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REDOX INTERACTIONS OF IRON WITH ORGANIC MATTER IN PEAT SOILS AND 
FE-ORGANIC COMPLEXES

A Dissertation in
Soil Science

by

Amrita Bhattacharyya

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The dissertation of Amrita Bhattacharyya was reviewed and approved* by the following:

Carmen Enid Martínez  
Associate Professor in Environmental and Soil Chemistry  
Dissertation Advisor  
Chair of Committee

Douglas Archibald  
Research Associate in Agricultural Analytical Chemistry

Jack Watson  
Professor of Soil Science

Brian Dempsey  
Professor of Environmental Engineering

Lasse Jensen  
Assistant Professor of Chemistry

Michael G. Messina  
Head and Professor of the Department of Ecosystem Science and Management

*Signatures are on file in the Graduate School
Natural organic matter (NOM) exerts a significant control on the oxidation state and coordination environment of Fe. Iron (Fe) complexes with organic matter (OM) represent an important class of structures whose chemistry and reactivity require further understanding under different environmental conditions since Fe speciation and mobility affect the availability, cycling, and transport of metals, OM and nutrients. The objective of this doctoral research was to increase scientific understanding of redox reactions of Fe with OM. Experimental studies were conducted both at molecular and field scales which are documented in the three manuscripts included in this dissertation.

The first study determined the oxidation state and coordination environment of Fe in peat soils under varying redox conditions using chemical techniques, spectroscopic analyses and theoretical CTM4XAS multiplet calculations. This is the first time that CTM4XAS has been used to quantify Fe oxidation states along with their coordination environments in complex, heterogeneous soil systems. The results from this investigation provide clear direct and indirect evidence that tetrahedral ferrous (Fe$^{2+}$) and octahedral ferric (Fe$^{3+}$) iron species co-exist in peat soils under oxic, suboxic and anoxic redox conditions with varying coordination environments. Fe showed a higher affinity to bind with heterocyclic N-containing molecules which have an available pair of electrons on their N atoms for donation to the Fe center compared to the more abundant O-containing functional groups. Complexation of Fe with soil organic matter (SOM) was considered to be the most important process in stabilizing the Fe$^{2+}$ and Fe$^{3+}$ species. In addition to the Fe-OM complexes, the findings of this chapter also aid in the identification of stable inorganic Fe species present in these peatlands.

The second study specifically focused on the electron exchange interactions between the redox active metal (Fe) center and the different functional groups of cysteine (carboxylic, amine
and thiol) in laboratory-synthesized Fe(II)-cysteine and Fe(III)-cysteine complexes as a function of time (0 and 12 months). Here, we detailed changes in oxidation states of Fe together with that of the elements in cysteine (C, N, O and S) under controlled conditions in a laboratory setup. The study of the oxidation state of Fe indicates preservation of Fe$^{2+}$ in Fe(II)-cysteine and initial reduction of Fe$^{3+}$ in Fe(III)-cysteine systems. Fe$^{2+}$ in the former underwent oxidation with time whereas Fe$^{3+}$ in the reduced complex underwent partial oxidation and retained most of its reduction features. The coordination environments and Fe-ligand bond character of Fe(II)- and Fe(III)-cysteine complexes were also altered with time. Besides the –COOH, -NH$_2$ and –SH functional groups, the C-backbone in cysteine also actively participated in the intramolecular electron transfer reactions stabilizing Fe$^{2+}$ and Fe$^{3+}$ in these complexes.

The third study further explored the binding of Fe with amino acids (cysteine, histidine and arginine) with different reactivity present in their side chains. While –SH was responsible for stabilizing Fe$^{2+}$ in Fe-cysteine complexes, the N present in the heterocyclic 5-membered imidazole group of histidine and the basic guanidine side chain of arginine stabilized Fe$^{2+}$ and Fe$^{3+}$ in the Fe-histidine and Fe-arginine complexes, respectively. Inner-sphere Fe-amino acid complexes were formed but the strength of Fe-O, Fe-N and Fe-S interactions varied greatly, depending on the electron shuttling capability of the donor atom of the ligand as well as that of the redox-active Fe. Fe-C interactions were observed in the second coordination shell of Fe(II)-histidine indicating that the cyclic C-chain in histidine can act as effective electron shuttles. The second coordination shell of Fe(III)-arginine and Fe(III)-histidine exhibited Fe-Fe interactions indicating the formation of stable ferrihydrite-like amorphous phases in these complexes.

