516.

Murphy, E. M.; Zachara, J. M.; Steven, C.; Smith S. C.; Phillips, J. L.; and Wletsma, T. W. Interaction of hydrobophic organic compounds with mineral-bound humic substances. *Environ. Sci. Technol.* 1994, 28: 1291-1299.

Nelson, D. W.; and Sommers, L. E. Total carbon, organic carbon and organic matter. In Methods of Soil Analysis. Part 3. D.L. Sparks et al. (Eds.) Soil Sci. Soc. Am. Book Series Number 5. SSSA and SAS, Madison, WI, 1996, pp 961-1010.

Omueti, J. A. I. Sodium hypochlorite treatment for organic matter destruction in tropical soils of Nigeria. *Soil Sci. Soc. Am. J*. 1980, 44: 878-880.

Pal, S.; Bollag, J. M. and Huang, P. M. Role fo abiotic and biotic catalyst in the transformation of phenolic compounds through oxidative coupling reactions. *Soil Biol.Biochem*. 1994, 26 (7): 813-820.

Parfitt, R. L.; Fraser, A. R.; and Farmer, V. C. Adsorption on hydrous oxides. III. Fulvic acid and humic acid on goethite, gibbsite and imogolite. J. Soil Sci. 1977, 28: 289-296.

Park, J-W.; Dec, J.; Kim, J-E. and Bollag, J. M. Effect of humic constituents on the transformation of chlorinated phenols and anilines in the presence of oxidoreductive enzymes or birnessite. *Environ. Sci. Technol.* 1999, 33:2028-2034.

Pignatello, J. J. Sorption dynamics of organic compounds in soils and sediments. In *B.L. Sawhney and K. Brown (ed). Reactions and movement of organic chemicals in soil.* SSSA Spec. Publ. 22. SSSA and ASA, Madison, WI, pg. 45-80,1989.

Ran, Y.; Xing, B.; Suresh, P.;Rao, C.; Sheng, G.; and Fu, J. Sorption kinetics of organic contaminants by sandy aquifers and its kerogen isolate. *Environ. Sci. Technol.* 2005, 39: 1649-1657.

Ruggiero, P; Sarkar, J.M and Bollag J-M. Detoxification of 2,4-dichlorophenol by laccase immobilized on soil or clay. *Soil Sci.* 1989, 147(5): 361-370.

Sanchez Martin, J. M. and Sanchez Camazano, M. Aspects of the adsorption of azinphos-methyl by smectites. *J. Agric. Food Chem*. 1984, 32,: 720-725.

Sannino, F.; Violante, A.; and Gianfreda, L. Adsorption-desorption of 2,4-D by hydroxyl aluminium montmorillonite complexes. *Pest. Sci.* 1997, 51: 429- 435.
Sarkar, J. M., and Bollag, J-M. Inhibitory effect of humic and fulvic acid on oxidoreductases as measured by the coupling of 2,4-dichlorophenol to humic substances. *Sci. Total Environ.* 1987, 62: 367-377.

Selig, H.; Keinath II, M. T.; and Weber, W. J. Jr. Sorption and manganese-induced oxidative coupling of hydroxylated aromatic compounds by natural geosorbents. *Environ. Sci. Technol.* 2003, 37: 4122-4127.

Shah, R. K; Choski, M. R.; and Joshi, B. C. Development studies of soil organic matter: humin. *Chem. Era*. 1975, 6:1-3.

Shannon, M. J. R.; and Bartha, R. Immobilization of leachable toxic soil pollutants by using oxidative enzymes. *Appl. Environ. Microbiol*. 1988, 54 (7): 1719-1723.

Sheng, G.; Johnston C. T.; Teppen, B. J. and Boyd, S. A. Potential contributions of smectite clays and organic matter to pesticide retention in soils. *J. Agric. Food Chem.* 2001, 49: 2899-2907.

Sheng, G.; Johnston C. T.; Teppen, B. J. and Boyd, S. A.Adsorption of dinitrophenol herbicides from water by montmorillonites. *Clays & Clay Miner*. 2002, 50 (1): 25-34.

Shuman, L. M. Sodium hypchlorite methods for extracting microelements associated with soil organic matter. *Soil Sci. Soc. Am. J*. 1983, 47: 656-660.

Specht, C. H.; Kumke, M. U.; and Frimmel, F. H. Characterization of NOM adsorption to clay minerals by size exclusion chromatography. *Water Res*. 34: 4063-4069.

Stone, A. T. and Morgan, J. J. Reduction and dissolution of manganese (III) and manganese (IV) oxides by organics: 2. Survey of the reactivity of organics. *Environ. Sci. Technol.* 1984, 617-624.

Theng, B. K. G. *Formation and properties of clay-polymers complexes.* Elsevier, Amsterdam, 1979, page 362.

Vaughan, D.; and Ord, B. G. An in vitro effect of soil organic matter and synthetic humic acids on the generation of free radicals. *Plant Soil.* 1982, 66: 113-116.

Wang, K.; and Xing, B. C Structural characteristics of adsorbed humic acid on clay minerals. *J. Environ. Qual.* 2005, 34: 342-349.

Wagner, M., and Nicell J. Impact of the presence of solids on peroxidase-catalyzed treatment of aqueous phenol. *J. Chem. Technol. Biotechnol*. 2003, 78: 694-702.

Williams, E. G; Scott N. M; and McDonald M. J. Soil properties and phosphate sorption. *J. Sci. Food Agr.* 9: 551-559.

Yost, E. C., Tejedor-Tejedor, M. I., and Anderson, M. A. In situ CIR-FTIR characterization of saliciylate complexes at the goethite/aqueous solution interface. *Environ. Sci. Technol.* 1900, 24: 822-828.

CHAPTER 5 - THE EFFECT OF SOLUTION IONIC STRENGTH ON DPP STABILIZATION

5.1 Abstract

Peroxidase-mediated oxidative polymerization has been proposed as an approach to stabilize phenolic contaminants, such as 2,4-dichlorophenol (DCP), in soils and aquifers. This process results in the generation of oligomeric colloids and particles that sorb or precipitate on soil surfaces. Variation in ionic strength of water can change the behavior of the precipitates and their interaction with soil particles. Ionic strength can also impact the catalytic activity of peroxidase enzyme. This study evaluated the effects of solution ionic strength on peroxidase-mediated formation of DCP polymerization products (DPP) in aqueous solutions and solutions containing geosorbents. Two geosorbents were studied: a natural surface soil and a soil organic matter (SOM)-free geomaterial. Sorption and stabilization of total solute were evaluated under different ionic strength conditions. While higher ionic strengths resulted in greater DPP precipitation in solid-free systems, no such effect was evident in the presence of geosorbents. Solute (DCP+ DPP) removed into soil surfaces remained constant at all ionic strengths after a 7-day contact period and was significantly greater than the solute removed by precipitation in the solid-free solutions. Extraction with low ionic strength buffers enhanced re-dissolution of solute in both soil-free and soil-containing systems. DCP stabilization under different ionic strength conditions was not impacted by the presence of SOM. Results from this study illustrate the general applicability of peroxidase-mediated stabilization of phenolic solutes by demonstrating effectiveness of DCP HRP-stabilization under a wide range of solution ionic strengths.

5.2 Background

5.2.1 Effect of Ionic Strength on Enzymatic Polymerization of Phenols

Agglomeration of charged soluble particles like polymers (Qasim et al., 2000), proteins (Olmsted and Borisy, 1975), bacteria (Mills et al., 1994, Abramson and Brown, 2007), colloids and fine soil particles (Elimelech and O'Melia, 1990; Chang and Vigneswaran, 1990; McDowell-Boyer, 1992; Gamerdinger and Kaplan, 2001;) and humic substances (Essignton, 2004) in high ionic strength solution is a well known phenomenon that has been utilized by

researchers in water/wastewater treatment, biotechnology, microbial soil transport and aggregation/desegregation of humic substances.

While increasing solution ionic strength produced no impact on the adsorption of nonionic pesticides (atrazine and isoproturon), it decreased adsorption of some acidic organic pesticides (mecoprop, 2,4-D and bentazone) in contact with iron oxides (Clausen and Fabricius, 2001). Similar results were reported for adsorption of 2,4-D onto goethite by Whatson et al. (1973). The reduced adsorption of pesticides was explained by the formation of Ca^{2+}-pesticide complexes and by the competition by chloride in salt with the negatively charged pesticide molecule for the positively charged iron oxide-sorption sites. Clausen et al. (2001) reported that at higher ionic strengths, adsorption of mecoprop onto quartz and kaolinite and of 2,4-D onto kaolinite increased. However, when calcite and ∝-alumina were used as sorbents, adsorption of the acidic pesticides diminished significantly upon increasing background solution salt concentration.

The oxidative polymerization of phenolic compounds is a chemical stabilization process that has been proposed as a remediation technique for contaminated soils (Simmons et al., 1987; Liu et al., 1987; Weber et al., 1996; Bhandari et al., 1996; Thorn et al., 1996; Bhandari et al., 1997; Park et al., 2000; Dec and Bollag, 2000; Bhandari and Xu, 2001; Xu and Bhandari, 2003 a and 2003 b; Palomo and Bhandari, 2005 and 2006). However, the effect of ionic strength on the formation of nonextractable polymerized residues in natural geosorbents has not been previously evaluated.

Few authors have addressed the effect of ionic strength on the formation and chemical behavior of phenolic polymerization products in aqueous solutions (Wagner and Nicell, 2002; Xu, 2002; Huang et al. 2005). Xu (2002) evaluated HRP-induced polymerization of 1-naphthol under aqueous conditions. The ionic strength of the solutions ranged from 10 and 160 mM. Increasing the solution ionic strength was found to reduce the transformation of naphthol. The reduced removal of parent naphthol was attributed to the inactivation or reduction of HRP activity due to enzyme denaturation at high ionic strengths or due to enzyme attachment to naphthol polymeric products. However, the distribution of soluble and precipitated polymeric products was not evaluated.

Evaluation of the effectiveness of enzymatic polymerization of phenol in soil-free systems and in the presence of inorganic salts (NaCl, $CaCl_2$, $MgCl_2$, NH_4Cl and $(NH4)_2SO_4$) was

reported by Wagner and Nicell (2002). These researchers focused their work on investigating the effect of dissolved wastewater constituents on the removal of phenolic contaminants by enzymatic polymerization. Polymerization was facilitated at high ionic strengths to determine the impact of salt concentrations (1 mM to 600 mM) on the activity of the enzyme, efficiency of the enzymatic process, solubility, and stability of the polymerization products. Polymerization induced in distilled water alone resulted in 0.33 M residual phenol. However, as the ionic strength was increased to 50 mM, conversion of phenol decreased producing a residual phenol of about 0.66 M. Ionic strengths beyond 50 mM did not further affect the conversion of phenol. These authors reported that the maximum phenol conversion was not affected by the type of salt used; however, for every salt used the maximum removal of phenol was observed at different ionic strength. Highest phenol removal occurred at the lowest salt concentration (10 mM) for the monovalent salts (NaCl and NH_4Cl). Precipitation of the polymerization products started at ionic strengths of 1 mM and 10 mM for the divalent and monovalent salts, respectively, while no precipitation was observed in salt-free systems. Although this study reported the precipitation of polymers, it did not quantify the amount of precipitated and soluble reaction products in the system, or whether the polymer production and distribution was affected by increasing the ionic strength.

Wagner and Nicell (2002) also reported the effect of ionic strength (at 50 mM NaCl, $(NH_4)_2SO_4$ and Na_3PO_4) on polymerization rate. The first hour of reaction in the salt-free system proceeded slower than in the salt-containing systems. However, after 3 hours, complete inactivation of the enzyme was observed in the salt-containing systems. Highest phenol removal was reported as 40% within three hours and remained constant until 24 hrs. In salt-free systems, the activity of the enzyme was prolonged up to 23 hours when 70 % of removal was measured. While precipitates were formed in the salt-containing systems, no insoluble polymeric products were reported for the controls. The authors suggested that while the salts promoted precipitation of the polymers, they also could indirectly cause inactivation of the enzyme since the enzyme could be attached to the polymers produced, thus reducing the removal of aqueous phenol.

Huang et al (2005) evaluated the precipitation of phenol polymerization products by addition of salts (zero to 200 mM of NaCl, KCl, $CaCl_2$ or $MgCl_2$) to the systems that contained the previously formed products. In contrast to what was reported by Wagner and Nicell (2002), Huang et al. (2005) reported formation of precipitated products in controls (no salt added), with

concentration equivalent to approximately 100 μM, i.e., one fifth of the initially added phenol concentration. The mass of polymers precipitated increased upon addition of salt to the reactors. For monovalent salts, the maximum polymer precipitation occurred at about 10 mM, while for the divalent salts, the maximum precipitation was observed at a lower ionic strength of about 0.5 mM. Beyond the ionic strength values mentioned above, no further variation in the amount of precipitated polymers was recorded. For all the salts studied, the maximum concentration of precipitated polymers was reported to be around 200 μM. For the systems that reached the maximum precipitation, irrespective of the salts used, the concentration of soluble polymers was reported to be close to 100 μM.

Another important finding was that the cation component of the added salts was responsible for the precipitation of the phenol products. Although divalent salts were observed to reach the maximum polymer precipitation faster than monovalent salts, both types of salts reached the same maximum value (around 200 μM). After treating the supernatant with different types of salts, the analysis of the soluble polymers left in solution once the maximum precipitation was reached, revealed that the products were similar in structure and concentration, independently of the salt used to induce the precipitation. These researchers suggested that addition of salt to the polymer-containing systems, caused changes in the structure of the soluble polymers. The structural changes, through electronic interactions and double layer compression, enhanced the precipitation of the soluble polymers.

In the context of the deposition of polymerized residues affected by the changes of the solution ionic strength, only the work done by Kim (2007) has been found. Kim (2007) evaluated the ability of the HRP-mediated polymerization to remove phenol from aqueous solution under simulated aquifer conditions. The aquifer conditions were simulated by utilizing a saturated column, packed with Ottawa sand, where no organic carbon was present in the porous media. The study included the effect of solution pH, ionic strength, and enzyme dose on the polymerization efficiency and removal of phenol inside the saturated porous media.

Polymerization of phenol resulted in precipitation and accumulation of the insoluble polymers in the porous media. The precipitation and accumulation of the phenolic polymers changed the pore volume of the media thus changing the hydraulic properties of the system. Removal of phenol was observed to be higher at the ionic strength of 20 mM. Further increase of solution ionic strength to 100 mM was observed to decrease the amount of soluble polymers

produced. Deposition of the polymers was also positively affected by increase in ionic strength from 5 to 20 and finally to100 mM. At 5 mM of ionic strength the system experienced a 5% decrease of pore volume, while at 20 and 100 mM, porosity was decreased by 8% due to polymer deposition.

Although Kim (2007) observed that increasing deposition of polymeric products as the solution ionic strength was increased, the study did not incorporate the washing out or desorption step to evaluate the formation of nonextractable or bound residues. Only one study has evaluated the effects of ionic strength on soil-deposited colloids by desorption with low ionic strength solution (McDowell-Boyer, 1991). McDowell-Boyer (1991) reported that low ionic strength solution mobilized or disturbed the deposited colloids.

Phenolic polymerization products are large macromolecules that bear a surface-charged area that can be affected by pH and ionic strength (Huang et al. 2005). These polymers can also be expected to behave similarly to colloidal particles. Hence, the stabilization/destabilization of polymerization products in aqueous solution can be significantly impacted by the soil-solution chemistry. A review of the scientific literature revealed a knowledge gap in our understanding of the role of solution ion strength on the stabilization-destabilization of phenol polymerization products in soil.

5.3 Research Goal and Hypotheses

The objective of this study was to evaluate the effect of varying solution ionic strength on the sorption and stabilization of dichlorophenol polymerization products (DPP) in the context of a surface soil (3.2 % SOM) and a SOM-free soil.

The hypotheses evaluated in this study were:

Hypothesis 10

Precipitation of soluble DPP in aqueous solution can be enhanced by increasing the solution ionic strength.

Hypothesis 11

HRP-mediated polymerization of DCP in the presence of a geosorbent is affected by the solution ionic strength.

Hypothesis 12

Redissolution of precipitated DPP occurs when the solution ionic strength is lowered.

5.4 Materials and Methods

5.4.1 Soils

5.4.1.1 Soils Background

The two soils utilized for this work include the parent agricultural surface soil described previously in section 3.3.1.1 and the oxidized or SOM-free soil described in section 4.3.1.1. Table 5.1 summarizes all the characteristics of the two sorbents. The agricultural soil was sterilized by three sequential cycles of autoclaving-incubation before storage and use. The two geomaterials were stored in the freezer until used.

Table 5.1. Properties of the agricultural surface soil before and after oxidation.

Property	Agricultural	
	Original	Oxidized
N_2 BET surface area m^2/g P/P_o=0.1, 0.2, 0.3	9.0	35.1
Organic matter %:		
Total	3.4 [#]	0.05 [+]
Alkali extractable (HA/FA)	1.45 [#]	
Non-extractable (humin)	1.95 [#]	
Texture:		
Sand %	32 [#]	8
Silt %	52 [#]	64
Clay %	16 [#]	28

[#] Walkley-Black Method (from Palomo and Bhandari, 2005), [+] LECO procedure

5.4.2 Chemicals

Working solutions of 10, 20, 70 and 100 mM ionic strength were prepared in distilled-

deionized water. These solutions contained 10 mM of ionic strength and pH 7 phosphate buffer (2.82 mM K_2HPO_4, 1.8 mM KH_2PO_4, and sodium azide, added to distilled-de-ionized water). The higher ionic strength solutions, i.e., 20, 70 or 100 mM were obtained by adding precise amounts of NaCl from two stock solutions of 0.84 and 5.0 M NaCl. Five different working solutions (0.5, 5, 50, 100 and 500 μM of DCP) with unlabeled and ^{14}C-labeled DCP were prepared in each of the 10, 20, 70, or 100 mM ionic strength buffers using the approach described previously in section 3.4.2.

In all experiments, except the one that evaluated the effect of HRP concentration on distribution of DPP, 2 AU HRP/mL were added to the reactors. Different HRP stock solutions were prepared to achieve 0.1, 0.25, 0.50, 0.75, 1.0, 1.5, and 2.0 AU/mL. The DCP: H_2O_2 ratio was 1.2 (Palomo and Bhandari, 2005) and five different H_2O_2 stock solutions (1.2 x DCP concentration) were prepared in distilled/de-ionized water one hour before initiating the experiment.

Accurate volumes of HRP and H_2O_2 stock solutions were added to the reactors to produce the desired HRP and H_2O_2 concentrations. The addition of H_2O_2 to the reactors initiated the oxidative polymerization reaction. Bovine liver catalase was purchased from Sigma Chemical Co; St. Louis, M. (EC1.11.1.6) and utilized to stop the polymerization reaction. One unit of catalase was defined by Sigma Chemical as the amount that decomposes 1μmol of H_2O_2 in one minute at pH 7, 25 °C. A sufficient amount of solid catalase was added to distilled-deionized water to prepare a 2500 AU/mL stock solution.

5.4.3 Preliminary Experiment

5.4.3.1 Effect of Enzyme Concentration on the Distribution of DPP

The effect of HRP concentration on the gravimetric removal of DPP was evaluated using completely mixed batch reactors (CMBRs). The reactors consisted of 10 mL centrifuge tubes that contained 8 mL of the working solution and the initial solution aqueous DCP concentration was 500 μM. Triplicate controls (no HRP or H_2O_2 added) were prepared along with working solutions. The reaction was allowed to take place in the absence of sorbent. Polymerization was initiated by adding the working solution to the test tubes followed by addition of corresponding volumes of HRP stock solution. The reaction was started by adding 10 μL of 0.48 mM H_2O_2 to the aqueous mixture in the CMBR. The CMBRs were mixed by hand several times and then

placed in an end-over-end tumbler (20 rpm) for 3 hours.

At the end of 3 hours, the CMBRs were removed from the tumbler and a precise volume of the 2500 AU/mL stock solution of catalase was added to each reactor to stop the reaction by degrading any residual H_2O_2. After 20 additional minutes of mixing, the CMBRs were centrifuged at 250g for 160 minutes to separate precipitated polymers from the aqueous phase.

Two samples were removed from the supernatant of each CMBR, one for LSC and one for HPLC analysis. HPLC analysis was used to quantify the amount of residual DCP in the solution after the polymerization reaction. LSC analysis was utilized to determine the total radioactivity remaining in solution (unreacted DCP + soluble polymers). A 250 μL sample was transferred to a 7 mL scintillation vial containing 5 mL of scintillation cocktail and analyzed in the LSC 24 hours later. One milliliter of the supernatant was removed with a gas-tight glass syringe and transferred into amber HPLC vials. Samples and controls were analyzed using reverse phase HPLC (RP-HPLC). HPLC samples were stored in the refrigerator when not analyzed on the same day. All samples were analyzed within 72 hours.

The disintegration-per-minute (dpm) data reported by the LSC were converted to equivalent concentrations (μM) of DCP. The concentration of precipitated polymers was determined from the LSC data by subtracting the residual aqueous radioactivity in the supernatant from the initially added radioactivity. Soluble polymers were determined by subtracting the concentration of un-reacted DCP in supernatant (HPLC analysis) from the residual radioactivity in supernatant (LSC analysis). These values were corrected to account for leakage or sorption to glassware obtained by measuring the aqueous solute concentration in the control CMBRs.

5.4.4 Effect of Ionic Strength on Polymerization of DCP and Extraction of Precipitated DPP in Soil-Free Systems

The first objective of this experiment was to determine the fate of DPP when enzymatic polymerization was induced under aqueous conditions at different ionic strengths. Secondly, re-dissolution of the precipitated DPP was studied by washing the precipitate with different ionic strength solutions.

Treatments- The effect of different ionic strengths on the fate DPP formed in soil-free systems was assessed in completely mixed batch reactors (CMBRs) that were similar to those

described in previous sections. Ten different treatments were used to evaluate the effect of ionic strength on the fate of DPP. These treatments are summarized in Figure 5.1. Polymerization was induced at pre-determined ionic strengths (10 mM for treatment numbers 1 and 5 to 10; 20 mM for treatment 2; 70 mM for treatment 3; and 100 mM for treatment 4). After 3 hours of reaction, the ionic strength of solution in treatments 6 to 10 was increased by addition of NaCl stock solution. The change in solution ionic strength in treatments 6 to 10 was as follows: treatment 6 and 7 were increased to 20 mM, treatment 8 and 9 were increased to 70 mM and treatment 9 and 10 were increased to 100 mM.

Polymerization- The procedure of the experiment was similar to that described in section 3.4.3. Reactors contained 8 mL of the working solution and were capped with Teflon-lined screw closures. The ionic strength of the initial solution was varied according to the treatment. The initial aqueous concentration of DCP was 500 μM. Triplicate controls (no HRP or H_2O_2 added) were included. No geosorbent was added. The polymerization experiment was initiated by adding a predetermined volume of HRP stock solution followed by 10 μL of 0.48 mM H_2O_2 to the aqueous mixture in the CMBR. The CMBRs were mixed by hand several times and then placed in the end-over-end tumbler (20 rpm) for 3 hours.

Precipitation- After the 3-hour period, a determined volume of 2500 AU/mL catalase stock solution was added to the CMBRs to stop the reaction. Samples were remixed for 20 minutes. The ionic strength of the reactors was increased by addition of an accurate volume of the corresponding NaCl stock solution to each CMBR. Equilibration was allowed to proceed by mixing the contents of the tubes for 30 minutes (Huang et. al, 2005). After the equilibration time, all reactors were centrifuged at 250g for 160 minutes to separate precipitated polymers from the aqueous phase. Reactors were allowed to sit overnight to ensure settling of precipitated polymers.

Water Extraction- LSC and HPLC samples were taken as described in the previous section. A pipette was utilized to remove an accurate volume of supernatant and the volume of supernatant removed and the weight of the reactor were recorded. A three-hour extraction with various ionic-strength buffers was performed to study the redissolution of the precipitated polymers. An exact volume of buffer was added to each tube to start the aqueous extraction. Weights of the reactors were documented to perform an accurate solute mass-balance. The contents of CMBR were centrifuged at 250g for 160 minutes to separate precipitated polymers

from the aqueous phase. Samples were taken from supernatant and were analyzed by LSC and HPLC right after collection.

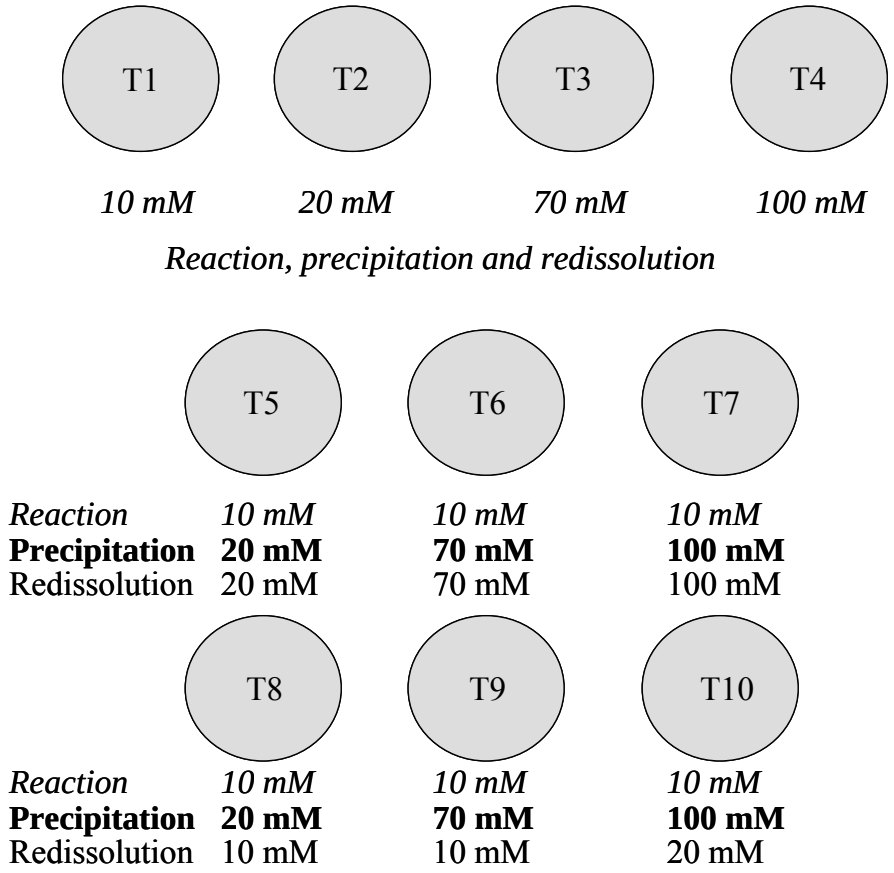

Figure 5.1 Experimental matrix, T= treatment.

Buffer solutions of different ionic strength were used for aqueous extraction. A calculated amount of NaCl salt was added to distilled-deionized water to prepare the fresh buffer solutions for extraction. To achieve the desired final ionic strength in the test tubes, the volume and ionic strength of the residual solution (left after removing the supernatant from sorption experiment) in the reactors was considered while calculating the NaCl concentration required for the buffer solution.

In treatments 1 to 4, the precipitates were extracted with buffer that had the same ionic strength as that used for the polymerization, i.e. for treatment 1, the polymerization was initiated at 10 mM, precipitation proceeded at 10 mM and buffer extraction was performed by adding 10

mM NaCl of buffer solution; for treatment 3 the polymerization was initiated at 20 mM, precipitation proceeded at 20 mM and buffer extraction was performed by adding 20 mM NaCl of buffer solution and so on. The precipitates associated with treatments 5 to 10 were extracted with buffer solutions that varied in ionic strength. Table 5.2 displays the ionic strength variation in every step of the experiment, as well as the treatment IDs that will be used hereafter to discuss the results obtained.

Table 5.2. Ionic strengths at the different stages of the solid-free system experiment.

Treatment #	Ionic strengths at the different stages of the experiment (mM)			
	Polymerization	Precipitation	Extraction	Treatment ID
1	10	10	10	10
2	20	20	20	20
3	70	70	70	70
4	100	100	100	100
5	10	20	20	10-20-20
6	10	20	10	10-20-10
7	10	70	70	10-70-70
8	10	70	10	10-70-10
9	10	100	100	10-100-100
10	10	100	20	10-100-20

5.4.5 Effect of Ionic Strength on Polymerization and Removal of DCP in Systems with Geosorbents and Subsequent Solute Extractability

The objective of this experiment was to investigate if the increase in solution ionic strength could enhance the sorption of solute in HRP-amended systems and decrease its extractability from soil; thereby augmenting the solute bound residue formation.

Polymer Formation and Sorption-The procedure of the experiment was similar to the previously described in section 3.4.3. Briefly, it consisted of the following steps. First the

reactors were weighed, and then 1.5 grams of the parent agricultural soil or SOM-free soil were placed in the test tubes, recording new weights. All treatments had 3 replicates and controls (no sorbent added). The working solution, with the corresponding ionic strength was added to each reactor and weights were recorded. Reaction in all test tubes was initiated after the addition of HRP and H_2O_2. Contents of the tubes were mixed in a vortex mixer and placed on the end-over-end tumbler (20 rpm) for a period of 3 hours. After the reaction time, tubes subjected to treatments 6 to 10 were removed from the tumbler and an aliquot of the NaCl stock solution was added to increase the ionic strength in the reactor, as indicated previously. The weights of reactors were recorded and after mixing, all tubes were replaced inside the tumbler. Sorption was allowed to proceed for 7 days.

Sample Analysis- After seven days of contact, all tubes were centrifuged at 250g for 45 minutes to separate soil from the aqueous phase. From each CMBR, a sample of 250 μL was transferred to a 7 mL scintillation vial containing 5 mL of scintillation cocktail and analyzed in the LSC 24 hours later. Approximately 1.5 milliliter of the supernatant was removed with a gas-tight syringe and filtered using a 0.45 μm glass microfiber (Millipore filters and Millex-LCR syringe driven filter unit) and transferred to an amber HPLC vial. Controls and the filtrate were analyzed using RP-HPLC under the same conditions described in previous chapters. HPLC samples were stored in the refrigerator when not analyzed the same day. Samples were analyzed within 72 hours

Solvent Extraction- After conclusion of the sorption step, the remaining supernatant inside the CMBRs was carefully removed with a pipette and discarded. The weights of tubes were recorded after removal of the supernatant to determine the remaining solution. Sorption was followed by two types of extractions. First an overnight buffer extraction was done. Buffer extraction was followed by three consecutive methanol extractions. As indicated before, the relative ease of sorbed DPP removed with water was determined using a single, overnight water extraction, while methanol extractions were conducted to measure the total extractable solute. Total extractable solute was described in the context of methanol as the extracting fluid. Methanol was selected as the extracting solvent to be consistent with other experiments performed in this research.

Aqueous and methanol extractions proceeded similarly as explained in section 3.4.4. However, the aqueous extraction differed in the fact that the ionic strength of the solution added

to test tubes varied depending on the treatment to study the extraction and re-dissolution of DPP at lower ionic strengths. A calculated amount of NaCl salt was added to distilled-deionized water to prepare the fresh buffer solutions. To achieve the desired final ionic strength in the test tubes, the volume and the ionic strength of the residual solution (left after removing supernatant from sorption experiment) in the reactors was considered while calculating the required NaCl concentration for the extracting buffer solution.

The solids associated with treatments 1 to 4 were extracted with buffer that had the same ionic strength as that used at the start of the experiment, i.e., for treatment 1 the reaction was initiated at 10 mM, sorption proceeded at 10 mM and buffer extraction was performed by adding 10 mM NaCl of buffer solution; for treatment 3 the reaction was initiated at 20 mM, sorption proceeded at 20 mM and buffer extraction was performed by adding 20 mM NaCl of buffer solution and so on. Treatments 5 to 10 were extracted with buffer solution that varied on ionic strength. Figure 5.1 and Table 5.2 illustrate the ionic strengths for each treatment at each stage of the experiment.

Three sequential fill-and-draw methanol extractions were performed on the soil immediately after the buffer extraction. After addition of the buffer or methanol to the CMBRs the weight was recorded. The exact volume of the solution added to each tube was calculated gravimetrically. The procedure was identical to that used for buffer extraction. Three methanol extractions were sufficient to reduce the measured radioactivity in the extract to close or below the detection limit (50 dpm). Once the methanol extractions were completed the soil in the CMBRs was air dried inside the fume hood.

The total solvent extractable solute included the water extractable and methanol extractable chemicals. A mass balance study was performed to verify that methanol-extractable DPP was not over or under estimated by experimental artifact. For this, the weight data for the tubes (weight recorded after removing supernatant and after adding new extractant) collected when conducting the extractions was utilized. For the majority of the tubes, the difference between values calculated from mass balance and actual measured values ranged from 1 to 6 %.

Soil Combustion- The soil in each CMBR was combusted at 925 °C in an OX-500 biological material oxidizer (BMO, R.J. Harvey Instrument). The exact mass of soil combusted was determined by weighing a combustion glass boat before and after addition of the soil. The glass boat containing the soil was introduced to the combustion chamber and combusted for 4

minutes at 925 °C. Any ^{14}C-DCP or ^{14}C-DPP associated with the soil was converted to ^{14}C- CO_2 that trapped in 20 mL scintillation vial containing a Carbon-14 cocktail (R.J. Harvey Instrument). The scintillation vials were analyzed by LSC after 24 hours.

5.5 Results and Discussion

5.5.1 Preliminary Experiment

5.5.1.1 Effect of Enzyme Concentration on Solute Distribution

The effect of HRP concentration on solute (DCP+DPP) production in sorbent-free 10 mM of ionic strength aqueous solutions was evaluated (Figure 5.2). As the enzyme concentration was increased, conversion of DCP was observed to be more efficient. The conversion of DCP increased from 6% to 98% at 0.1 AU HRP/mL and 2 AU HRP/mL, respectively. Improvement of aqueous phenol removal efficiency by increasing HRP dose was reported by Nicell et al. (1992). Similarly, in solid-free solutions, Xu (2002) reported that naphthol removal efficiency at pH 7, for 0.1 AU HRP/mL and 1.6 AU HRP/mL was approximately of 5 % and 90%, respectively. Similarly, Kim (2007) reported 90 % reduction of phenol concentration at 2 AU HRP/mL.

The formation of soluble polymers was enhanced with an increased HRP concentration from 0.1 AU HRP/mL to 1 AU HRP/mL. Beyond 1 AU HRP/mL, the formation of soluble polymers appeared to level off, between 86 to 91% of the total DCP initially added. Thus, beyond 1 AU HRP/mL DCP removal occurred mainly as a result of insoluble DPP generation. The total precipitated polymers increased from 1.0% to 12% as HRP activity increase from 0.1 AU HRP/mL to 2 AU HRP/mL, respectively. Similarly to our results, Kim (2007) reported that the amount of soluble polymer increased by incrementing the HRP dose.

Minard et al. (1981) reported the formation of oligomers as large as pentamers. Utilizing MS and NMR spectroscopy, these researchers identified two dimers of DCP, 2-(2,4-dichlorophenoxy)-6-chloro-1,4-benzoquinone, and 2-(2,4-dichlorophenoxy)-1,4-benzoquinone. Dec and Bollag, (1994) described the molecular structure of the formed soluble polymers under aqueous conditions. These researchers proposed a simple model where ten different dimers of DCP were formed from the participation of the four DCP free radicals formed during polymerization reactions. These researchers invoked the idea of subsequent growth of the polymers through the reaction of radicals from the monomers and oligomers. Growth of

polymerization products has been reported to be terminated upon formation of insoluble tetramers and pentamers (Dec and Bollag, 1990), thus, the model was expanded to include the formation of oligomers not larger than pentamers.

In the current study experimental results indicated that 98% DCP conversion did not really imply 98% removal of the chemical from aqueous solution. Greater than 90% of the parent DCP or DPP remained in solution. An initial DCP concentration of 500 μM, an ionic strength of 10 mM and a 1.2 DCP:H_2O_2 molar ratio were used to conduct all the remaining experiments reported in this chapter since the maximum conversion of DCP and maximum DPP formation were achieved under these conditions.