The research findings presented in this dissertation contribute to our understanding of Fe-OM interactions and to the factors which stabilize the Fe redox species in terrestrial and aquatic systems where such complexes are abundant.
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Chapter 1

INTRODUCTION

1.1. Background

Iron (Fe) is one of the 3d transition elements and the chemical forms of Fe in soils, sediments, and surface waters have a large influence on its mobility and availability.\(^1\) Soil Fe is present in inorganic (mineral) forms and as organically-bound Fe. Inorganic forms of iron in soils include Fe oxide and hydroxide minerals, Fe in manganese minerals, and structural Fe in primary and secondary aluminosilicate.\(^2\) Iron can also be found in sulfides, sulfates, phosphates, and carbonates. Organically bound Fe includes complexation of Fe with natural organic matter (NOM) to form inner sphere complexes or Fe oxide nanoparticles getting sorbed onto the organic matrix through weak electrostatic attraction to form outer sphere complexes. These solid phases (minerals and organic) contain ferric and/or ferrous iron.

From the perspective of chemical reactivity, Fe is extremely redox-active and the charge on the metal is extremely sensitive to the surrounding reduction–oxidation conditions (such as oxygen concentrations and available moisture content), resulting in changes in the valence state.\(^3,4\) The determination and quantification of the valence of Fe in aquatic and terrestrial ecosystems can, therefore, provide a great insight into redox mechanisms and transformations occurring as a result of both biotic and abiotic chemical reactions. Electrode-measured ranges of soil potentials indicate redox transformations occur between -100 and +100 mV for Fe species. This means that most of Fe redox transformations can occur under suboxic (wet) conditions and do not require anoxic (waterlogged) environments (the formation of FeS\(_2\) may be an exception—due to the need of S\(^2-\)). We can therefore expect variations in the chemical species of Fe in the
majority of soils. Substantial changes in Fe speciation may occur with variations in water and organic matter (OM) content. For example, iron in the form of sparingly soluble oxides may be reduced and Fe solubility enhanced by the generation of water-soluble Fe(II) species.\(^5\)

Iron availability in soils has been linked to the strength of interaction between Fe and organic matter since these organic ligands can act as active electron shuttles which, via chemically and microbially driven transformations, may donate and accept electrons, thereby reducing and oxidizing redox-active metals like Fe.\(^6\)\(^9\) The different functionalities present in the OM interact differently with Fe\(^{2+}/Fe^{3+}\) to form stable inner sphere or outer sphere complexes having potential Fe-O, Fe-N or Fe-S bonds with varying metal-ligand bond valence characteristics. Some of the OM functional groups may stabilize Fe(III) in reduced conditions whereas some others may presumably stabilize Fe(II) in oxidized conditions through complexation, sorption/desorption and dissolution/precipitation reactions. Few studies have been reported on the Fe speciation (oxidation state and coordination environment) with NOM.\(^10\)\(^-\)\(^12\) There are still gaps in the knowledge about the in-situ coordination environment of Fe in redox stratified natural systems with elevated organic matter content such as the peatlands. It is clear that to accurately assess these ligand-mediated processes one must understand, at a molecular-level, the electron exchange mechanism between Fe and the different organic functional groups found in the natural systems. Key parameters which need to be identified are the identity and the distance of the ligand atom from the metal center along with their coordination environment and the proportions in which that particular oxidation state is stabilized in association with OM.

Some of the questions that need to be addressed regarding Fe interactions with organic matter in soils are: (1) What are the chemical forms of Fe(II) and/or Fe(III) present in oxic organic-rich soils? Do they differ from the chemical forms of Fe(II) and/or Fe(III) present in suboxic and anoxic organic-rich soils? If so, then how? (2) What are the possible electron exchange pathways which follow when Fe form complexes with organic molecules and how do
the elements in the functional groups (O, N, C and S) participate in such electron exchange reactions? To address these questions, in my dissertation I use a combination of chemical analyses together with synchrotron studies and molecular modeling approaches to explore the speciation of Fe in large scale natural systems like peatlands where we expect great heterogeneity and in laboratory-synthesized Fe-organic molecular systems.

1.2. Dissertation structure

This dissertation consists of three main chapters that will be submitted for publication in peer reviewed journals. Chapter 2 titled “Co-existence and Stabilization of Fe(II) and Fe(III) in Oxic and Anoxic Peats: An Insight into Fe Coordination Chemistry” focuses on studying the presence, proportion and local chemical environment of Fe$^{2+}$ and Fe$^{3+}$ in the various redox zones of organic-rich peat soils, and the different factors which lead to the stabilization of Fe$^{2+}$ in the oxic layers and Fe$^{3+}$ in the anoxic layers. My hypothesis was the following: Fe(II) and Fe(III) co-exist in both the oxic and anoxic layers of organic rich soils suggesting the coexistence of Fe$^{2+}$ and Fe$^{3+}$ in the oxic and anoxic layers of the peats. I have used traditional chemical approaches together with sophisticated synchrotron and molecular modeling studies to investigate the redox interactions between Fe and organic matter in these peat soils.