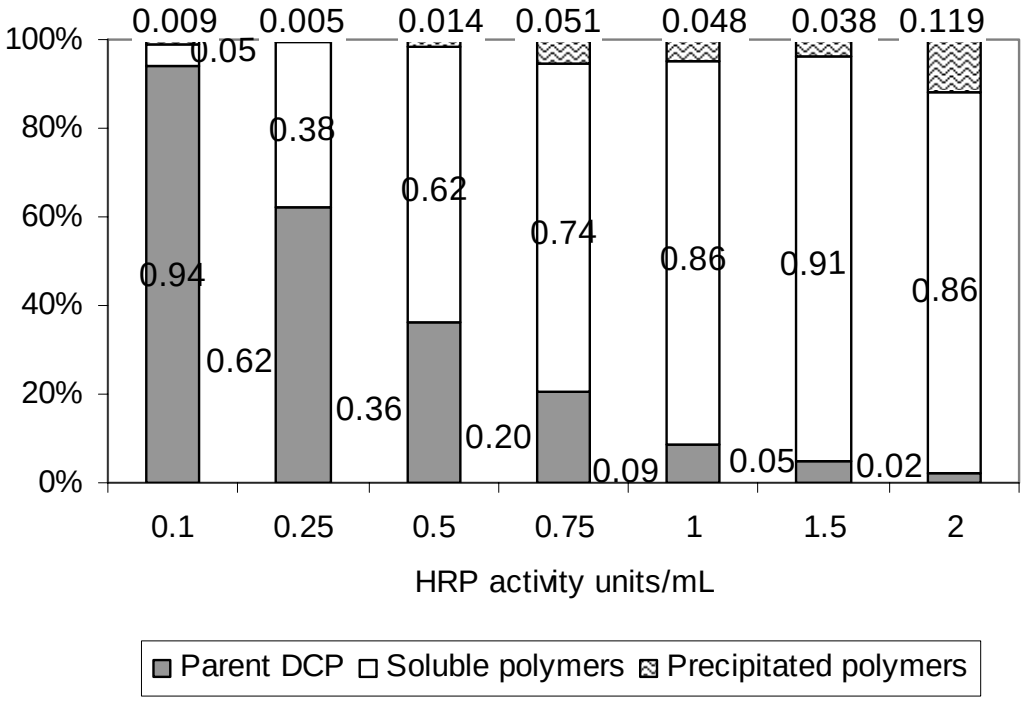

Figure 5.2. Effect of enzyme concentration on the distribution of polymerization products, 10mM ionic strength, 500μM of initially added DCP and 1.2 DCP:H_2O_2 molar ratio.

5.5.2. Effect of Ionic Strength on DPP Formation and Re-dissolution of Precipitated Polymers in Soil-Free Systems

The extent of DCP polymerization and distribution of residual DCP and DPP in soil-free systems is presented in this section. Re-dissolution or extraction of polymers from the precipitate is also discussed. The first objective of this study was to observe the effect of solution ionic strength on the distribution of DPP. Secondly, this study intended to elucidate how modification of solution ionic strength may impact the extractability or re-dissolution of the formed polymers.

Two different conditions were utilized to study the effect of ionic strength on the formation, distribution and extraction/re-dissolution of DPP, 1) polymerization was induced at the base line condition of 10 mM ionic strength and the ionic strength was subsequentially increased, and 2) ionic strength of the solutions was constant at 10, 20, 70 and 100 mM throughout the different phases of the experiment. The purpose of these experiments was to determine the most favorable solution chemistry to enhance the transformation of DCP and the removal of DPP from solution.

5.5.2.1 Distribution of Polymerization Products

The distribution of the polymerization products in soil-free systems is presented in Figure 5.3. Values on the x-axis indicate the ionic strength of the aqueous solution, all with units of mM. Values in the y-axis correspond to the ratio of mass of chemical as DCP, soluble or precipitated polymer to the total initial mass of DCP added to the reactor. The series labeled as 10, 20, 70 and 100 represents the reactors where the ionic strength was maintained constant through the experiment. The series labeled as 10-20, 10-70 and 10-100, represents reactors where reaction was induced at 10 mM ionic strength and the ionic strength was subsequently increased to 20, 70 and 100 mM, respectively, by addition of NaCl stock solution.

Greater than 95% of transformation of DCP was observed for all treatments. The highest ionic strength, 100 mM, showed the lowest conversion, with 5% of the original DCP mass remaining unreacted in the reactor.

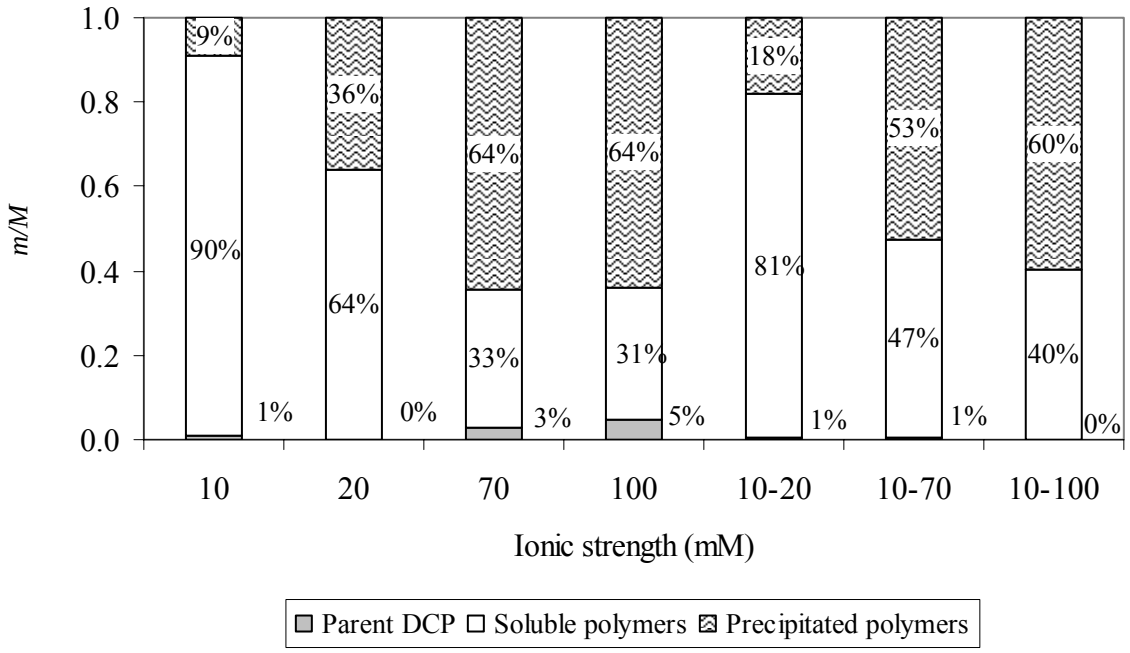

Figure 5.3. Effect of the ionic strength on the distribution of polymerization products in soil free systems, 500 μM of initial DCP concentration, 1.2 DCP:H_2O_2 molar ratio, and 2AU HRP/mL. Where *M*- total concentration of DCP added initially to the reactor, *m*- measured concentration of DPP.

Under both conditions (constant ionic strength and increased ionic strength to enhance precipitation) the concentration of soluble polymers declined as the solution ionic strength was increased. When the experiments were conducted at a constant ionic strength, the soluble polymer in the aqueous phase reduced from 90 to 31% of initial DCP added, at 10 and 100 mM ionic strength, respectively. When the ionic strength was raised to induce precipitation after polymer formation at 10 mM ionic strength, the soluble DPP fraction was reduced from 81 to 40% of total DCP added, at 10-20 and 10-100, respectively. Production of precipitated polymers increased as the ionic strength of the aqueous phase was increased.

Increased precipitation of molecules containing reactive moieties as a consequence of the increased solution ionic strength is a known phenomenon. Enhanced precipitation of phenol polymerization products has also been observed and reported previously in the literature (Wagner and Nicell, 2002; Huang et al., 2005). Wagner and Nicell (2002) attributed the precipitation to the interaction of salt ions with the formed phenol polymerization products. Additionally, these authors found that the enhanced precipitation of oligomers by increasing

solution ionic strength had a negative impact on the conversion of phenol, because the enzyme was attached to the insoluble polymeric products thus causing its inactivation. This conclusion was in agreement with the findings of Dec and Bollag, (1990), who reported that growth of polymers stopped upon formation of insoluble polymers. Huang et al. (2005) also attributed increased precipitation of polymers to change in their electrical and structural configuration upon interaction with the added salt ions.

Results from the 20 and 10-20 treatments are interesting since at 20 mM fixed ionic strength, about 36% and 64% of the parent DCP was converted into precipitated and soluble DPP, respectively. While for the 10-20 study, percentages corresponding to precipitated and soluble polymers were 18 and 81%, respectively. The reaction taking place at 20 mM ionic strength doubled the precipitated polymer compared to that observed for the 10-20 condition. At the higher initial ionic strength, it is likely that salt ions facilitated the precipitation of polymerization products by physically changing the conformation of the forming polymers into denser structures in a manner analogous to what has been reported to occur in the case of natural organic matter macromolecules (Essignton, 2004).

The same trend was observed for 70 and 10-70 treatments, where the condition of constant 70 mM ionic strength resulted in higher DPP precipitation (64%). For 100 mM and 10-100, the amount of precipitated polymers was relatively similar, 64 and 60%, respectively. Thus, in general, under higher ionic strength a higher fraction of the DCP was converted to precipitated polymers for all treatments evaluated.

Under constant ionic strength treatments, soluble polymers amounted to 64, 33, and 31% of total DCP initially added, at the 20, 70 and 100 mM, respectively. While at 10-20, 10-70 and 10-100 treatments soluble polymers represented 81, 47 and 40 % of the initial DCP, respectively. Enhanced DPP precipitate formation, at higher ionic strengths, significantly reduced the concentration of soluble DPP.

It is important to observe that, at high ionic strength, the conversion of DCP was significantly affected. At 70 and 100 mM the DCP conversion efficiencies were 97 and 95 %, respectively, while at 10 and 20 mM, the conversion efficiency was > 99%. Reduced transformation of phenol and 1-napththol at high ionic strengths was also reported by Xu (2002) and Wagner and Nicell (2002). These researchers attributed the reduced conversion efficiency, to possible changes in the conformational structure of HRP responsible for enzyme inactivation.

Such modifications in the structural conformations of HRP at high ionic strengths have been reported by Laurenti et al., (2000). Decrease in the polymerization of DCP at higher ionic strength could also be attributed the increased proportion of ionized DCP in solution. However, despite the reduction in DCP conversion, the increase in solution ionic strength in the current study appeared to have a positive impact on the distribution and overall removal of DPP from the aqueous solution.

5.5.2.2 Re-Dissolution of Precipitated Polymer

The objective of water extraction of precipitated DPP with different ionic strength solutions was to assess the relative ease of polymer re-dissolution from the precipitate. Re-dissolution of the polymers was achieved by adding DCP-free buffer solutions to the reactors containing precipitated polymers. Re-dissolution (extraction) of DPP was conducted with buffers identical in ionic strength as the DCP solution used during the polymerization/sorption stage of the experiment. As well as, buffers with lower ionic strengths. The experimental matrix in Figure 5.1 shows in detail the ionic strength condition for all the treatments at each step of the experiment. The objective of reducing the ionic strength was to evaluate whether the extent of DPP re-dissolution is altered when the precipitate comes in contact of low ionic strength water as may be likely in natural environments.

No DCP was detected by HPLC in the supernatant after the single buffer extraction, i.e., no reverse-transfer of DCP was noted during the 24-hr water extraction. Thus, the discussion that follows refers only to extracted or re-dissolved polymers. Figure 5.4 shows the experimental results of the re-dissolution of DPP under different ionic strength conditions. DPP that re-dissolved during buffer extraction accounted for 1 to 3% of the total DCP added initially to the systems. In treatments where the ionic strength was maintained constant at all steps of the experiment, no overall trend was observed for the extent of re-dissolution as a function of ionic strength even though the amount of precipitated polymers increased with the ionic strength of the initial solution.

For the treatments where the ionic strength of the extracting solution was lowered, i.e., 10-100-10, 10-70-20 and 10-100-20, a greater percent of polymers were re-dissolved. For example, at 10-70-10 re-dissolved DPP constituted 3.1 % of initial DCP added to reactors, while at 10-70-70 only 1.9% of the total DCP added was recovered as re-dissolved polymers. However, even after re-dissolution of polymers, the precipitated polymers formed under constant

high ionic strengths remained higher than that formed at 10 mM of ionic strength and precipitated at different ionic strengths.

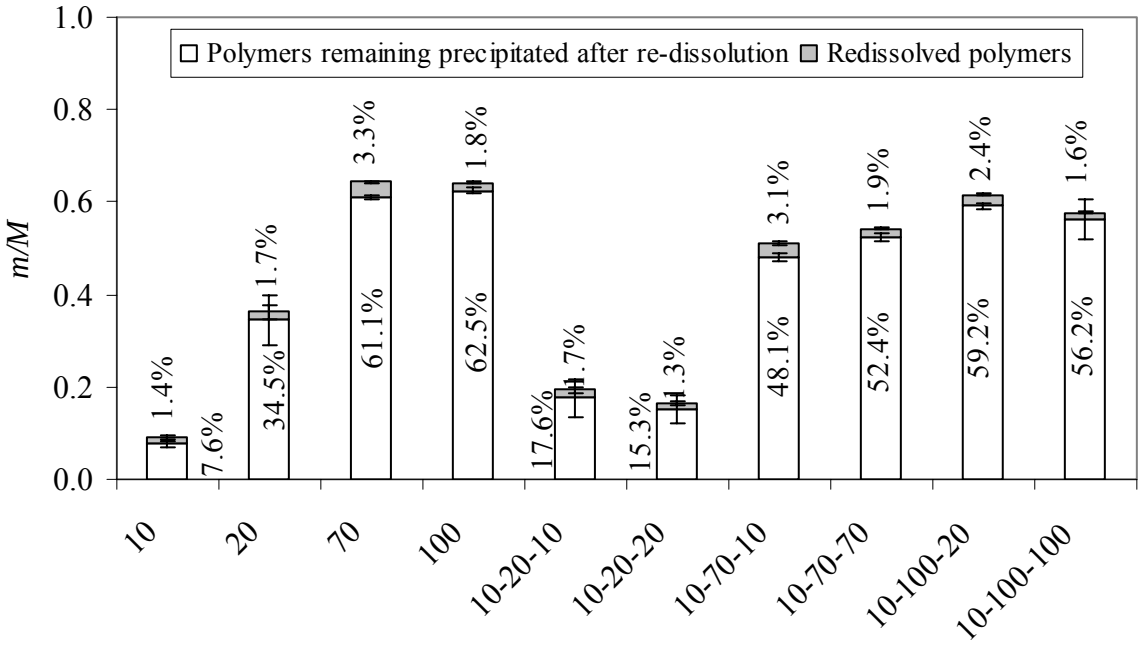

Figure 5.4. Redissolution of precipitated polymers. Where *M*- total concentration of DCP initially added to the reactor, *m*- measured concentration of precipitated polymer.

5.5.3 Effect of Ionic Strength on DCP Enzymatic Trasformation, Sorption, and Re-dissolution in the Presence of Geosorbents

The impact of increasing ionic strength on the enzymatic transformation of DCP and on the polymer-precipitate formation in soil-free systems was discussed in the previous section of this chapter. Understanding formation and precipitation of DPP in aqueous solution of varying ionic strengths is important as it can impact the chemical nature of the synthesized polymers and consequently their ultimate characteristics. This knowledge also forms the basis to explain how the presence of geosorbents can influence the physical-chemical behavior of the oligomeric products. The purpose of this experiment was, therefore, to determine if solutions containing higher ionic strengths could enhance solute sorption in HRP-amended systems, decreasing solute extraction from the sorbent, thereby, enhancing contaminant stabilization.

This experiment evaluated two soils, a SOM-free soil material (described in Chapter 4)

and a surface agricultural soil containing 3.4 % of SOM. The former geosorbent was derived from the latter by chemically oxidizing the SOM associated with the soil. The rationale behind selecting these materials was that they represent common and representative types of natural sorbents, i.e., inorganic and organic soils.

5.5.3.1 DCP Enzymatic Polymerization in the Presence of SOM-Free Soil

The enzymatic polymerization process, in the presence of SOM-free soil, was studied under varying DCP concentrations and solution ionic strengths. Results describing sorption, binding and distribution of solute among the solid and liquid phases are discussed in the following sections.

5.5.3.1.1 Effect of Ionic Strength on the Solute Phase Distribution

Solute phase distribution curves were developed for solution of varying ionic strength (10, 20, 70, 10-20 and 10-70 mM) and compared. These are illustrated in Figure 5.5. Although a notable effect of ionic strength on the distribution of solute was seen in solid-free systems, in the presence of SOM-free soil no significant impact was noted. The phase distribution data obtained at different ionic strength conditions obtained in presence of SOM-free geosorbent were not significantly different suggesting that the amount of solute associated with SOM-free soil was independent of solution ionic strength.

Freundlich parameters obtained from the phase distribution data illustrated in Figure 5.5 are summarized in Table 5.3. Phase distribution was Type S since the value of n was in the order of 1.20 in all cases. A value of n higher than unity suggests that solute (DCP+ DPP) sorption was enhanced at higher solute concentrations most likely as a result of sorbent surface modification caused by sorption or deposition of polymers. The K_F parameter varied from 4.2 to 4.8 $(\mu mole/kg)*(\mu M)^{-n}$, however, no significant difference was observed at the studied ionic strengths. The values of n and K_F were similar to those resulting for the time-dependent PDR study discussed in the previous chapter, where the $n(t)$ and $K_F(t)$ values were equal to 1.22 and 4.10 $(\mu mole/kg)*(\mu M)^{-1.219}$, respectively. Thus, total solute in the system exhibited similar phase distribution behavior irrespective of the solution ionic strength.

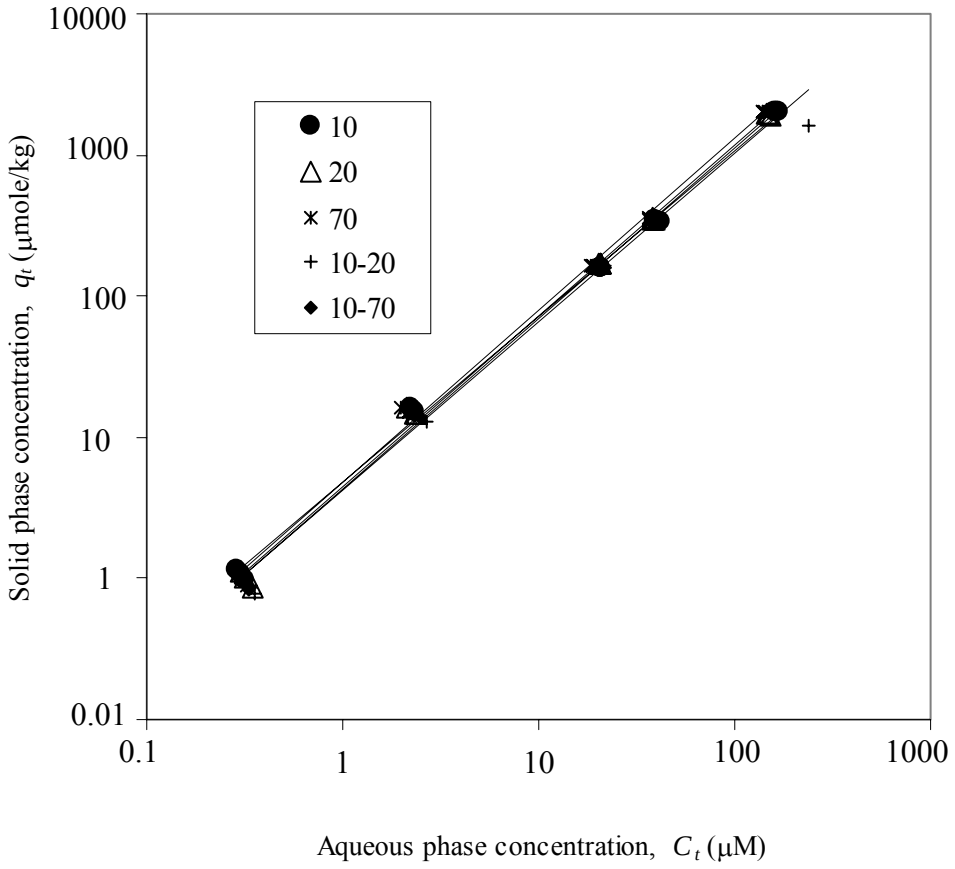

Figure 5.5. Effect of ionic strength on solute phase distribution in HRP-amended systems containing SOM-free soil.

Table 5.3. Freundlich parameters for HRP-amended systems containing SOM-free soil. 95% confidence limits are included in parenthesis.

Treatment	*n*	K_F	R^2
10	1.18 *0.034*	4.81 *0.743*	0.997
20	1.21 *0.038*	4.49 *0.759*	0.997
70	1.22 *0.042*	4.82 *0.883*	0.996
10-20	1.20 *0.034*	4.18 *0.922*	0.995
10-70	1.21 *0.026*	4.30 *0.703*	0.997

* Units for $K_F(t)$ are $\frac{\mu mol}{kg}(\mu M)^n$

5.5.3.1.2 Re-dissolution of Solute Associated with SOM-Free Soil

Re-dissolution of solute was evaluated by extracting the sorbent containing sorbed solute (DCP+DPP) with buffers of varying ionic strengths. After a single buffer extraction, no DCP was detected by HPLC in the supernatant indicating no reverse-mass transfer of DCP from the soil. The re-dissolved solute consisted only of DPP. This was likely due to negligible sorption of unreacted DCP on the soil surfaces. The mass fraction of DPP removed from the soil during extraction with buffers of varying ionic strength is illustrated in Figure 5.6.

The mass fraction of re-dissolved DPP varied from 2.8 to 7.9% of the total mass of DCP initially added to the reactors. However, no consistent trend with respect to ionic strength was observed. For treatments where the ionic strengths were varied in the course of the experiment, increased ionic strength appeared to have no effect on the amount of DPP remaining sorbed to soil (60%, 60% and 65% for10-20-10, 10-70-10 and 10-100-20, respectively or 66%, 66% and 66% for10-20-20, 10-70-70 and 10-100-100, respectively).

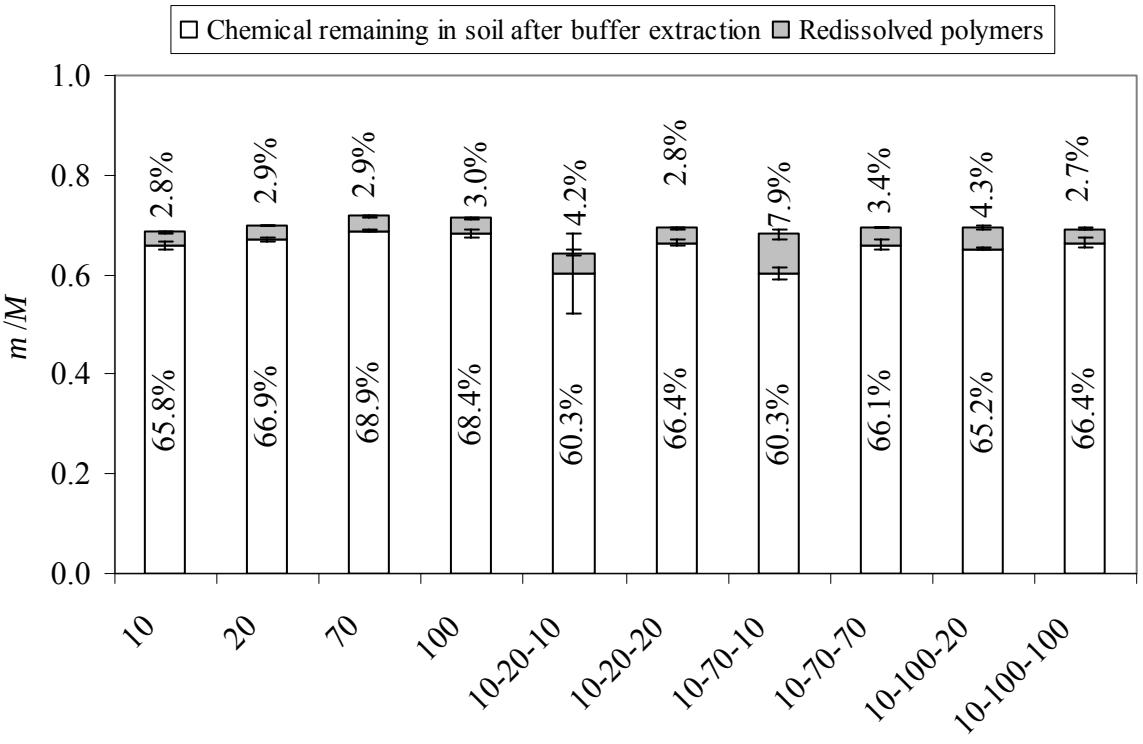

Figure 5.6. Redissolution or extraction of precipitated/sorbed polymers. Where *M*- total concentration of DCP initially added to the reactor, *m*- measured concentration of precipitated polymer.

For the treatments where the ionic strength was lowered in the extraction step, the mass fraction of re-dissolved DPP was higher than when the ionic strength was kept constant. For example, comparing treatments 10-20-10 and, 10-20-20 the percent of re-dissolved polymers were 4.2 and 2.8 %, respectively. A similar behavior was observed for other treatments (10-70-10 versus 10-70-70, and 10-100-20 versus 10-100-100). The amount of polymers remaining in soil was lower when the soil was extracted with low ionic strength buffers. Hence, this results support our suggestion that lowering the ionic strength of the solution removes or re-dissolves some soil associated polymers, although a significant amount of DPP continues associated with the solids.

5.5.3.1.3 Effect of Ionic Strength of Solute Stabilization in SOM-Free Soil

Contaminant stabilization was evaluated in the context of solution ionic strength. The stabilized fraction of DPP was defined as the DPP mass that remained bound with the sorbent after one buffer extraction followed by three consecutive methanol extractions. Figure 5.7 illustrates the equivalent mass of DCP that was bound to the SOM-free soil.

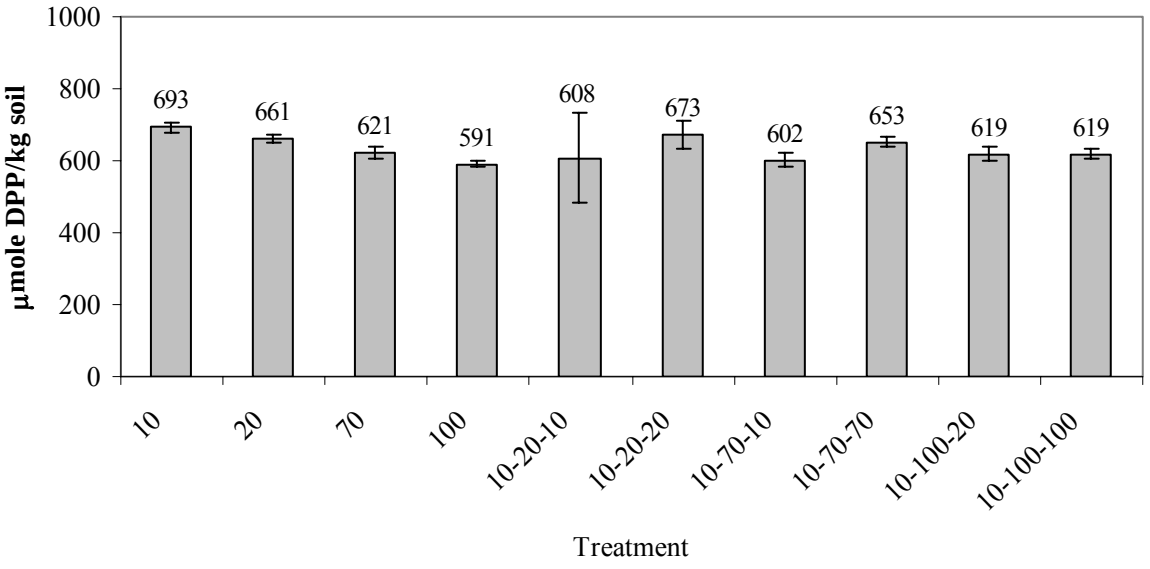

Figure 5.7. Mass of chemical bound to SOM-free soil. Data corresponds to 500 µM of initially added DCP.

For treatments where ionic strength was maintained constant through the course of the experiment, the observed results were opposite of expected ones. It was hypothesized that higher ionic strength would produce greater bound residues. However, Figure 5.7 shows that the bound residue decreased from 693 to 591 μmole /kg of soil as ionic strength increased from 10 to 100 mM. This occurred despite the fact that more DPP associated with the sorbent at higher solution ionic strengths. Thus, it appears that methanol was able to dissolve and extract significant amounts of polymers especially at the higher ionic strength. Since at higher ionic strength the polymers are suggested to be denser, their interaction with the soil phase may have been reduced, thus, making the polymers more available and susceptible to methanol extraction. Consequently, polymers were more easily dissolved and removed from soil by methanol.

In the case of treatments with varying ionic strength no constant trend was observed in the stabilization of DCP. No significant differences were observed for treatments 10-20-10 and 10-20-20, 10-100-20 and 10-100-100, where the mass of stabilized DPP was similar when the soil was extracted with low ionic strength solutions. However, the bound residues for treatments 10-70-10 and 10-70-70 were significantly different.

These results suggest that methanol had less difficulty in dissolving the stabilized DCP at higher ionic strength. At varying ionic strength, the stabilized DCP seemed to be more strongly associated with the SOM-free soil than at constant ionic strength, i.e., at varying ionic strength greater mass of DPP remained in soil after the extraction cycle.

5.5.3.1.4 Distribution of Polymerization Products

The distribution of total solute in the different experimental phases is presented in Figure 5.8. The unreacted DCP corresponds to the DCP that was not converted during the initial polymerization stage; the term “soluble DPP” indicates the residual polymers remaining in solution once the reaction has been completed. The term “extractable DPP” designates the total sum of polymers removed by buffer and methanol extraction. The HPLC analysis of the methanol extracts did not reveal any presence of any DCP. Thus, all extracted chemical corresponded to sorbed polymers.

In contrast to the results observed in the soil-free experiments, in this study, DCP conversion was not affected at higher ionic strengths. Residual DCP varied from 2.2 to 4.4% of total DCP initially added. Results for the experiment at constant ionic strength showed that the

percent of soluble DPP decreased with increasing ionic strength. Soluble DPP decreased from 28 to 24 % for 10 and 100 mM, respectively. The amount of extractable DPP increased while the bound residue decreased at higher ionic strengths.

Results suggest that solution ionic strength had no significant impact on distribution of the initial DCP among the four fractions (unreacted DCP, soluble DPP, extractable DPP and stabilized residue). Unreacted DCP, soluble DPP, extractable and stabilized DPP varied from 2.2 to 8.5 %, 27 to 29%, 43 to 48 %, and 20 to 23 %, respectively. About half of the initially added DCP was recovered as extractable DPP while only about one quarter remained stabilized in soil. Thus, the effect of ionic strength observed in soil-free systems was not as evident in the presence of soil particles. These results suggest that solution ionic strength does not play a significant role in the stabilization of phenolic contaminants in soils and aquifers via HRP-mediated polymerization. However, solution ionic strength does not appear to impact the DCP precipitation from solution, as it does in solid-free systems.

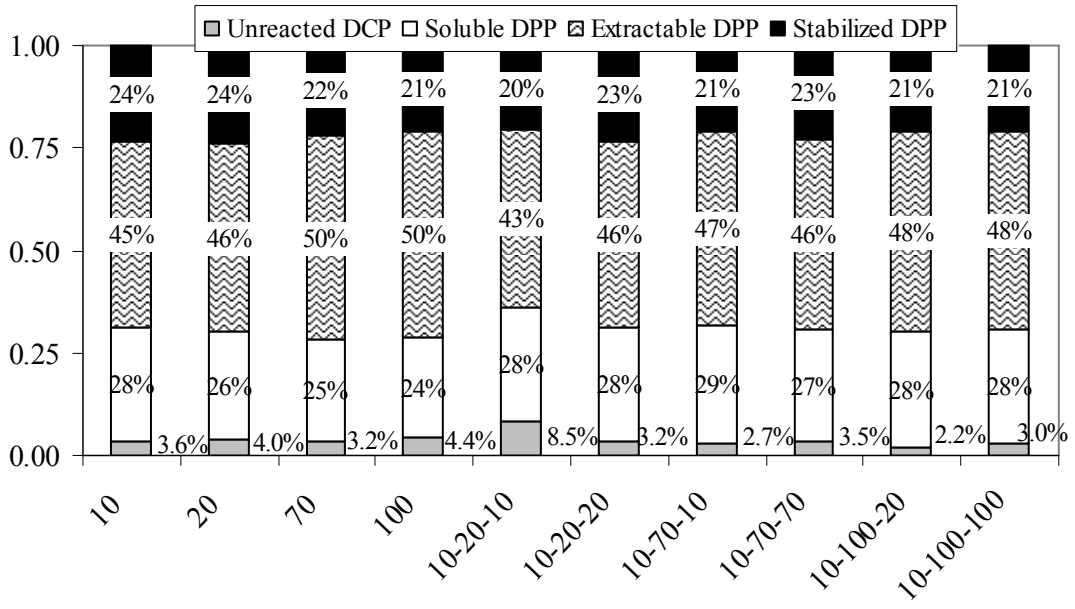

Figure 5.8. Effect of the ionic strength on the distribution of polymerization products in the presence of SOM-free soil.

5.5.3.2 DCP Enzymatic Polymerization in the Presence of SOM-Containing Soil

HRP-mediated DCP polymerization was evaluated in the presence of an agricultural soil under varying solution ionic strength and with an initial DCP concentration of 500 μM. Results for sorption/desorption, distribution and binding of the solute in the system are presented in the following sections. The purpose of using a surface soil with significant SOM content was to contrast the efficacy of the enzymatic polymerization process in the presence of inorganic and organic soils.

5.5.3.2.1 Extraction of Unreacted DCP and DPP from SOM-Containing Soil

Extraction of solute (unreacted DCP+DPP) was performed using buffers of varying ionic strength as explained in previous sections. The presence of SOM in the system makes it necessary to recall the operational definition of DPP. DPP consists of all polymerization reaction products including: soluble, sorbed and precipitated polymer, and DCP or polymers cross-coupled to SOM. After a single buffer extraction, DCP was detected in the supernatant. Thus, in this case the redissolved solute included unreacted DCP and DPP. The total mass fraction of DCP + DPP removed from soil with aqueous solutions of varying ionic strengths is illustrated in Figure 5.9.

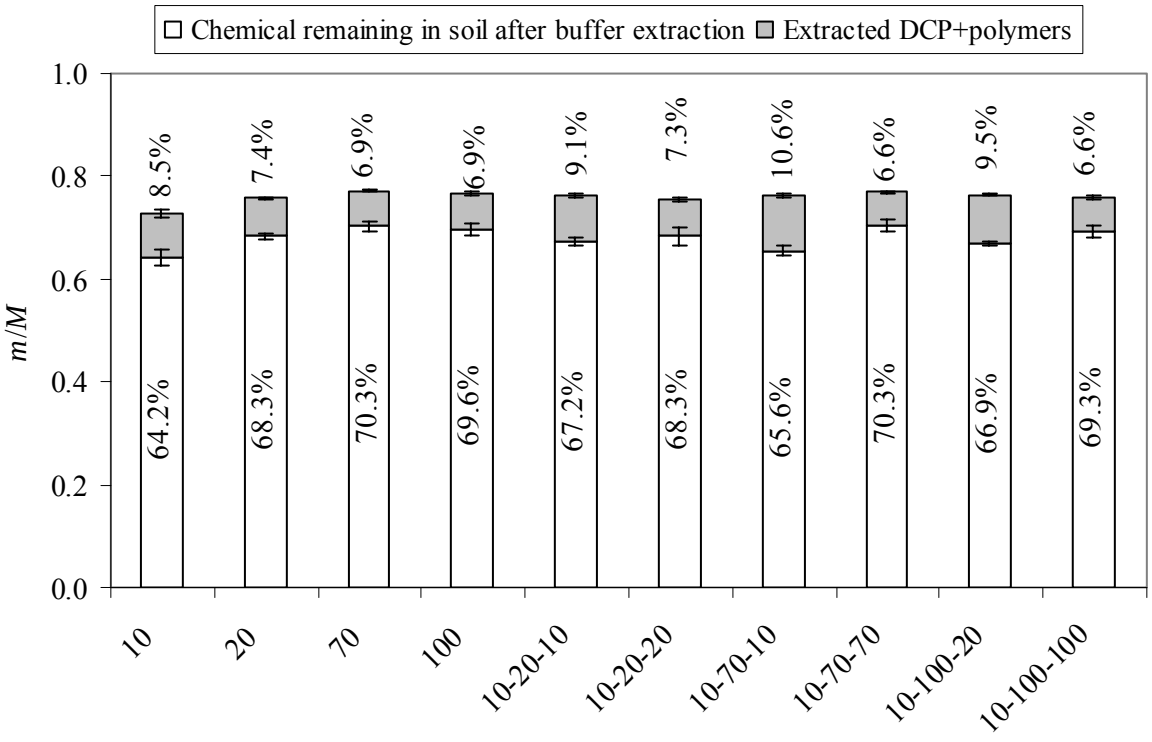

Figure 5.9. Unreacted DCP and DPP extracted with water from SOM-free soil.

In contrast to what was observed for the SOM-free soil (percent of chemical extracted remained constant), a decrease in the amount of extractable DCP + DPP was noted with increasing ionic strength for SOM-containing soil, for experiments where ionic strength was maintained constant at various stages of treatment. Consequently, increasing amounts of DCP + DPP remained associated with the soil at higher ionic strengths.

For treatments where the ionic strength was varied in the course of the experiment, more DCP + DPP were extracted when lower ionic strength buffers were used for extraction. The assumption of enhanced extraction of DCP + DPP with lower ionic strength buffers was found to be true and in agreement with the results of McDowell-Boyer, (1991) who observed that low ionic strength solutions disturbed and mobilized soil-deposited colloids. Observed extraction or re-dissolution of DCP + DPP was greater for the SOM-containing soil than for the SOM-free soil (Figure 5.6).