Chapter 3, “Redox Interactions between Fe and Cysteine: Spectroscopic studies and Multiplet Calculations” focuses on how the redox state of Fe (+2 and +3) change when it complexes with the amino acid cysteine as a function of time. Here I test the following hypothesis: Cysteine, with three potential metal binding groups (carboxyl, amine and thiol), acts as a true redox ligand, capable of oxidizing or reducing Fe with any of its own elements (C, N, O, S) getting reduced or oxidized simultaneously. In order to test this hypothesis, I have used experimental spectroscopic data together with theoretical molecular modeling to develop a
molecular level understanding of the electron exchange mechanism between Fe and the –COOH, -NH₂ and –SH functional groups in cysteine.

Finally, Chapter 4, “Binding of Fe(II) and Fe(III) with the N-containing basic amino acids histidine and arginine: Spectroscopic studies and Multiplet Calculations” is an attempt to understand the potential role of N-containing functional groups in Fe retention and chemical forms. The hypothesis is that: The mode and strength of binding (or retention) of Fe²⁺ and Fe³⁺ to the functional groups of histidine and arginine differs between themselves and differs from that of cysteine. To test the hypothesis, I used Fe L₃,₂-edge XANES and Fe K-edge EXAFS spectroscopy and CTM4XAS modeling techniques to explore the binding mode and oxidation state of Fe in these model systems.

Chapter 5 gives an overall summary and the conclusions of my research. It further reinforces the fact that the chemical bonding between Fe and the different organic functional groups depends on the donor interactions between the occupied orbitals of the ligand and the unoccupied and partially occupied orbitals of the metal, and acceptor interactions between the occupied or partly occupied orbitals of the metal and the unoccupied orbitals of the ligand. Further, since cysteine, histidine, and arginine collectively contain the most abundant functional groups (O > N > S) present in organic matter, I tried to use the results from these model organic complexes to understand and explain what is observed in the organic-rich peat soils.

References:


Chapter 2

CO-EXISTENCE OF IRON (II) AND IRON (III) IN OXIC AND ANOXIC PEATS: AN INSIGHT INTO Fe-ORGANIC MATTER COORDINATION AND STABILIZATION

Abstract

Iron interactions with organic matter are presumed to determine its biological availability. We report on the solid-phase speciation of naturally occurring Fe in the peatlands of western New York. Soil layers in these peatlands (i.e., organic-matter-rich wetlands) present various redox conditions (oxic, anoxic, suboxic) with depth thus facilitating the determination of the chemical forms, oxidation states and local coordination environment of organically-bound Fe. Chemical (selective extractions and 0.5M HCl extraction tests), spectroscopic (Fe L-edge and K-edge XAS experiments), and theoretical computational (CTM4XAS analyses of the Fe L-edge XANES spectra) methods are used in this investigation of Fe chemical forms in peat soils. The results from this investigation provide clear direct and indirect evidence ferrous (Fe$^{2+}$) and ferric (Fe$^{3+}$) iron species co-exist in peat soils under oxic, suboxic and anoxic redox conditions. Fe showed a higher affinity to bind with heterocyclic N-containing molecules which have an available pair of electrons on their N atoms for donation to the Fe center, thereby stabilizing the complex, compared to the O-containing functional groups. The mineral forms of Fe$^{3+}$ included the ‘amorphous’ ferrihydrite while Fe$^{2+}$ was mostly present as ‘residual’ siderite or ‘amorphous’ pyrite in its mineral form. CTM4XAS calculations further point to the fact that Fe$^{3+}$ and Fe$^{2+}$ exist in octahedral and tetrahedral coordination environments respectively with the ligand atoms, the ionic strength of the metal-ligand interaction depending on the electronegativity difference between the two. The stabilization of Fe$^{2+}$ in oxic peats and Fe$^{3+}$ in the anoxic peats was thought
to be the result of a combination of factors such as complexation with soil organic matter, sorption-desorption and precipitation-dissolution reactions. Also it is to be noted that this is the first time that the theoretical multiplet calculation program CTM4XAS has been used to model the Fe L-edge XANES spectra of natural samples. Overall, the results help to develop an understanding of how variations in redox condition dictates the way Fe interacts in organic-rich systems, and therefore how changes in redox influence its biological availability and potential mobility. The insights we provide on the chemical bonding environment that might lead to the stabilization of Fe$^{2+}$ in oxic and of Fe$^{3+}$ in anoxic environments can be translated to other systems besides peatlands, for example, surface layers of forest soils (litter layer, O-horizon) and in composted organic materials used in restoring mining-impacted lands.