The mass fraction of DCP + DPP extracted from the surface soil was significantly higher than that extracted from the SOM-free soil, and the amount of solute remaining on soil was 1 to 7 % larger than that observed for the SOM-free soil. Sorption of parent DCP was noted in the surface soil suggesting that transformation of DCP could have been reduced by the presence of SOM. Geng et al. (2004) reported a reduction in the transformation of chlorophenols due to the sorption of soybean seed hull peroxidase (SBP) to the SOM. Thus, the reduction of DCP conversion in presence of SOM-containing soil might be attributed to HRP inactivation by blockage of the enzyme active site (HRP sorbed to SOM), by direct DCP sorption to SOM molecules, or by H_2O_2 scavenging by SOM.

5.5.3.2.2 Effect of Ionic Strength on Contaminant Stabilization in SOM-Containing Soil Systems

The extent of solute stabilization observed in the SOM-containing surface soil is illustrated in Figure 5.10. Stabilized solute was defined as the mass of solute remaining bound to the solid phase after four consecutive extractions (one with buffer and three with methanol). Result for the treatments where ionic strength was maintained constant through the course of the experiment, did not show any significant trends in contaminant stabilization (bound-residue formation). The amount of solute stabilized varied from 746 to 773 μmole/kg. The extent of contaminant stabilization was significantly greater in the presence of the SOM-containing

surface soil than that observed in SOM-free soil. For example, at 10 mM ionic strength, bound residue was 693 and 773 μmole/kg in the SOM-free soil and surface soil, respectively; at 100 mM ionic strength, the bound residue was 591 and 756 μmole/kg for the SOM-free and agricultural soil, respectively. Thus, although the conversion of DCP was reduced in presence of SOM, the extent of contaminant stabilization was higher in SOM-containing soil.

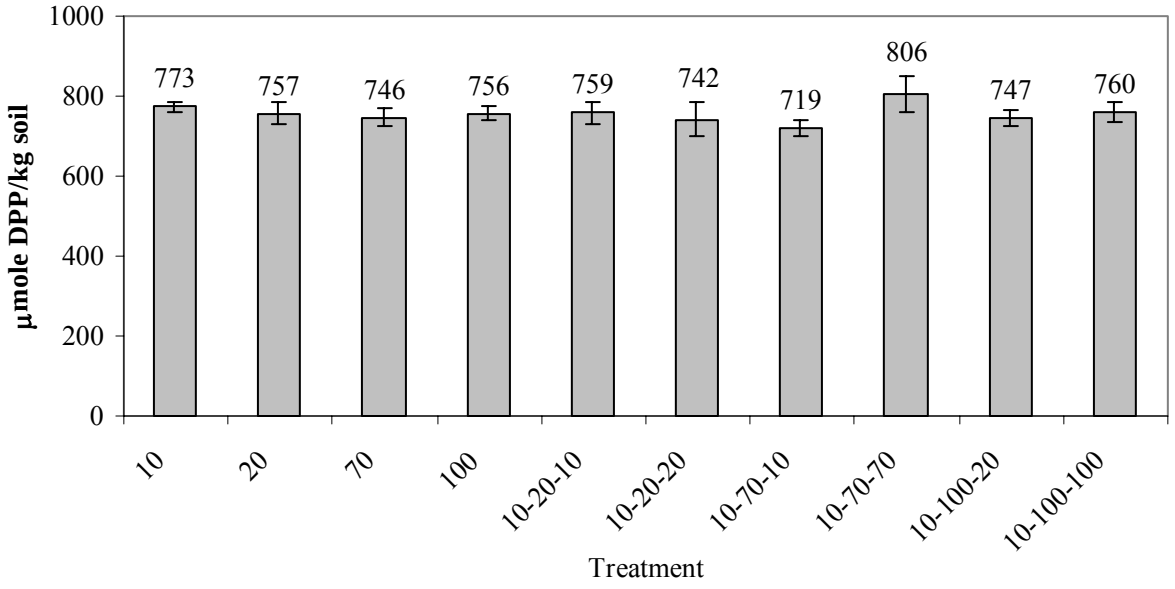

Figure 5.10. Mass of chemical bound to the agricultural soil. Data corresponds to 500 μM of initially added DCP.

In treatments with varying ionic strength, the effect of desorption with lower ionic strength solutions as seen after the buffer extraction, was no longer evident after three consecutive methanol extractions. Recall that bound residue was determined right after the completion of the methanol extractions. Thus, the effects of solution ionic strength were diminished by the use of methanol. However, the magnitude of the formed bound residue remained significantly larger than that reported for the SOM-free soil. Like in the case of the SOM-free soil, polymer binding at higher ionic strength was attenuated by the presence of soil particles.

5.5.3.2.3 Distribution of Total Solute in HRP-Amended Systems

The distribution of the total solute (DCP+ DPP) in presence of the SOM-containing surface soil was different from that resulting in the presence of SOM-free soil. The term "solvent extractable" was used to refer to the sum of water extractable solute (DCP + DPP) plus methanol extractable solute (DCP + DPP). The distribution of the solute among the aqueous and solid phases used throughout the experiment is presented in Figure 5.11. All the values corresponding to the distribution of solute are shown in Table 5.4. The amount of contaminant stabilized was higher in surface soil than in SOM-free soil. The percent of bound residue formed in the SOM-containing soil varied from 26 to 28 % of the initial DCP mass added. Solution ionic strength did not have a significant impact on bound residue formation.

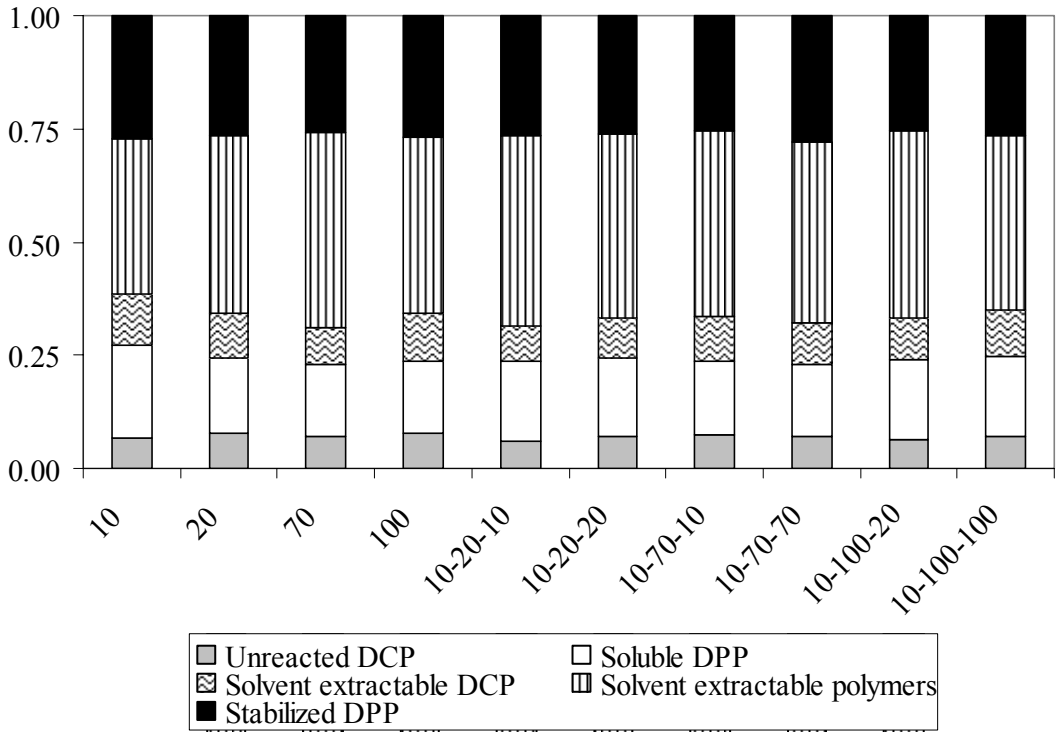

Figure 5.11. Effect of the ionic strength on the distribution of solute in presence of agricultural soil.

The unreacted DCP in supernatant, varied from 6.0 to 7.7 %. Unreacted DCP did not show any trend as function of solution ionic strength indicating that conversion was not affected by ionic strength. In contrast, the percent of unreacted DCP left in supernatant when the reaction

took place in the presence of SOM-free soil, was lower varying from 2.2 to 4.4 % as described in section 5.4.3.1.4.

For the treatments subjected to constant ionic strength, the soluble polymers in supernatant after completion of the reaction showed a decreasing trend with ionic strength increasing from 10 to 70 mM. Thus, for ionic strength s from 10 to 70 mM, the mass fraction of soluble DPP decreased from 0.21 to 0.16, respectively. However, when the solution ionic strength was varied, no significant differences were observed in the mass of soluble DPP, which varied from 16 to 18%. More unreacted DCP was left after completion of the reaction and less soluble DPP was produced with the SOM-containing surface soil than in SOM-free soil systems.

Total solvent extractable DCP (sum of DCP extracted by buffer and methanol) appeared to be independent of solution ionic strength. The percent of solvent extractable DCP ranged from 8 to 11% of total added parent DCP. Solvent extractable DPP fluctuated from 34 to 43%. Recall that when SOM-free soil was used, no DCP was extracted and solvent extractable DPP was as high as 50% of the initially added solute. These results show that in the presence of SOM-containing soil the transformation of DCP was reduced but SOM exhibited affinity to strongly associate with the parent DCP.

Table 5.4. Distribution of the DCP polymerization products. Standard deviation in italics (units are μmole).

	Treatment									
Type	**10**	**20**	**70**	**100**	**10-20-10**	**10-20-20**	**10-70-10**	**10-70-70**	**10-100-20**	**10-100-100**
Unreacted DCP	0.285 *0.090*	0.329 *0.034*	0.306 *0.040*	0.327 *0.040*	0.255 *0.031*	0.300 *0.032*	0.313 *0.015*	0.300 *0.030*	0.275 *0.014*	0.299 *0.072*
Soluble polymers	0.873 *0.062*	0.707 *0.032*	0.683 *0.044*	0.667 *0.023*	0.761 *0.047*	0.745 *0.020*	0.698 *0.051*	0.693 *0.028*	0.784 *0.072*	0.759 *0.056*
Solvent extractable polymer	1.444 *0.191*	1.676 *0.050*	1.872 *0.067*	1.643 *0.089*	1.802 *0.088*	1.741 *0.039*	1.740 *0.036*	1.723 *0.012*	1.817 *0.077*	1.638 *0.079*
Solvent extractable DCP	0.481 *0.151*	0.419 *0.080*	0.351 *0.046*	0.456 *0.053*	0.330 *0.013*	0.381 *0.062*	0.418 *0.021*	0.384 *0.032*	0.407 *0.043*	0.440 *0.031*
Bound	1.155 *0.017*	1.131 *0.043*	1.119 *0.035*	1.134 *0.027*	1.135 *0.041*	1.113 *0.066*	1.075 *0.032*	1.203 *0.065*	1.116 *0.031*	1.137 *0.035*

5.6 Summary and Conclusions

The objective of this study was to evaluate the effect of varying solution ionic strength on the sorption and stabilization of dichlorophenol polymerization products (DPP) in the context of a surface soil (3.4 % SOM) and a SOM-free soil.

The following hypotheses were evaluated:

Hypothesis 10: Precipitation of soluble DPP in aqueous solutions can be enhanced by increasing the solution ionic strength;

Hypothesis 11: HRP-mediated polymerization of DCP in the presence of a geosorbent is affected by the solution ionic strength; and

Hypothesis 12: Re-dissolution of precipitated DPP occurs when the solution ionic strength is lowered.

Hypothesis 10 was proven true by observing that when peroxidase enzyme and H_2O_2 were added to DCP solutions, the distribution of soluble DPP, precipitated DPP and unreacted DCP were dependent on the solution ionic strength. Higher ionic strengths resulted in more precipitated products and fewer soluble DPP.

Hypothesis 11 was proven false when DPP association with the SOM-free geomaterial and natural soil was found to be unaffected by solution ionic strength. Solution ionic strength appeared to have no impact on unreacted DCP, soluble polymers, and soil-associated polymers. The mass fraction of DPP that associated with SOM-free soil was significantly greater than that precipitated in soil-free systems.

Hypothesis 12 was proven true in sorbent-free systems by observing that extraction with reduced ionic strength water resulted in greater re-dissolution of the precipitated products.

Hypothesis 12 in presence of solids was proven true since the amount of water extractable polymers increased when using lower ionic strength buffers to re-dissolve the precipitated polymers.

However, increase of solution ionic strength appeared to have no impact in the amount of stabilized DPP.

5.6.1 Real-World Implications

Enzyme-mediated oxidative polymerization of phenolic contaminants can be an effective process to stabilize the organic contaminants in soil. However, groundwater and surface waters

can have a broad range of ionic strength. Variation of the ionic strength in the surface and surbsurface water could change the sorption behavior of soil particles and contaminants.

The impact of solution ionic strength on HRP-mediated polymerization of DCP, in contact with inorganic and organic geosorbents demonstrated that the contaminant stabilization was effective independently of the solution background concentration.

In the case of surface soils, even if the remediated zone is irrigated by lower ionic strength water the results of this experiment suggested that although some desorption or redissolution of the polymer could occur most of the chemical would remain strongly associated with the soil. Thus, seems that the solute (DCP+DPP) would be stable in soil.

However, a limitation of the current study is that, the experimental set up did not evaluate the situation where constant flow of low ionic strength is continuously drained through the soil that has been subjected to the HRP-stabilized treatment, since it could affect the soil stabilized DPP.

5.7 Literature Cited

Abramson, A. and Brown, D. G. Influence of solution ionic strength on the collision efficiency distribution and predicted transport distance of a Sphingonomonas sp. flowing through porous media. *Water Res.* 2007, 41: 4435- 4445.

Bhandari, A.; Novak, J. T. and Berry, D. F. Binding of 4-monochlorohenol to soil. *Environ. Sci. Technol.* 1996, 30:2305-2311.

Bhandari, A.; Novak, J. T.; Burgos, W. D.; and Berry, D. F. Irreversible binding of chlorophenols to soils and its impact on bioavailability. *J. Environ. Eng*. 1997, 506-513

Bhandari, A. and Xu, F. Impact of peroxidase addition on the sorption-desorption behavior of phenolic contaminants in surfaces soils. *Environ. Sci. Technol.* 2001, 35:3163-3168.

Chang, J. S.; and Vigneswaran,S. Ionic strength in deep filtration. *Water Res*. 1990, 24 (11): 1425-1430.

Clausen, L.; and Fabricius, I. Atrazine, Isoproturon, mecoprop, 2,4-D, and betazone adsorption onto iron oxides. *J. Environ. Qual.* 2001, 30: 858-869.

Clausen, L.; Fabricius, I.; and Madsen, L. Adsorption of pesticides onto quartz, calcite, kaolinite and ∝-alumina. *J. Environ. Qual.* 2001, 30: 846-857.

Dec, J. and Bollag, J. M. Detoxification of substituted phenols by oxidoreductive enzymes through polymerization reactions. *Arch. Environ. Contam. Toxicol.* 1990, 19: 543-550.

Dec, J. and Bollag, J. M. Phenoloxidase-mediated interactions of phenols and anilines with humic materials. *J. Environ. Qual.* 2000; 29(3): 665-676.

Elimelech, M. and O'Melia, C. R. Kinetics of deposition of colloidal particles in porous media. *Environ.Sci.Technol.* 1990, 24:1528-1536.

Essington, M. E. *Soil and water chemistry. An integrative approach.* CRC Press, 2004.

Gamerdinger, A. P.; and Kaplan, D. I. Colloid transport and deposition in water-saturated Yucca Mountain tuff as determined by ionic strength. *Environ. Sci. Technol.* 2001, 35:3326-3331.

Geng. Z.; Bassi, A.; and Gijzen, M. Enzymatic treatment of soils contaminated with phenol and chlorophenols using soyben seed hulls. *Water, Air, and Soil Pollution.* 2004, 154: 151–166.

Huang, Q.; Tang, J.; and Weber Jr., W. J. Precipitation of enzyme-catalyzed phenol oxidative coupling products: Background ion and pH effects. *Water Res.*. 2005, 39:3021-3027.

Kim, W. Phenol removal from saturated porous media using horseradish peroxidase mediated oxidative polymerization process. Ph.D. dissertation, Department of Civil Engineering, Kansas State University, 2007.

Laurenti, E.; Suriano, G.; Ghibaudi, E. M.; and Ferrari, R. P. Ionic strength and pH effect on the Fe(III)-imidazolate bond in the heme pocket of horseradish peroxidase: an EPR and UV-visible combined approach. *J. Inorganic Biochem*. 2000, 81: 259-266.

Liu, S-Y.; Minard, R. D. and Bollag J. M. Soil-catalyzed complexation of the pollutant 2,6-diethylaniline with syringic acid. *J. Environ. Qual*. 1987, 16(1): 48-53.

McDowell-Boyer, L. M. Chemical mobilization of micron-sized particles in saturated porous media under steady flow conditions. *Environ. Sci. Technol.* 1992, 26: 586-593.

Mills, A. L.; Herman, J. S.; Hornberger, G. M.; and DeJesús, T. H. Effect of solution ionic strength and iron coatings on mineral grains on the sorption of bacterial cells to quartz sand. *Appl. Environ. Microbiol.* 1994, 60 (9): 3300-3306.

Minard, R. D.; Liu, S-Y; and Bollag, J.-M. Oligomers and Quinones from 2,4-Dichlorophenol. *J. Agric. Food Chem.* 1981, *29*, 250-253.

Nicell, J. A.; Bewtra, J. K.; C.C. St. Pierre; N. Biswas; and K.E. Taylor. Enzyme catalyzed polymerization and precipitation of aromatic compounds from wastewater. *Water Sci. Technol.* 1992, 25:157-164.

Olmsted, J. B.; and Borisy, G. G. Ionic and nucleotide requirements for microtubule polymerization in vitro. *Biochem.* 14(13): 2996-3005.

Palomo, M.; and Bhandari, A. Time-dependent sorption-desorption behavior of 2,4-dichlorophenol and its polymerization products in surface soils. *Environ. Sci.Technol*. 2005, 39: 2143-2151.

Palomo, M.; and Bhandari, A.Impact of aging on the formation of bound residues after peroxidase-mediated treatment of 2,4-DCP contaminated soils. *Environ. Sci. Technol*. 2006, 40:3402-3408.

Park, J-W.; Dec, J.; Kim, J-E.; and Bollag, J. M. Transformation of chlorinated phenols and anilines in the presence of humic acid. *J. Environ. Qual*. 2000, 29:214-220.