2.1 INTRODUCTION

The chemical form, oxidation state and local coordination environment of iron (Fe) have a great influence on its biological availability and biogeochemical cycling in terrestrial and aquatic environments. Iron is a micronutrient taken up by plants and microorganisms in its ferrous (Fe$^{2+}$) and ferric (Fe$^{3+}$) oxidation states; it is involved in photosynthesis, chlorophyll formation, respiration and enzymatic reactions. Iron is present in mineral (i.e., phyllosilicates, sulfides, oxides, carbonates) and organic (i.e., outer- and inner- sphere complexes) chemical forms, while these chemical forms exist in solution (mineral and organic nano-colloids, and dissolved Fe-organic complexes, for example) and as the solid phases of soils and sediments. Yet, most of the biologically reactive Fe arises from the coupling of the organic matter and Fe biogeochemical cycles; perhaps the result of the low solubility of Fe-containing mineral phases but most likely due to the oxidation of organic matter that leads to the generation of the more soluble ferrous (Fe$^{2+}$) iron.
The biogeochemical cycle of iron is greatly affected by the stoichiometry of the Eh-pH dependent oxidation/reduction reactions. Iron redox transformations occur at Eh values between -100 and +100 eV thus indicating that most of Fe redox transformations can occur under suboxic (wet) conditions and do not require anoxic (waterlogged) environments (the formation of FeS$_2$ may be an exception due to the need of S$^{2-}$). In other words, ferric (Fe$^{3+}$) iron is the redox species expected in environments with Eh values greater than +100 eV whereas ferrous (Fe$^{2+}$) iron is expected in environments where Eh values are lower than -100 eV. Recent studies, however, suggest a higher level of complexity$^{2,3,4}$ For example, Hunter et al. (2008) found that both Fe(II) and Fe(III) are present in oxic-circumneutral biofilms but that these redox species are heterogeneously distributed: Fe(III) in association with cell surfaces and Fe(II) in the extracellular space.$^2$ Furthermore, the authors tested and then discarded the possibility that anaerobic respiration would have generated Fe(II) in the extracellular region of the biofilms, and suggested extracellular electron transfer as a possibility for the reduction of ferric (Fe$^{3+}$) to ferrous (Fe$^{2+}$) iron. Another study reported the presence of small amounts of Fe(II) in organic aggregates collected from an oxygenated hydrothermal plume and the authors hypothesized complexation with organic matter might have stabilized Fe(II).$^3$ In addition to electron shuttling and complexation, the iron-to-organic carbon (Fe/OC) ratio is considered a variable for the stabilization of Fe(II) in oxic stream waters; the authors reported an inverse relationship between Fe(II) and Fe/OC ratios where Fe(II) constituted 40-70% of the total Fe in stream waters with 0.333 to 0.05 Fe/OC ratios.$^4$ Since the functional groups (i.e., carboxyls, phenolic, thiols, amines) present in organic matter can act as effective electron shuttles through both chemical and microbial processes, these functional groups are capable of oxidizing and reducing redox active metals like Fe.$^5,6,7$ Although the above-mentioned studies implicate Fe/OC ratios, chemical bonding and electron shuttling as determinants in the stabilization of ferrous and/or ferric iron no
studies have been conducted which accurately determine the chemical forms, oxidation states and local coordination environment of in situ organically-bound Fe under various redox conditions.

Iron binds strongly to organic matter, however, determination of the local coordination environment (possibility of bonding to O-, N-, and/or S- ligand atoms) and of the oxidation state of organically-bound Fe has proven challenging and is still unresolved. Studies using organic matter fractions (i.e., extracted humic and fulvic acids) have shown that ferric iron binds to carboxylic and phenolic functional groups and spectroscopic (EPR and Mössbauer) evidence has indicated that Fe(III) forms inner-sphere complexes (tetrahedral and octahedral coordination) with soil humic substances. Using undisturbed field samples, Gustafsson et al. (2007) reported that Fe in organic soils occurred either as monomeric or polymeric (a mixture of \((\text{O}_5\text{Fe})_3\text{O}\) and \((\text{O}_5\text{Fe})_2\text{O}\)) organically bound species depending on the pH of the system. On the other hand, several studies have indicated that Fe was poorly polymerized in natural fresh waters due to complexation with organic matter. In general, however, the involvement of phenolic functional groups in Fe(III)-organic complex formation is presumed to stabilize the 3+ oxidation state of Fe while sulfur (e.g., thiol) and nitrogen (e.g., pyridines, pyrroles) functional groups are presumed to stabilize Fe(II)-organic complexes.

The peatlands of western New York State show vertical redox stratification. In this study, soil samples from these peatlands (i.e., organic-rich wetlands) are used to investigate iron-organic matter interactions under redox variable (oxic, suboxic, anoxic) conditions. Ferric and/or ferrous iron species might be present in the peats, and the potential exists for the formation of Fe(II)- and/or Fe(III)-organic complexes containing Fe-O, Fe-N, and/or Fe-S local coordination environments. The goal of this study is to determine, using field collected samples, the chemical forms, oxidation states and local coordination environment of iron (Fe) in organic (i.e., peat) soils under various redox conditions. This general goal led to the hypotheses that Fe(II) and Fe(III) co-exist in all redox zones (oxic, anoxic, suboxic) albeit in different proportions, and that
complexation with organic matter (i.e., organically-bound Fe) is the primary chemical form in which both Fe(II) and Fe(III) are present in the peats. Furthermore, it is hypothesized that variations in the local coordination environment (i.e., geometry, identity of ligand atoms, bond distance), and therefore in the presumed stability of the chemical bond, are associated— or can be used to explain the persistence of Fe(II) in oxic peats and the persistence of Fe(III) in anoxic peats.