Qasim, S. R., Motley, E. M and Zhu, G. *Water Works Engineering. Planning, Design and Operation*. Prentice Hall PTR. 2000.

Simmos, K. A.; Minard R. D.; and Bollag, J. M. Oligomerization of 4-chloroaniline by oxidoreductases. *Environ. Sci. Technol.* 1987, 21: 999-1003.

Thorn, K. A.; Pettigre, P.J.; Goldenberg, W. S. and Weber, E. J. Covalent binding of aniline to humic substances. 2. ^{15}N NMR studies of nucleophilic addition reactions. *Environ. Sci. Technol*. 1996, 30:2764-2775.

Wagner, M. and Nicell, J. Impact of dissolved wastewater constituents on peroxidase-catalyzed treatment of phenol. *J. Chem. Technol. Biotechnol*. 2002, 77:419-428.

Whatson, J. R.; Posner, A. M. and Quirk, J. P. Adsorption of the herbicide 2,4-D on goethite. *J. Soil. Sci.* 1973, 24: 503-511

Weber, E. J.; Spidle, D. L. and Thorn, K. A. Covalent binding of aniline to humic substances 1. Kinetic studies. *Environ. Sci. Technol*. 1996, 30:2755-2763.

Xu, F. Horseradish peroxidase mediated sorption and binding of phenol, o-cresol, 2,4-dichlorophenol and 1-naphthol to two surface soils. Ph.D. dissertation, Department of Civil Engineering, Kansas State University, 2002.

Xu, F.; and Bhandari, A. Retention and distribution of 1-naphthol and naphthol polymerization products on surface soils. *J. Environ .Eng*. 2003a, *129,* 1041-4050.

Xu, F.; and Bhandari, A. Retention and extractability of phenol, cresol, and dichlorophenol exposed to two surfaces soils in the presence of horseradish peroxidase. *J. Agric. Food Chem.* 2003b, *51*, 183-188.

CHAPTER 6 - SUMMARY AND CONCLUSIONS

6.1 DCP Stabilization in a Humin-Mineral Soil

The work reported in this dissertation illustrates the effectiveness of horseradish peroxidase mediated oxidative polymerization in stabilizing phenolic contaminants in humin-mineral soils. This work is distinctive from previous investigations since it addresses the impact of contact time on the association of solute (DCP+DPP) with two humin-mineral soils and it subsequent extractability with polar solvents such as water and methanol.

HRP-mediated oxidative coupling of DCP was induced in presence of two humin-mineral soils and formation of DPP was observed (Hypothesis 1). Addition of HRP to the system enhanced the removal of DCP from the aqueous solution and increased the amount of solute associated with the humin-mineral soils. (Hypothesis 1 and Hypothesis 2). Solute phase distribution for both sorbents exhibited a nonlinear sorption behavior for DCP and linear phase distribution of solute in HRP-amended systems. Single point organic matter-normalized distribution coefficient, $K_{OM,C\,t}$ for DCP, showed that at low residual solute concentrations AHM exhibited a higher sorption capacity. As residual aqueous concentration increased, WHM sorbed more DCP than AHM. In contrast, in the HRP-amended systems, $K_{OM,\,Ct}$ values indicated that AHM exhibited a higher capacity for DPP at all the residual aqueous concentrations evaluated. These results suggested that enzymatic polymerization of DCP enhanced solute affinity for the humin-type organic matter present in AHM.

Aqueous extractability (*EI*) of DCP was observed to decrease at higher residual solute concentrations and contact times, suggesting that DPP precipitation and solute diffusion into the exposed humin matter were mechanisms that over surface adsorption predominantly removed solute from the aqueous phase. Extractability of DCP from AHM was slightly higher from WHM *EI*. Aqueous extractability of solute (DCP+DPP) decreased as contact time progressed and residual aqueous concentration increased. Total solute (DCP+DPP) associated with WHM was more readily extracted with water than that associated with AHM, indicating that the SOM content did not increase oligomer binding (Hypothesis 3).

Overall, extractability of DCP and DPP with water and methanol decreased with contact time. The enhancement in bound residue formation was significant in the presence of HRP. Bound residue in both AHM and WHM was similar at all initial aqueous concentrations except

at C_o= 500 μM where significantly more solute was associated WHM soil (Hypothesis 4).

The amount of solute associated after 28 days of contact, independently of the initial aqueous concentration, consisted of approximately 80% and 78% of the total mass of DCP initially added for the WHM and AHM, respectively. The residual solute in supernatant consisted of soluble DPP and unreacted DCP. The amount of soluble polymers increased slightly at higher initial aqueous concentrations.

The amount of bound residue increased slightly with contact time for WHM, but remained steady for the AHM soil. The extractable solute consisted of sorbed DPP and DCP. While extractable DPP increased at higher initial solute concentrations, due to greater DPP formation, the amount of extractable DCP was reduced.

6.1.1 Real-World Implications

The stabilization of phenolic contaminants through enzymatic polymerization is a potential remediation technique for contaminated surface soil and subsurface material. Successful application of engineered humification requires a complete visualization of the fate of the target contaminants and their polymerization products. The significance of studying polymerization of DCP in the context of humin-enriched geosorbents is the fact that phenolic contaminants can be present in the subsurface where organic matter present is dominated by the humin type. Interactions between DCP polymerization products (DPP) with the humin fraction and their effect on the sorption, extraction and binding of DPP are, therefore, of interest in order to evaluate the effectiveness of this method under conditions different from that observed in a surface soil.

Results from this study suggest that, though the amount of organic matter present in soil is an important factor to determine the amount of organic chemicals sorbing and binding to the soil phase, the chemical characteristics of the SOM should also being taken into account to predict sorption behavior of the contaminant. This study showed that the mass of chemical associated with geosorbents with low SOM content and chemical character different from that predominant in surfaces soils, can be as high as that observed when high SOM contents are present in the geosorbent.

So, the application of the HRP-mediated stabilization of aqueous phase DCP was observed to be independent of the amount of SOM present in soil but favored by the chemical

composition and physical arrangement of the humin fraction. The results from this work suggest that HRP-mediated stabilization would be successfully applied in a subsurface environment.

Lastly, phase distribution data implies that 24 hrs would be enough time to get significant mass of contaminant associated with the soil, since sorption equilibrium seem to be achieved after 1 day without further increase on the amount of mass sorbed to the geosorbent. Thus, field application of this remediation technology would not require prolonged amount of time.

6.2 DCP Stabilization in a SOM- free Soil

Enzymatic polymerization of DCP in SOM-free soil can be as high as that observed in SOM containing systems (Hypothesis 5). Polymerization reactions in SOM-free soils results in significant DCP removal from aqueous phase in HRP-amended soils (Hypothesis 6). Sorption contact time does not have an impact on the extent of solute sorption. SOM-free soil surface is modified upon coverage with polymers, thus enhancing subsequent solute (DCP+ DPP) sorption.

Solute extractability from SOM-free soil decreased with contact time and was lower at smaller residual solute aqueous concentrations (Hypothesis 7). Total solvent extractable solute consists entirely of DPP and no unreacted DCP. The interactions between DPP and minerals in the SOM-free soil appeared to be strong since DPP resist extraction with methanol (Hypothesis 7). Contact time increased bound residue formation at all initial DCP concentrations (Hypothesis 7) and a greater fraction of the solute was converted to DPP bound residues at lower initial concentrations (Hypothesis 8).

In SOM-free soils, the precipitation of DPP appears to be the main mechanism causing the removal of the solute from aqueous phase. In the case of sorbents rich in SOM a combination of precipitation, sorption and cross-coupling of DPP are the processes that contribute to the solute retention in the solid phase (Hypothesis 9).

Thus, HRP mediated-oxidative polymerization of DCP is an effective process that is capable of stabilizing DCP in the presence of predominantly mineral materials.

6.2.1 Real-World Implications

Mineral soils are abundant in the earth. Inorganic soils might be present in the surface or in the subsurface. Contamination of soils is not exclusive of those containing SOM. Understanding of the interaction of DPP with mineral soils is important to extend the application of HRP mediated-oxidative polymerization of phenolic contaminants as a remediation

technology for soil containing none or negligible SOM.

Results from this study are important because they support the idea that even in the absence of SOM, enzyme-mediated oxidative polymerization of phenolic contaminants can be an effective process to stabilize the organic contaminants in soil. It was reported that the HRP-stabilization of DCP was not dependent of the amount of SOM present in the sorbent. Thus, this remediation treatment could be effectively used to remediate predominantly mineral contaminated soils.

Similarly to what was observed in the case of the humin-mineral geosorbent, about 24 hrs were required to achieve significant sorption of the DPP with the mineral phase, suggesting that in a real situation the amount of time required to conduct the treatment would be relatively short.

Contact time appeared to be important in the bound residue formation of DPP in mineral soils. In a real situation these results would imply that, time will allow the increase of the amount of stabilized contaminant within the mineral soil. The HRP-stabilization of phenolic chemicals in a mineral soil can be related to the formation of synthetic SOM coverage that can provide potential sorption capacity to the geosorbent.

6.3 Ionic Strength Effect on the Stabilization of DPP

In soil-free systems, the distribution of soluble DPP, precipitated DPP and unreacted DCP was dependent on the solution ionic strength. Higher ionic strength resulted in more precipitated products and fewer soluble DPP (Hypothesis 10). Redissolution with water containing low ionic strengths resulted in higher extraction of DPP (Hypothesis 12). The amount of precipitated polymers after redissolution with water remained greater at higher ionic strengths.

In soil-containing systems retention of solute under varying ionic strength conditions, was studied in the context of a mineral and a SOM-containing soil.

SOM-free soil-The mass fraction of solute (DCP+DPP) associated with SOM-free soil was significantly higher that the mass fraction precipitated in soil-free systems. Solute association with soil was not affected by ionic strength (Hypothesis 11). Extraction with low ionic strength removed or redissolved significant amount of soil associated solute (Hypothesis 12), but a major DPP mass fraction continued to be associated with the solids. Bound residue formation was observed to decrease as ionic strength increased. Solution ionic strength had no impact in unreacted DCP, soluble polymers, extractable polymers and bound residue. Nearly half

of the formed DPP was removed through solvent extractions. From the initially added DCP about one quarter remained bound to the soil.

Agricultural soil- The definition of DPP includes all the polymerization reaction products (soluble, sorbed, precipitated DPP and those cross-coupled to SOM). Increase in solutions ionic strength did not impact the amount of solute (DCP+DPP) associated with the agricultural soil. When solution ionic strength was maintained constant no change in the formation bound residue was observed. DCP polymerization appeared to be reduced in presence of SOM. However, the amount of DCP stabilized in the agricultural soil was higher than in SOM-free geosorbent. Enhancement of removal of colloids or polymers from aqueous solutions, at high ionic strengths, was attenuated in the presence of both geosorbents (Hypothesis 11). Utilization of low ionic strength solution resulted in greater DPP extraction from the soil (Hypothesis 12).

6.3.1 Real-World Implications

Enzyme-mediated oxidative polymerization of phenolic contaminants can be an effective process to stabilize the organic contaminants in soil. However, groundwater and surface waters can have a broad range of ionic strength. Variation of the ionic strength in the surface and surbsurface water could change the sorption behavior of soil particles and contaminants.

The impact of solution ionic strength on HRP-mediated polymerization of DPP, in contact with inorganic and organic geosorbents demonstrated that the contaminant stabilization was effective independently of the solution background concentration.

In the case of surface soils, even if the remediated zone is irrigated by lower ionic strength water the results of this experiment suggested that although some desorption or redissolution of the polymer could occur most of the chemical would remain strongly associated with the soil. Thus, seems that the DPP would be stable in soil.

However, a limitation of the current study is that, the experimental set up did not evaluate the situation where constant flow of low ionic strength is continuously drained through the soil that has been subjected to the HRP-stabilized treatment, since it could affect the soil stabilized DPP.

6.4 Overall Conclusions

The results show that even in the absence of SOM, a significant amount of DCP can be stabilized in minerals soils or soils that have negligible SOM content. In the past, enzymatic polymerization was broadly studied in soils that were rich in SOM to take advantage of the cross-coupling reactions between the SOM and the phenolic molecules. However, as demonstrated in this study, even in the absence of SOM but in presence of a mineral soil, the self-coupling of phenolic molecules can result in significant removal of the parent phenol from the aqueous phase. The amount solute (DCP+DPP) associated with the SOM-free soil was comparable to that observed with humin-mineral or surface agricultural soils. Thus, stabilization of DCP through HRP-mediated polymerization was enhanced in the present of solids and seemed to be independent of the type of geosorbent.

The difference in sorption capacity of the humin-mineral geosorbents was attributed to the difference in SOM content of both sorbents. However, higher affinity of the oligomers for the organic matter in AHM than that in WHM, was credited to AHM chemical characteristics, which exhibited higher aliphaticity and lower polarity when compared toWHM.

For both humin-mineral and mineral geosorbents, contact time was observed to be important for the increased formation of stabilized solute residues. Therefore, though sorption equilibrium seemed to be achieved with 24 hrs, the mass transfer at the particle level appeared to continue throughout the experimental time, favoring the formation of stabilized solute. It is suggested that as time passes the precipitated DPP are arranged in such way that they form stronger interactions with the geosorbent surface and/or diffuse into the soil matrix (mineral or organic), consequently hindering them from being extracted and dissolved, and favoring their stabilization.

This work also suggests that while changing solution ionic strength has a slight impact on the distribution of the polymerization products, the overall effect on the stabilized contaminant is practically independent of the ionic strength utilized at any stage of the experiment.

Appendix A - DATA FOR CHAPTER 3

Table 6.1. Data for Figure 3.2.

Initial aqueous concentration (μM)	Contact Time (Days)					
	1		7		28	
	C **(t)**	***q*** **(t)**	***C*** **(t)**	***q*** **(t)**	***C*** **(t)**	***q*** **(t)**
0.5	-0.753	0.267	-0.835	0.307	-0.861	0.307
0.5	-0.951	0.349	-0.902	0.329	-0.869	0.315
0.5	-0.773	0.270	-0.844	0.305	-0.844	0.296
5	0.246	1.246	0.186	1.284	0.202	1.291
5	0.296	1.220	0.159	1.296	0.221	1.286
5	0.341	1.189	0.162	1.284	0.121	1.324
50	1.424	2.127	1.381	2.191	1.398	2.144
50	1.404	2.112	1.391	2.189	1.398	2.138
50	1.461	2.107	1.385	2.195	1.403	2.139
100	1.763	2.356	1.749	2.403	1.735	2.421
100	1.761	2.369	1.743	2.398	1.729	2.430
100	1.754	2.380	1.744	2.397	1.733	2.441
500	2.543	2.927	2.528	2.968	2.524	2.998
500	2.540	2.945	2.503	3.010	2.531	2.985
500	2.524	2.965	2.522	2.987	2.530	2.987

Table 6.2. Data for Figure 3.3.

Initial aqueous concentration (μM)	Contact Time (Days)					
	1		7		28	
	C (t)	q (t)	C (t)	q (t)	C (t)	q (t)
0.5	-0.819	0.326	-0.884	0.321	-0.868	0.310
0.5	-0.815	0.323	-0.868	0.308	-0.854	0.311
0.5	-0.822	0.316	-0.888	0.320	-0.841	0.306
5	0.362	1.159	0.330	1.200	0.329	1.220
5	0.360	1.157	0.362	1.173	0.326	1.224
5	0.358	1.166	0.360	1.119	0.346	1.211
50	1.517	1.993	1.511	2.043	1.502	1.998
50	1.527	1.970	1.521	2.027	1.494	2.011
50	1.517	1.993	1.498	2.065	1.505	1.995
100	1.853	2.201	1.841	2.242	1.835	2.266
100	1.827	2.254	1.844	2.185	1.833	2.265
100	1.843	2.213	1.834	2.261	1.850	2.227
500	2.607	2.730	2.597	2.778	2.608	2.726
500	2.621	2.657	2.597	2.781	2.600	2.760
500	2.598	2.778	2.612	2.712	2.602	2.772

Table 6.3. Data for Figure 3.4.

Initial aqueous concentration (μM)	Contact Time (Days)							
	1				7			
	C (t)	q (t)	C (t)^e	q (t)^e	C (t)	q (t)	C (t)^e	q (t)^e
0.5	-0.753	0.267	-1.028	0.149	-0.835	0.307	-1.038	0.202
0.5	-0.951	0.349	-0.999	0.235	-0.902	0.329	-1.066	0.240
0.5	-0.773	0.270	-1.002	0.146	-0.844	0.305	-1.021	0.199
5	0.246	1.246	0.003	1.108	0.186	1.284	-0.011	1.169
5	0.296	1.220	0.024	1.066	0.159	1.296	-0.031	1.185
5	0.341	1.189	0.043	1.019	0.162	1.284	-0.021	1.177
50	1.424	2.127	1.067	1.929	1.381	2.191	1.045	2.051
50	1.404	2.112	1.064	1.925	1.391	2.189	1.056	2.063
50	1.461	2.107	1.061	1.890	1.385	2.195	1.086	2.050
100	1.763	2.356	1.380	2.125	1.749	2.403	1.369	2.216
100	1.761	2.369	1.367	2.132	1.743	2.398	1.366	2.200
100	1.754	2.380	1.375	2.143	1.744	2.397	1.379	2.192
500	2.543	2.927	2.033	2.642	2.528	2.968	2.095	2.759
500	2.540	2.945	2.045	2.669	2.503	3.010	2.090	2.809
500	2.524	2.965	2.053	2.705	2.522	2.987	2.050	2.768

Initial aqueous concentration (μM)	28			
	C (t)	q (t)	C (t)^e	q (t)^e
0.5	-0.861	0.307	-1.034	0.205
0.5	-0.869	0.315	-1.034	0.210
0.5	-0.844	0.296	-1.041	0.191
5	0.202	1.291	-0.024	1.189
5	0.221	1.286	-0.002	1.196
5	0.121	1.324	-0.050	1.231
50	1.398	2.144	1.071	1.970
50	1.398	2.138	1.058	1.966
50	1.403	2.139	1.063	1.974
100	1.735	2.421	1.388	2.240
100	1.729	2.430	1.354	2.271
100	1.733	2.441	1.371	2.252
500	2.524	2.998	2.055	2.787
500	2.531	2.985	2.062	2.777
500	2.530	2.987	2.041	2.780

Table 6.4. Data for Figure 3.5.