Whereas traditional wet chemical methods used to determine the Fe chemical forms allow for the quantification of operationally-defined fractions, synchrotron-based X-ray absorption spectroscopy yields more detailed information about the electronic oxidation state and coordination environment of Fe. Using Fe L$_{3,2}$-edge XANES spectroscopy we identify Fe(II) and Fe(III) species and the bonding geometry in which Fe is present in the peats. The intensities of the L$_3$ peaks also give an estimation of the covalent nature of the partially occupied valence $t_{2g}$ and $e_g$ orbitals of the metal center (Fe) with the ligands. To model the Fe L$_{3,2}$-edge experimental data we have used a computational approach which employs theoretical charge transfer multiplet calculations (within the computer program CTM4XAS, Charge-Transfer Multiplet for X-ray Absorption Spectroscopy) to obtain a quantitative estimation of Fe(II) and Fe(III) in the peat layers. In addition, the Fe K-edge EXAFS spectra are used to determine the local coordination environment and potential chemical forms of Fe. Collectively, the results obtained from the various methods have helped us to develop a molecular level understanding of the interactions of Fe with organic matter and have provided insights into their coupled biogeochemical cycles.
2.2 MATERIALS AND METHODS

2.2.1 Peat Samples.

The peat cores used in this study were collected from the Manning peatland region of western New York during dry (D series; water table was absent) and wet (W series; water table at ~40 cm depth) seasons. Chemical and spectroscopic analyses were performed for samples from three depths (per season, for a total of six peat samples). Surface peats are the soils within the 0-5 cm section of each peat core and represent the oxic zone. The anoxic zone is represented by peat samples collected at 35-70 cm depths. A peat sample collected at 75-80 cm depth represents the suboxic zone; this peat sample was directly above the underlying mineral marl layer. Redox zones were identified based on concentration profiles of redox sensitive elements (S, Mn, Fe) and microbiological analyses (detection of dsrAB genes coding for dissimilatory (bi)-sulfite reductase) that corresponded to variations in water content and indicated vertical redox stratification in peat cores. Details on study site characteristics, sample collection, and peat characterization can be found in Martínez et al. (2007) and Yoon et al. (2012). 

2.2.2 Fe forms by wet chemical methods.

2.2.2.1. Selective Chemical Extractions.

Peat soils were treated using a selective dissolution procedure to estimate the amount of Fe in various chemical forms (i.e., solid-phase pools or fractions). These are operationally defined fractions and include treatment of the soil with Na-pyrophosphate (for “organically-bound” Fe), ammonium oxalate in the dark (for Fe in poorly-crystalline or “amorphous” inorganic materials),
and dithionate-citrate (for free, non-silicate, “crystalline” Fe). For the sodium pyrophosphate (SP, Fe\textsubscript{p}) extraction, soil samples were ground to pass a 100 mesh sieve, weighed (0.3 g) into 50 ml centrifuge tubes, and 30 ml of 0.1 M Na-pyrophosphate solution added. The suspensions were shaken overnight (14h), centrifuged at 10,000 rpm for 10 min, and the supernatants filtered (0.45 μm). For the acid ammonium oxalate (AO, Fe\textsubscript{o}) extraction soil samples (0.250 g) were weighed into foil-covered centrifuge tubes, 10 ml of the oxalate solution (700 ml of 0.2 M oxalate solution and 535 ml of 0.2 M oxalic acid solution, adjusted to pH 3) were added to the tubes, and the suspensions were shaken for 4 hours. The suspensions were then centrifuged at 10,000 rpm for 20 min, and the supernatants filtered (0.45 μm). For the dithionite-citrate (DC, Fe\textsubscript{d}) extraction, homogenized soil samples (< 2mm) were ground to pass a 35-mesh sieve, weighed (0.5 g) into 50-ml centrifuge tubes, and 25 ml of 0.68 M sodium citrate solution and 0.4 g of dithionite powder were added to the tubes. The tubes were shaken overnight (14h), and then centrifuged for 20 min at 10,000 rpm and the supernatants filtered (0.45 μm). All extraction procedures were done in triplicate and the average was taken. The extracts were analyzed for Fe by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES). It should be noted that pyrophosphate extracts organically-bound Fe by peptization, however, it may also extract some inorganic amorphous Fe. Ammonium oxalate extraction does not remove any particular mineral phase, rather it extracts dominantly non-crystalline Fe along with some crystalline material, during the short reaction time employed. The content of crystalline Fe was estimated as the difference between DC-extractable Fe and AO-extractable Fe (Fe\textsubscript{d} – Fe\textsubscript{o}). The difference between AO-extractable Fe and SP-extractable Fe is used to estimate the contents of poorly-crystalline (amorphous) inorganic Fe (Fe\textsubscript{o} – Fe\textsubscript{p}). The fraction of Fe obtained from the difference between the total Fe (from acid digestion) and the calculated sum of the contents of organically bound, crystalline and amorphous Fe from the above extractions gives an estimate of the “residual” Fe (Fe\textsubscript{r}) in the soil. Residual Fe refers to the non-labile Fe associated with the silicate matrix of the
soil and not extracted by the above-mentioned procedures. This fraction would likely include the mineral marl which is present as the underlying material in these peats.

2.2.2.2 Determination of Ferrous Iron by the 0.5 N HCl Method.

One gram (1g) of air-dried peat soil was weighed and an aliquot of 5mL of 0.5N HCl was added to digest the soil samples. The mixture was swirled for 30s and left in the dark for 24h to ensure complete digestion of the soil samples.\(^\text{36}\) The supernatant was then filtered using a 20μm cellulose acetate filter. An aliquot (20ul) of the filtrate was then taken in a 6ml glass tube and 0.25ml each of ferrozine (0.01M) and acetic acid buffer (pH 4) along with 5ml of deionized water were added. The test tubes were covered and kept in the dark for 24h. The absorbance was measured at 562nm using the Shimadzu UV-310-1PC UV-VIS-NIR Scanning Spectrophotometer and the concentration of ferrous (Fe\(^{2+}\)) iron calculated and reported in g Fe\(^{2+}\) (kg peat soil\(^{-1}\). In addition, total Fe concentrations obtained from HNO\(_3\)/HClO\(_4\) digests\(^\text{17}\) are used to calculate the concentration of Fe\(^{3+}\) in the peats as follows:

\[
[\text{Fe}^{3+}] = [\text{Total Fe}]_{(\text{HNO}_3/\text{HClO}_4 \text{ digests})} - [\text{Fe}^{2+}]_{(0.5\text{N HCl digests})}
\]

2.2.3 Fe K-edge EXAFS (Extended X-Ray Absorption Fine Structure) Spectroscopy.

The iron K-edge (7112 eV) EXAFS spectra for peat soils, Fe-containing minerals, and Fe(II, III)-organic complexes were collected at Beamline X-10C at the National Synchrotron Light Source (NSLS), Brookhaven National Laboratory (Upton, NY) under standard operating conditions (2.584 GeV and 100-250 mA). The monochromator used in these experiments consisted of two parallel Si\{220\} crystals with an entrance slit of 0.5mm. Each sample was pressed into a 0.5-mm-thick acrylic holder with a Mylar film (2.5mm thick, Chemplex Industries,
NY) window. The spectra were recorded in fluorescence mode using a Stern-Heald ionization
detector filled with N$_2$ gas and positioned 90° to the incident beam. The monochromator was
detuned 70% at the Fe K-edge in order to reduce fluorescence induced by high-order harmonics.
The elemental Fe K-edge spectrum (assigned a value of 7112 eV) was used for energy
calibration. Scans ranged from 100 eV below to 500 eV above the Fe absorption edge with 0.2 eV
step size. Each spectrum represents the average of three scans. Data reduction and normalization
of the original spectra followed standard procedures. Data reduction and analyses were
performed using the computer program Athena (Athena 0.8.041, Bruce Ravel, 2005). A smooth
pre-edge background was removed from each averaged spectrum by fitting a first-order
polynomial to the pre-edge region and subtracting this polynomial from the entire spectrum. The
Fourier transforms were calculated over a range of 2-12 Å$^{-1}$ using a Kaiser-Bessel window
function.