Initial aqueous concentration (μM)	Contact Time (Days)							
	1				7			
	C (t)	q (t)	C (t)^e	q (t)^e	C (t)	q (t)	C (t)^e	q (t)^e
0.5	-0.819	0.326	-1.029	0.221	-0.884	0.321	-1.033	0.218
0.5	-0.815	0.323	-1.034	0.221	-0.868	0.308	-1.036	0.217
0.5	-0.822	0.316	-1.038	0.214	-0.888	0.320	-1.070	0.232
5	0.362	1.159	0.050	0.973	0.330	1.200	0.064	1.033
5	0.360	1.157	0.048	0.969	0.362	1.173	0.040	1.016
5	0.358	1.166	0.038	0.988	0.360	1.119	0.053	0.932
50	1.517	1.993	1.052	1.728	1.511	2.043	1.061	1.833
50	1.527	1.970	1.033	1.704	1.521	2.027	1.079	1.841
50	1.517	1.993	1.042	1.725	1.498	2.065	1.044	1.855
100	1.853	2.201	1.335	1.881	1.841	2.242	1.313	2.000
100	1.827	2.254	1.336	1.685	1.844	2.185	1.339	1.890
100	1.843	2.213	1.332	1.900	1.834	2.261	1.306	2.027
500	2.607	2.730	1.965	2.353	2.597	2.778	1.978	2.478
500	2.621	2.657	1.946	2.243	2.597	2.781	1.944	2.526
500	2.598	2.778	1.975	2.407	2.612	2.712	1.963	2.423

Initial aqueous concentration (μM)	28			
	C (t)	q (t)	C (t)^e	q (t)^e
0.5	-0.868	0.310	-1.043	0.212
0.5	-0.854	0.311	-1.043	0.208
0.5	-0.841	0.306	-1.016	0.193
5	0.329	1.220	0.050	1.078
5	0.326	1.224	0.043	1.088
5	0.346	1.211	0.046	1.054
50	1.502	1.998	1.028	1.777
50	1.494	2.011	1.031	1.793
50	1.505	1.995	1.045	1.758
100	1.835	2.266	1.313	2.037
100	1.833	2.265	1.323	2.007
100	1.850	2.227	1.276	2.058
500	2.608	2.726	1.942	2.424
500	2.600	2.760	1.932	2.562
500	2.602	2.772	1.946	2.364

Table 6.5. Data for Figure 3.6.

DCP	Woodland			Agricultural		
t (days)	EI_1	EI_{10}	EI_{100}	EI_1	EI_{10}	EI_{100}
1	0.894	0.697	0.555	1.034	1.075	1.119
7	0.814	0.610	0.463	0.836	0.549	0.362
28	0.781	0.560	0.403	0.776	0.501	0.316

Table 6.6. Data for Figure 3.7.

Initial Concentration (μM)	0.5			5			50		
Time (days)	**1**	**7**	**28**	**1**	**7**	**28**	**1**	**7**	**28**
Bound	0.04 *0.00*	0.10 *0.01*	0.14 *0.01*	0.29 *0.03*	0.86 *0.09*	1.01 *0.08*	2.21 *0.42*	4.00 *0.28*	5.42 *0.06*
Solvent Extractable	1.95 *0.22*	1.96 *0.06*	1.89 *0.05*	16.27 *1.10*	18.54 *0.34*	18.97 *0.95*	128.27 *3.39*	151.48 *1.24*	132.72 *1.03*
Initial Concentration (μM)	**100**			**500**					
Time (days)	**1**	**7**	**28**	**1**	**7**	**28**			
Bound	3.90 *0.37*	6.44 *0.98*	8.57 *0.47*	11.60 *0.49*	19.12 *3.36*	26.63 *2.51*			
Solvent Extractable	229.58 *6.78*	244.42 *1.93*	261.00 *6.43*	871.66 *38.07*	955.14 *43.15*	950.32 *12.93*			

Table 6.7. Data for Figure 3.8.

Initial Concentration (μM)	**0.5**			**5**			**50**		
Time (days)	**1**	**7**	**28**	**1**	**7**	**28**	**1**	**7**	**28**
Bound	0.04 *0.01*	0.08 *0.00*	0.10 *0.01*	0.24 *0.02*	0.46 *0.04*	0.76 *0.04*	1.64 *0.13*	2.77 *0.25*	3.54 *0.34*
Solvent Extractable	2.06 *0.03*	1.99 *0.03*	1.94 *0.01*	14.24 *0.14*	14.17 *1.33*	15.77 *0.28*	95.06 *3.05*	108.26 *5.02*	96.84 *1.56*
Initial Concentration (μM)	**100**			**500**					
Time (days)	**1**	**7**	**28**	**1**	**7**	**28**			
Bound	2.85 *0.25*	3.29 *1.01*	5.98 *0.82*	10.60 *1.34*	14.22 *0.98*	17.55 *0.51*			
Solvent Extractable	164.36 *10.88*	166.78 *16.32*	173.00 *9.02*	519.80 *71.79*	558.88 *50.90*	548.89 *29.92*			

Table 6.8. Data for Figure 3.9.

Initial aqueous concentration (μM)	**Contact Time (Days)**					
	1		**7**		**28**	
	C **(t)**	***q*** **(t)**	***C*** **(t)**	***q*** **(t)**	***C*** **(t)**	***q*** **(t)**
0.5	-1.188	0.384	-1.180	0.383	-1.154	0.384
0.5	-1.154	0.375	-1.195	0.388	-1.102	0.370
0.5	-1.172	0.381	-1.187	0.376	-1.079	0.372
5	-0.116	1.400	-0.145	1.390	-0.131	1.381
5	-0.098	1.389	-0.159	1.380	-0.120	1.383
5	-0.125	1.392	-0.086	1.373	-0.099	1.377
50	0.842	2.390	0.892	2.384	0.888	2.378
50	0.860	2.381	0.861	2.386	0.926	2.376
50	0.850	2.384	0.846	2.380	0.884	2.385
100	1.191	2.688	1.229	2.671	1.276	2.683
100	1.213	2.686	1.251	2.677	1.456	2.615
100	1.201	2.691	1.192	2.695	1.259	2.678
500	1.972	3.358	2.015	3.365	2.021	3.358
500	1.964	3.371	2.028	3.365	2.022	3.353
500	1.974	3.361	1.999	3.354	1.977	3.369

Table 6.9. Data for Figure 3.10.

Initial aqueous concentration (μM)	Contact Time (Days)					
	1		7		28	
	C (t)	q (t)	C (t)	q (t)	C (t)	q (t)
0.5	-1.043	0.369	-1.086	0.369	-1.056	0.358
0.5	-1.030	0.352	-1.066	0.369	-0.979	0.344
0.5	-1.040	0.351	-1.059	0.366	-1.003	0.348
5	-0.017	1.372	-0.035	1.355	-0.006	1.354
5	0.031	1.358	-0.023	1.358	0.043	1.346
5	-0.015	1.371	-0.007	1.364	0.039	1.346
50	0.965	2.366	1.027	2.350	1.017	2.349
50	0.977	2.343	1.031	2.355	1.025	2.345
50	0.984	2.366	1.012	2.354	1.062	2.336
100	1.359	2.523	1.375	2.638	1.383	2.643
100	1.312	2.670	1.392	2.646	1.421	2.639
100	1.336	2.653	1.381	2.647	1.401	2.645
500	2.040	3.349	2.086	3.346	2.092	3.347
500	2.044	3.352	2.080	3.349	2.073	3.351
500	2.040	3.353	2.081	3.352	2.088	3.336

Table 6.10. Data for Figure 3.11.

Initial aqueous concentration (μM)	Contact Time (Days)							
	1				7			
	C (t)	q (t)	C (t)^e	q (t)^e	C (t)	q (t)	C (t)^e	q (t)^e
0.5	-1.188	0.384	-1.363	0.345	-1.180	0.383	-1.347	0.342
0.5	-1.154	0.375	-1.332	0.331	-1.195	0.388	-1.383	0.352
0.5	-1.172	0.381	-1.365	0.341	-1.187	0.376	-1.371	0.337
5	-0.116	1.400	-0.306	1.357	-0.145	1.390	-0.351	1.351
5	-0.098	1.389	-0.264	1.341	-0.159	1.380	-0.345	1.340
5	-0.125	1.392	-0.300	1.347	-0.086	1.373	-0.253	1.322
50	0.842	2.390	0.621	2.353	0.892	2.384	0.582	2.353
50	0.860	2.381	0.638	2.343	0.861	2.386	0.558	2.356
50	0.850	2.384	0.644	2.345	0.846	2.380	0.589	2.348
100	1.191	2.688	0.984	2.646	1.229	2.671	0.915	2.637
100	1.213	2.686	0.970	2.646	1.251	2.677	0.928	2.642
100	1.201	2.691	0.949	2.652	1.192	2.695	0.928	2.659
500	1.972	3.358	1.666	3.318	2.015	3.365	1.615	3.333
500	1.964	3.371	1.669	3.330	2.028	3.365	1.579	3.335
500	1.974	3.361	1.660	3.324	1.999	3.354	1.611	3.320

Initial aqueous concentration (μM)	28			
	C (t)	q (t)	C (t)^e	q (t)^e
0.5	-1.154	0.384	-1.255	0.332
0.5	-1.102	0.370	-1.253	0.320
0.5	-1.079	0.372	-1.219	0.314
5	-0.131	1.381	-0.411	1.349
5	-0.120	1.383	-0.322	1.341
5	-0.099	1.377	-0.295	1.331
50	0.888	2.378	0.563	2.347
50	0.926	2.376	0.602	2.343
50	0.884	2.385	0.584	2.353
100	1.276	2.683	0.937	2.644
100	1.456	2.615	1.124	2.556
100	1.259	2.678	0.924	2.646
500	2.021	3.358	1.611	3.325
500	2.022	3.353	1.602	3.321
500	1.977	3.369	1.617	3.336

Table 6.11. Data for Figure 3.12.

Initial aqueous concentration (μM)	Contact Time (Days)							
	1				7			
	C (t)	q (t)	C (t)^e	q (t)^e	C (t)	q (t)	C (t)^e	q (t)^e
0.5	-1.043	0.369	-1.292	0.318	-1.086	0.369	-1.284	0.321
0.5	-1.030	0.352	-1.266	0.299	-1.066	0.369	-1.279	0.320
0.5	-1.040	0.351	-1.263	0.297	-1.059	0.366	-1.336	0.325
5	-0.017	1.372	-0.269	1.321	-0.035	1.355	-0.295	1.310
5	0.031	1.358	-0.242	1.304	-0.023	1.358	-0.281	1.310
5	-0.015	1.371	-0.285	1.327	-0.007	1.364	-0.293	1.316
50	0.965	2.366	0.653	2.325	1.027	2.350	0.650	2.314
50	0.977	2.343	0.683	2.296	1.031	2.355	0.636	2.317
50	0.984	2.366	0.663	2.323	1.012	2.354	0.667	2.311
100	1.359	2.523	1.002	2.475	1.375	2.638	0.963	2.597
100	1.312	2.670	1.002	2.624	1.392	2.646	0.987	2.606
100	1.336	2.653	0.995	2.608	1.381	2.647	0.965	2.607
500	2.040	3.349	1.615	3.314	2.086	3.346	1.584	3.318
500	2.044	3.352	1.626	3.316	2.080	3.349	1.567	3.318
500	2.040	3.353	1.626	3.317	2.081	3.352	1.565	3.325

Initial aqueous concentration (μM)	28			
	C (t)	q (t)	C (t)^e	q (t)^e
0.5	-1.056	0.358	-1.298	0.311
0.5	-0.979	0.344	-1.202	0.281
0.5	-1.003	0.348	-1.235	0.290
5	-0.006	1.354	-0.343	1.314
5	0.043	1.346	-0.258	1.294
5	0.039	1.346	-0.266	1.295
50	1.017	2.349	0.642	2.312
50	1.025	2.345	0.667	2.304
50	1.062	2.336	0.604	2.301
100	1.383	2.643	0.928	2.607
100	1.421	2.639	0.965	2.598
100	1.401	2.645	0.961	2.606
500	2.092	3.347	1.567	3.317
500	2.073	3.351	1.562	3.323
500	2.088	3.336	1.530	3.311

Table 6.12. Data for Figure 3.13.

DPP	Woodland			Agricultural		
t (days)	EI_1	EI_{10}	EI_{100}	EI_1	EI_{10}	EI_{100}
1	0.350	0.236	0.139	0.142	0.006	-0.112
7	0.234	0.052	-0.096	0.073	-0.115	-0.273
28	0.244	0.027	-0.145	0.014	-0.186	-0.355

Table 6.13. Data for Figure 3.14.

Initial Concentration (μM)	0.5			5			50		
Time (days)	**1**	**7**	**28**	**1**	**7**	**28**	**1**	**7**	**28**
Bound	0.53 *0.04*	0.71 *0.05*	0.49 *0.01*	7.45 *1.47*	8.67 *1.03*	10.11 *1.22*	70.24 *17.12*	120.22 *9.22*	122.83 *12.39*
Solvent Extractable	1.86 *0.05*	1.70 *0.03*	1.88 *0.05*	17.30 *1.20*	15.36 *0.89*	13.91 *1.08*	172.27 *17.84*	121.49 *8.03*	116.80 *10.22*
Initial Concentration (μM)	**100**			**500**					
Time (days)	**1**	**7**	**28**	**1**	**7**	**28**			
Bound	173.23 *9.25*	223.70 *13.05*	198.56 *26.85*	575.08 *177.60*	801.57 *102.38*	1032.61 *168.72*			
Solvent Extractable	314.72 *7.11*	256.00 *15.06*	258.42 *14.86*	1734.31 *154.48*	1497.81 *83.72*	1259.03 *127.35*			

Table 6.14. Data for Figure 3.15.

Initial Concentration (μM)	0.5			5			50		
Time (days)	**1**	**7**	**28**	**1**	**7**	**28**	**1**	**7**	**28**
Bound	0.70 *0.10*	0.90 *0.08*	0.68 *0.04*	7.03 *2.46*	10.19 *0.87*	9.32 *1.64*	89.74 *16.05*	109.73 *9.01*	95.84 *13.23*
Solvent Extractable	1.58 *0.05*	1.44 *0.08*	1.56 *0.05*	16.27 *2.74*	12.66 *1.07*	13.00 *1.46*	138.60 *9.20*	115.61 *10.16*	124.62 *12.22*
Initial Concentration (μM)	**100**			**500**					
Time (days)	**1**	**7**	**28**	**1**	**7**	**28**			
Bound	146.42 *24.47*	159.56 *32.96*	185.24 *18.54*	511.76 *96.28*	714.69 *77.48*	636.01 *15.39*			
Solvent Extractable	270.48 *58.45*	280.64 *32.71*	253.64 *15.51*	1735.00 *58.45*	1519.52 *32.71*	1575.79 *15.51*			

Table 6.15. Data for Figure 3.16.

	1 day			7 day		
	0.43	**0.86**	**4.3**	**0.43**	**0.86**	**4.3**
Sorbed DCP+DPP	0.857 *0.003*	0.843 *0.004*	0.816 *0.002*	0.852 *0.008*	0.834 *0.011*	0.798 *0.007*
Aqueous DPP	0.091 *0.005*	0.107 *0.004*	0.145 *0.024*	0.106 *0.008*	0.128 *0.016*	0.167 *0.013*
Aqueous DCP	0.052 *0.002*	0.050 *0.006*	0.039 *0.023*	0.042 *0.000*	0.038 *0.005*	0.035 *0.007*

	28 day		
	0.43	**0.86**	**4.3**
Sorbed DCP+DPP	0.842 *0.010*	0.786 *0.061*	0.800 *0.017*
Aqueous DPP	0.117 *0.009*	0.166 *0.043*	0.164 *0.009*
Aqueous DCP	0.042 *0.001*	0.048 *0.018*	0.035 *0.008*

Table 6.16. Data for Figure 3.17.

	1 day			7 day		
	0.43	**0.86**	**4.3**	**0.43**	**0.86**	**4.3**
Sorbed DCP+DPP	0.810 *0.004*	0.786 *0.012*	0.783 *0.001*	0.788 *0.005*	0.761 *0.005*	0.764 *0.002*
Aqueous DPP	0.138 *0.005*	0.165 *0.015*	0.175 *0.004*	0.161 *0.005*	0.194 *0.001*	0.202 *0.004*
Aqueous DCP	0.052 *0.007*	0.050 *0.005*	0.042 *0.004*	0.050 *0.000*	0.045 *0.005*	0.034 *0.003*

	28 day		
	0.43	**0.86**	**4.3**
Sorbed DCP+DPP	0.782 *0.012*	0.753 *0.011*	0.759 *0.006*
Aqueous DPP	0.170 *0.014*	0.210 *0.010*	0.212 *0.008*
Aqueous DCP	0.048 *0.003*	0.037 *0.002*	0.028 *0.004*

Table 6.17. Data for Figure 3.18.

	1 day			7 day			28 day		
	283	**567**	**2833**	**283**	**567**	**2833**	**283**	**567**	**2833**
Bound DPP	0.248 *0.060*	0.306 *0.016*	0.203 *0.063*	0.424 *0.033*	0.395 *0.023*	0.283 *0.036*	0.434 *0.044*	0.350 *0.047*	0.364 *0.060*
Extractable DPP	0.357 *0.066*	0.397 *0.011*	0.553 *0.054*	0.156 *0.025*	0.298 *0.038*	0.450 *0.030*	0.196 *0.033*	0.295 *0.036*	0.389 *0.049*
Extractable DCP	0.251 *0.009*	0.158 *0.008*	0.059 *0.004*	0.273 *0.003*	0.154 *0.012*	0.079 *0.001*	0.216 *0.004*	0.161 *0.012*	0.056 *0.005*

Table 6.18. Data for Figure 3.19.

	1 day			7 day			28 day		
	283	**567**	**2833**	**283**	**567**	**2833**	**283**	**567**	**2833**
Bound DPP	0.317 *0.057*	0.258 *0.043*	0.181 *0.034*	0.387 *0.032*	0.282 *0.058*	0.252 *0.027*	0.338 *0.047*	0.327 *0.033*	0.224 *0.005*
Extractable DPP	0.255 *0.038*	0.351 *0.083*	0.572 *0.031*	0.156 *0.035*	0.388 *0.052*	0.485 *0.026*	0.198 *0.057*	0.315 *0.018*	0.497 *0.019*
Extractable DCP	0.234 *0.009*	0.126 *0.021*	0.040 *0.002*	0.252 *0.004*	0.133 *0.001*	0.051 *0.003*	0.229 *0.004*	0.133 *0.009*	0.059 *0.008*

Appendix B - DATA FOR CHAPTER 4

Table 6.19. Data for Figure 4.1.

Oxidation Cycle	SOM %
1	0.600
3	0.300
5	0.133
7	0.052
9	0.028

Table 6.20. Data for Figure 4.4.