The experimental Fe K-edge EXAFS spectra were analyzed using the linear combination
fit (LCF) procedure included in the program Athena. In essence, the LCF procedure involves the
comparison of XAS spectra collected for a sample with the spectra of model compounds. The
LCF procedure yields information on the likely or potential identity of Fe chemical forms present
in the soils. A spectral library of reference compounds was created that include Fe(II)- and
Fe(III)- containing organic and mineral standards; the K-edge EXAFS spectra of the standards
were collected and used to reconstruct the original soil spectra. Mineral standards included:
ferrihydrite, goethite, pyrite, siderite and magnetite. Organic standards included: Fe(II,III)-
histidine. Fe(II,III)-cysteine, Fe(II,III)-arginine, Fe(III) acetate, Fe(III) citrate. The complexes
Fe(II,III) histidine, Fe(II,III)-arginine and Fe(II,III)-cysteine were prepared in an anaerobic glove
box by mixing the ligand and Fe$^{2+}$ or Fe$^{3+}$ salts at a molar ratio of 5:1. The ligand solution was
dissolved in water and the pH was brought to 7 by adding acid/base, as needed. Fe$^{2+}$/Fe$^{3+}$ salts
were added to the ligand solution and throughout the addition of the salt the pH was maintained at
7. The Fe-organic solution was then freeze dried and kept in sealed vials for spectroscopic analyses. The standards used in the LCF procedure were selected based on what we know about our system from previous investigations\textsuperscript{16,17} and based on current Fe L-edge XANES analysis. The amino acids, citrate and acetate collectively represent the most common functional groups (and therefore potential Fe binding sites) present in organic matter, whereas the mineral standards represent Fe minerals expected to form in oxidized and reduced environments. Thus, the standards used mimic potential chemical forms for Fe in organic-rich environments. The experimental Fe K-edge EXAFS spectra were fitted by combining normalized spectra of up to eight Fe standards, with a k-fitting range of 2-12 Å\textsuperscript{-1} relative to the Fe K-edge. The only physical constraints in our LCF procedure were that the weights of the standards should be between 0 and 1 and that a maximum of eight standards could be selected. The weights were not forced to sum to one and the energy was fixed. Thus, compliance with the physical constraints and a sum of components (weights) close to one validates our LCF procedure.

The EXAFS scattering curves were fitted using FEFF included within the Artemis program for the atomic model to determine the number and identity of atoms bonded to Fe in the first coordination shell of the peats. Iron K-edge EXAFS data were processed using IFEFFIT package.\textsuperscript{37,38} Interatomic distances of Fe from representative O, N and S atoms were calculated from crystallographic data of ferrihydrite and pyrite (structures obtained as .cif files from the American Mineralogist Crystal Structure Database) using the ATOMS program within the IFEFFIT package. No inorganic compound containing Fe-N bond was found in the database. Since Fe-O and Fe-N bond distances occur at similar distances in the RSF plots, it was decided that if the fits picked up Fe-O bond then it could mean that a Fe-N bond was also present in the peats. The initial estimate of the Debye-Waller factor for every atomic shell was 0.004 Å\textsuperscript{2}. Backscattering paths and phase corrections were calculated by the FEFF6 program. EXAFS data were Fourier transformed across a $k$ range of 2.0 – 12.0 Å\textsuperscript{-1} for an $R$-space fitting between 0 and
5.0 Å, which was conducted in the Artemis program. When fitting the data in Artemis, k = 1, k = 2, and k = 3 were chosen to optimize the fits over all three weighting factors. When constructing the model, all paths with a computed amplitude of <20% were discarded because these paths were not needed to fit spectral features across the chosen k-space and R-space fitting ranges. To simplify the model, paths that are composed of the same atoms and whose difference of \( R_{\text{eff}} \) (from FEFF 6 calculation based on the crystal structure) is within 0.1 Å are grouped together. Typically, for each group of paths, the path whose \( R_{\text{eff}} \) is the closest to the \( R_{\text{eff}} \) average is included in the structure model to represent other paths. If all paths in that group have the same difference to the \( R_{\text{eff}} \) average, then the path with higher amplitude is chosen. For each chosen path, the parameter coordination number (CN) is fixed as the combination of the degeneracy of all paths in that group, except for the Fe-O paths in the first coordination shell, due to its most dominant contribution to the overall EXAFS spectrum. An amplitude reduction factor (s) of 0.86 was kept fixed for the fits.

2.2.4 Fe L\(_{3,2}\)-edge XANES (X-ray Absorption Near Edge Structure) spectroscopy.

The Fe L\(_{3,2}\)-edge spectrum arises from the dipole-allowed 2p-to-3d transition when excited Fe 2p core electrons move to the empty 3d orbitals, and thus provide a direct probe of the Fe 3d electrons. Iron L\(_{3,2}\)-edge (706.8 eV) XANES experiments were performed at Beamline U4B of the National Synchrotron Light Source (NSLS), Brookhaven National Laboratory under ring operating conditions of 700 mA and 0.808 GeV and under ultrahigh vacuum (UHV) conditions (~10\(^{-7}\) Torr). The peat soils were finely ground and spread across Fe-free double-sided adhesive conductive graphite tape attached to a copper paddle. The paddle was affixed to a rotary stage and inserted into the experimental chamber aligned at 45° to the incident defocused beam. Spectra at the Fe L\(_{3,2}\)-edge were collected over the energy range 680-738 eV
with 0.5 eV steps in the pre-edge (680-695-395 eV) and post-edge (725-738 eV) regions, and with a step size of 0.1 eV between 695-725 eV. A low energy grating (600 lines/mm) monochromator was used to collect the spectra and entrance and exit slits were set to 50/50. The total electron yield (TEY) signals were recorded. The Fe L₃,₂-edge XANES spectral signal was normalized to the incident photon flux (I₀), measured simultaneously. The photon energy was calibrated using the spectrum of Fe₂O₃ as an internal reference, which was acquired simultaneously with each scan of every sample. The second peak of the Fe₂O₃ spectrum is assigned a value of 706.8 eV and is used for Fe energy calibration. Ten to fifteen scans were collected per sample (as needed) at room temperature and averaged. Sample decay was not observed as evidenced by identical spectra initially and at the end of data collection for each sample. Data reduction of the spectra includes baseline removal, energy calibration and normalization. The spectra were processed using the Athena software.³⁷