	Woodland soil		Agricultural soil	
Soil mass (g)	***C*** (μmole/kg)	***q*** (μmole/kg)	***C*** (μmole/kg)	***q*** (μmole/kg)
1.0	46.73	28.28	47.71	18.55
1.0	48.04	16.18	49.02	7.69
1.5	49.35	3.14	48.04	10.73
1.5	49.02	4.92	49.35	3.12
2.0	46.38	9.99	45.42	13.66
2.0	45.57	13.44	45.65	12.84
2.0	48.28	2.31	45.56	13.81
2.5	46.11	9.10	45.46	11.14
2.5	47.07	5.89	46.57	7.60
2.5	44.35	14.73	46.78	6.90
3.0	45.50	8.95	45.36	9.32
3.0	44.44	11.68	45.65	8.36
3.0	47.11	4.01	45.34	9.29

Table 6.21. Data for Figure 4.5.

	Woodland soil		Agricultural soil	
C_o (μM)	Average C (μmole/kg)	Average q (μmole/kg)	Average C (μmole/kg)	Average q (μmole/kg)
50	17.08	181.09	20.10	164.75
	0.94	*6.00*	*3.72*	*21.94*
500	167.98	1929.22	168.45	1969.03
	3.84	*28.02*	*0.53*	*20.52*

Table 6.22. Data for Figure 4.6.

Initial aqueous concentration (μM)	**1 day**		**7 day**		**28 day**	
	***C* (t) (μmole/kg)**	***q* (t) (μmole/kg)**	***C* (t) (μmole/kg)**	***q* (t) (μmole/kg)**	***C* (t) (μmole/kg)**	***q* (t) (μmole/kg)**
0.5	-0.419	-0.106	-0.444	-0.049	-0.441	-0.119
0.5	-0.495	0.052	-0.485	0.034	-0.454	-0.088
0.5	-0.465	-0.003	-0.450	-0.033	-0.461	-0.067
5	0.339	1.234	0.371	1.196	0.396	1.164
5	0.376	1.201	0.351	1.212	0.386	1.172
5	0.326	1.237	0.342	1.215	0.383	1.176
50	1.287	2.268	1.302	2.246	1.346	2.204
50	1.300	2.264	1.342	2.215	1.358	2.188
50	1.262	2.283	1.336	2.221	1.347	2.204
100	1.585	2.544	1.617	2.542	1.636	2.529
100	1.582	2.548	1.606	2.551	1.620	2.540
100	1.585	2.545	1.606	2.544	1.621	2.534
500	2.193	3.317	2.226	3.293	2.249	3.276
500	2.195	3.316	2.225	3.297	2.230	3.285
500	2.206	3.317	2.228	3.288	2.249	3.272

Table 6.23. Data for Figure 4.7.

Initial aqueous concentration (μM)	Contact Time (Days)							
	1				7			
	C (t) (μmole/kg)	q (t) (μmole/kg)	C (t)^e (μmole/kg)	q (t)^e (μmole/kg)	C (t)^e (μmole/kg)	q (t)^e (μmole/kg)	C (t)^e (μmole/kg)	q (t)^e (μmole/kg)
0.5	-0.419	-0.106	-1.231	-0.145	-0.444	-0.049	-1.133	-0.096
0.5	-0.495	0.052	-1.212	0.011	-0.485	0.034	-1.165	-0.002
0.5	-0.465	-0.003	-1.225	-0.043	-0.450	-0.033	-1.183	-0.050
5	0.339	1.234	-0.295	1.199	0.371	1.196	-0.266	1.166
5	0.376	1.201	-0.309	1.170	0.351	1.212	-0.295	1.183
5	0.326	1.237	-0.316	1.205	0.342	1.215	-0.261	1.177
50	1.287	2.268	0.659	2.237	1.302	2.246	0.683	2.220
50	1.300	2.264	0.648	2.233	1.342	2.215	0.689	2.189
50	1.262	2.283	0.703	2.249	1.336	2.221	0.652	2.200
100	1.585	2.544	0.956	2.513	1.617	2.542	0.962	2.516
100	1.582	2.548	0.964	2.517	1.606	2.551	0.955	2.525
100	1.585	2.545	0.981	2.510	1.606	2.544	0.964	2.517
500	2.193	3.317	1.648	3.283	2.226	3.293	1.638	3.274
500	2.195	3.316	1.696	3.276	2.225	3.297	1.636	3.265
500	2.206	3.317	1.682	3.280	2.228	3.288	1.689	3.258

Initial aqueous concentration (μM)	28			
	C (t) (μmole/kg)	q (t) (μmole/kg)	C (t)^e (μmole/kg)	q (t)^e (μmole/kg)
0.5	-0.441	-0.119	-1.178	-0.161
0.5	-0.454	-0.088	-1.199	-0.121
0.5	-0.461	-0.067	-1.205	-0.096
5	0.396	1.164	-0.292	1.141
5	0.386	1.172	-0.241	1.143
5	0.383	1.176	-0.263	1.149
50	1.346	2.204	0.671	2.180
50	1.358	2.188	0.682	2.165
50	1.347	2.204	0.690	2.179
100	1.636	2.529	0.971	2.509
100	1.620	2.540	0.975	2.516
100	1.621	2.534	0.958	2.511
500	2.249	3.276	1.663	3.251
500	2.230	3.285	1.675	3.259
500	2.249	3.272	1.657	3.250

Table 6.24. Data for Figure 4.8.

	Soil bound (μmole/kg)			Solvent extracted (μmole/kg)		
	Time (days)					
Co (μM)	**1**	**7**	**28**	**1**	**7**	**28**
0.5	0.4	0.5	0.6	0.5	0.4	0.2
5	7.2	8.6	9.4	9.5	7.5	5.4
50	64.6	77.3	87.1	122.5	91.6	71.0
100	119.2	148.0	167.3	232.2	203.3	175.1
500	519.7	642.9	745.4	1552.0	1319.3	1149.6

Table 6.25. Data for Figure 4.9.

Initial Concentration (μM)	**0.5**			**5**			**50**		
Time (days)	**1**	**7**	**28**	**1**	**7**	**28**	**1**	**7**	**28**
Bound (μmole/kg)	0.44 *0.08*	0.53 *0.02*	0.56 *0.03*	7.24 *0.43*	8.65 *0.20*	9.39 *0.48*	64.56 *1.62*	77.31 *2.58*	87.10 *0.78*
Solvent Extractable (μmole/kg)	0.53 *0.10*	0.44 *0.11*	0.25 *0.02*	9.52 *0.42*	7.49 *0.18*	5.43 *0.60*	122.45 *3.38*	91.61 *5.39*	70.95 *2.51*
Initial Concentration (μM)	**100**			**500**					
Time (days)	**1**	**7**	**28**	**1**	**7**	**28**			
Bound (μmole/kg)	119.17 *4.62*	148.01 *4.09*	167.30 *1.10*	519.73 *1.50*	642.93 *10.13*	745.37 *9.95*			
Solvent Extractable (μmole/kg)	232.17 *6.55*	203.29 *5.14*	175.07 *4.70*	1552.02 *1.96*	1319.27 *20.49*	1149.65 *26.20*			

Table 6.26. Data for Figure 4.10.

	1 day			7 day		
	0.43	**0.86**	**4.3**	**0.43**	**0.86**	**4.3**
Sorbed DCP+DPP	0.624 *0.017*	0.613 *0.002*	0.692 *0.005*	0.578 *0.020*	0.596 *0.006*	0.668 *0.001*
Aqueous DPP	0.376 *0.017*	0.387 *0.002*	0.245 *0.004*	0.422 *0.020*	0.404 *0.006*	0.269 *0.002*
Aqueous DCP	0.000 *0.000*	0.000 *0.000*	0.064 *0.008*	0.000 *0.000*	0.000 *0.000*	0.064 *0.003*
	28 day					
	0.43	**0.86**	**4.3**			
Sorbed DCP+DPP	0.548 *0.007*	0.580 *0.009*	0.650 *0.009*			
Aqueous DPP	0.452 *0.007*	0.420 *0.009*	0.276 *0.008*			
Aqueous DCP	0.000 *0.000*	0.000 *0.000*	0.073 *0.073*			

Appendix C - DATA FOR CHAPTER 5

Table 6.27. Data for Figure 5.2.

HRP dose AU/mL	Fraction of souble polymers	Fraction of DCP	Fraction of precipitated polymers
0.1	0.05	0.94	0.01
0.3	0.38	0.62	0.00
0.5	0.62	0.36	0.01
0.8	0.74	0.20	0.05
1.0	0.86	0.09	0.05
1.5	0.91	0.05	0.04
2.0	0.86	0.02	0.12

Table 6.28. Data for Figure 5.3

IS Treatment (mM)	Fractional Distribution of DCP added to the Reactors		
	Residual DCP	**Soluble DPP**	**Precipitated DPP**
10	0.01 *0.007*	0.90 *0.002*	0.09 *0.007*
20	0.00 *0.000*	0.64 *0.068*	0.36 *0.068*
70	0.03 *0.010*	0.33 *0.012*	0.64 *0.003*
100	0.05 *0.005*	0.31 *0.013*	0.64 *0.009*
10-20	0.01 *0.010*	0.81 *0.034*	0.18 *0.039*
10-70	0.01 *0.011*	0.47 *0.019*	0.53 *0.020*
10-100	0.00 *0.004*	0.40 *0.031*	0.60 *0.035*

Table 6.29. Data for Figure 5.4.

Treatment	Fraction of re-dissolved polymers	Fraction of polymer remaining precipitated
10	0.014 *0.0047*	0.076 *0.0079*
20	0.017 *0.0143*	0.345 *0.0535*
70	0.033 *0.0022*	0.611 *0.0040*
100	0.018 *0.0027*	0.625 *0.0066*
10-20-10	0.017 *0.0070*	0.176 *0.0406*
10-20-20	0.013 *0.0042*	0.153 *0.0300*
10-70-10	0.031 *0.0035*	0.481 *0.0086*
10-70-70	0.019 *0.0032*	0.524 *0.0103*
10-100-20	0.024 *0.0023*	0.592 *0.0075*
10-100-100	0.016 *0.0034*	0.562 *0.0427*

Table 6.30. Data for Figure 5.5.

Initial aqueous concentration	10		20		70	
(μM)	*C*	*q*	*C*	*q*	*C*	*q*
0.5	-0.498	-0.005	-0.502	0.012	-0.527	0.031
0.5	-0.543	0.059	-0.461	-0.059	-0.486	-0.055
0.5	-0.519	0.029	-0.519	0.051	-0.524	0.012
5	0.372	1.181	0.376	1.160	0.337	1.178
5	0.344	1.208	0.369	1.162	0.332	1.180
5	0.360	1.196	0.326	1.205	0.295	1.207
50	1.321	2.202	1.308	2.234	1.279	2.209
50	1.314	2.203	1.317	2.230	1.265	2.221
50	1.314	2.204	1.313	2.233	1.280	2.209
100	1.595	2.537	1.577	2.552	1.558	2.543
100	1.619	2.521	1.591	2.534	1.558	2.552
100	1.613	2.526	1.582	2.547	1.570	2.545
500	2.216	3.301	2.179	3.279	2.149	3.304
500	2.222	3.311	2.173	3.288	2.140	3.305
500	2.202	3.309	2.167	3.290	2.146	3.310

Initial aqueous concentration	10-20		10-70	
(μM)	*C*	*q*	*C*	*q*
0.5	-0.473	-0.049	-0.512	-0.017
0.5	-0.445	-0.113	-0.495	-0.044
0.5	-0.470	-0.058	-0.477	-0.086
0.5	-0.503	0.017	-0.507	-0.026
0.5	-0.508	0.024	-0.501	-0.029
0.5	-0.526	0.051	-0.533	0.029
5	0.374	1.168	0.394	1.135
5	0.426	1.108	0.364	1.151
5	0.376	1.164	0.368	1.150
5	0.341	1.201	0.324	1.195
5	0.387	1.146	0.353	1.170
5	0.361	1.180	0.348	1.180
50	1.319	2.210	1.326	2.204
50	1.324	2.204	1.331	2.194
50	1.335	2.197	1.330	2.198
50	1.314	2.218	1.321	2.206
50	1.333	2.213	1.306	2.220
50	1.328	2.205	1.334	2.202
100	1.604	2.519	1.601	2.530
100	1.602	2.518	1.611	2.513
100	1.609	2.519	1.580	2.525
100	1.595	2.518	1.587	2.534
100	1.618	2.513	1.584	2.542
100	1.596	2.517	1.589	2.538
500	2.208	3.315	2.182	3.309
500	2.212	3.319	2.210	3.285
500	2.378	3.210	2.188	3.305
500	2.205	3.314	2.199	3.294
500	2.218	3.298	2.196	3.289
500	2.206	3.302	2.226	3.291

*Units for *C* are μM and for *q* are μmole/kg of soil

Table 6.31.Data for Figure 5.6.

Treatment	Fraction of re-dissolved polymers	Fraction of chemical remaining in soil after buffer extraction
10	0.0276 *0.0011*	0.6578 *0.0082*
20	0.0289 *0.0011*	0.6691 *0.0039*
70	0.0287 *0.0005*	0.6886 *0.0025*
100	0.0296 *0.0014*	0.6839 *0.0084*
10-20-10	0.0417 *0.0055*	0.6026 *0.0792*
10-20-20	0.0285 *0.0022*	0.6645 *0.0071*
10-70-10	0.0788 *0.0091*	0.6029 *0.0109*
10-70-70	0.0344 *0.0017*	0.6606 *0.0103*
10-100-20	0.0425 *0.0025*	0.6524 *0.0037*
10-100-100	0.0272 *0.0023*	0.6641 *0.0090*

Table 6.32. Data for Figure 5.7.

Treatments	Mass of bound DPP (μmol/kg)	Standard Deviation
10	693	14
20	661	12
70	621	17
100	591	6
10-20-10	608	124
10-20-20	673	37
10-70-10	602	20
10-70-70	653	13
10-100-20	619	19
10-100-100	619	13

Table 6.33.Data for Figure 5.8.

Treatments	Unreacted DCP	Soluble polymers	Solvent extractable	Bound
10	0.036	0.278	0.450	0.236
20	0.040	0.261	0.458	0.241
70	0.032	0.250	0.496	0.221
100	0.044	0.242	0.505	0.209
10-20-10	0.085	0.276	0.435	0.204
10-20-20	0.032	0.279	0.457	0.233
10-70-10	0.027	0.290	0.471	0.211
10-70-70	0.035	0.274	0.463	0.228
10-100-20	0.022	0.283	0.484	0.212
10-100-100	0.030	0.279	0.481	0.210

Table 6.34.Data for Figure 5.9.

Treatment	Fraction of extracted DCP+polymers	Fraction of chemical remaining in soil after buffer extraction
10	0.0853 *0.0065*	0.6417 *0.0152*
20	0.0743 *0.0025*	0.6832 *0.0060*
70	0.0692 *0.0033*	0.7028 *0.0104*
100	0.0689 *0.0037*	0.6963 *0.0119*
10-20-10	0.0911 *0.0053*	0.6721 *0.0074*
10-20-20	0.0726 *0.0038*	0.6835 *0.0176*
10-70-10	0.1064 *0.0043*	0.6556 *0.0101*
10-70-70	0.0664 *0.0024*	0.7032 *0.0121*
10-100-20	0.0947 *0.0016*	0.6688 *0.0048*
10-100-100	0.0659 *0.0027*	0.6929 *0.0123*

Table 6.35.Data for Figure 5.10.

Treatments	Mass of bound DPP (μmol/kg)	Standard Deviation
10	773	12
20	757	29
70	746	23
100	756	19
10-20-10	759	28
10-20-20	742	43
10-70-10	719	21
10-70-70	806	44
10-100-20	747	20
10-100-100	760	24

Table 6.36. Data for Figure 5.11.

Fractional distribution of DPP in agricultural soil.

	Treatment									
Type	**10**	**20**	**70**	**100**	**10-20-10**	**10-20-20**	**10-70-10**	**10-70-70**	**10-100-20**	**10-100-100**
Unreacted DCP	0.067	0.077	0.071	0.077	0.060	0.070	0.074	0.070	0.063	0.070
Soluble polymers	0.206	0.166	0.158	0.158	0.178	0.174	0.165	0.161	0.178	0.178
Solvent extractable polymers	0.341	0.393	0.432	0.389	0.421	0.407	0.410	0.401	0.413	0.383
Solvent extractable DCP	0.114	0.098	0.081	0.108	0.077	0.089	0.099	0.089	0.092	0.103
Bound	0.273	0.265	0.258	0.268	0.265	0.260	0.253	0.280	0.254	0.266

Appendix D - PICTURES OF DPP PRODUCED AT DIFFERENT IONIC STRENGTHS

Figure 6.1. Polymers produced at 10 mM ionic strength.

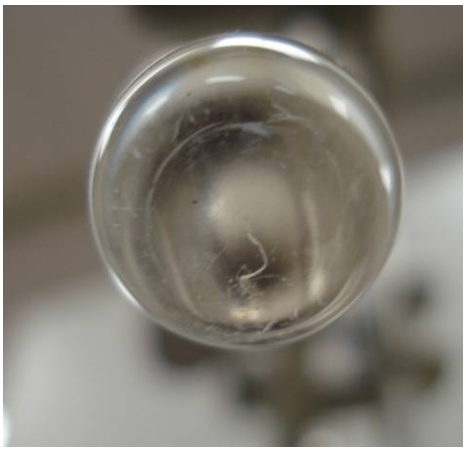

Figure 6.2. Polymers produced at 20 mM ionic strength.

Figure 6.3. Polymers produced at 70 mM ionic strength.

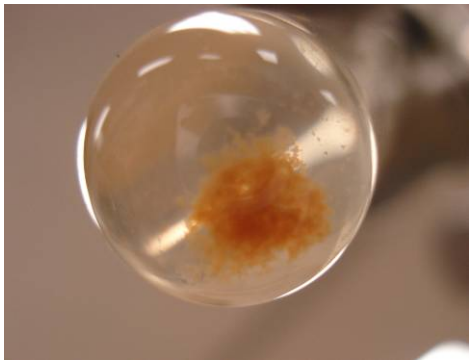

Figure 6.4. Polymers produced at 100 mM ionic strength.

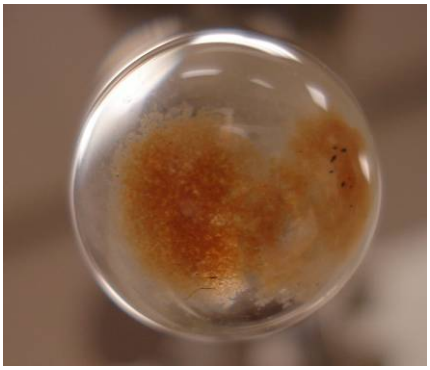

Figure 6.5. Polymers produced at 10-20 mM ionic strength.

Figure 6.6. Polymers produced at 10-70 mM ionic strength.

Figure 6.7. Polymers produced at 10-100 mM ionic strength.