2.2.5 Multiplet Calculations of Fe L₃,₂-edge XANES spectra.

Quantitative information about bonding, back-bonding, charge-transfer and spin character can be obtained by modeling the Fe L₃,₂-edge XANES spectra using the Charge-Transfer Multiplet program for X-ray Absorption Spectroscopy (CTM4XAS) developed by Frank de Groot and Eli Stavitski.⁴⁰ CTM4XAS utilizes a semi-empirical method to simulate the L-edges of transition metal compounds which is based on a multiplet model developed by Theo Thole and with contributions by Ogasawara as the basis for the CTM4XAS calculations. This method takes into consideration the important core-valence, two-electron integrals (multiplet effects) and the core hole induced charge transfer effects, thereby helping to interpret the interactions in an L-edge spectrum. The CTM4XAS simulated intensities have been matched with those of the experimental spectra to model the coordination environment and oxidation state of Fe in peat
soils. Till date, CTM4XAS had proven its validity in interpreting transition metal L-edge in systems such as pure molecules and condensed matter. 41,42 This is, to our knowledge, the first time CTM4XAS has been used to determine Fe oxidation states and coordination environments in natural systems like soils where we expect heterogeneity and a myriad of Fe coordination environments and oxidation states.

2.3 RESULTS AND DISCUSSION

Samples from the oxic and suboxic peat layers had pH values ranging from 4.8 to 5.3 whereas anoxic peats had pH values between 5.3 and 6.9 (Table 2-1). Most peats had organic matter contents of 75-83\%, with the exception of one sample (D16) which was mixed with the underlying marl layer and contains 25\% organic matter. Total Fe and Al concentrations were at the low end of values reported for mineral soils (2-550 g kg\(^{-1}\) Fe, 10-300 g kg\(^{-1}\) Al\(^{3+}\)) but were similar to values reported for peatlands.43,44 Somewhat higher Fe and Al levels in the suboxic peat (D16) results from physical mixing of the peat with the marl.

2.3.1 Chemical forms of iron in peats.

Selective chemical extractions and linear combination fit (LCF) analyses of the Fe K-edge EXAFS spectra were used to investigate the chemical forms in which Fe is present in the peat soils. Selective chemical extractions (Table 2-1) showed that 73-100\% of the total Fe in all but one of the peats was present in organic and amorphous forms. Specifically, results from sodium pyrophosphate extractions revealed that from 43 to 60\% of the total Fe was organically bound Fe in oxic and anoxic peats for both dry and wet season samples. Since these peat soils contain ~80\% organic matter, the high percentage of organically bound Fe is expected, however,
differing oxidation states and local coordination environments of Fe are shown in the subsequent X-ray absorption spectroscopic data. Results from the AO extractions showed a notable difference with 17-20% and 52-58% of the total Fe found in amorphous Fe forms in dry and wet peats, respectively, independent of redox condition. Furthermore, the lack of crystalline Fe phases is striking (only the suboxic peat, D16, showed 6%), suggesting the high percentage of organic matter present in these peats impedes the formation of stable crystalline phases that persist in these highly fluctuating redox-dynamic systems which are frequently exposed to alternating wet-dry cycles. The residual Fe fraction was only significant for the dry season peats and increased with depth from 19% to 66% in peat D16 where the peat is mixed with the underlying mineral marl layer. In general, selective chemical extractions suggest that the most important- and perhaps the most rapid- changes occur between the organically-bound and the amorphous forms of Fe.

The likely chemical forms (i.e., potential chemical bonding environment) in which Fe is present in the peat soils were also investigated using linear combination fit (LCF) analyses of the Fe K-edge EXAFS spectra. The Fe K-edge EXAFS spectra of the six peat samples, their Fourier-transform magnitudes (radial structure functions, RSF), and their $k^3$-weighted EXAFS spectra and fits are shown in Figure 2-1. The fitting results are presented in Table 2-2. Consistent with selective chemical extractions, the results from LCF analyses suggest 56-100 % of the total Fe in all of the peats is present in Fe(II)- and Fe(III)- complexes with organic molecules and in amorphous (i.e., ferrihydrite) forms (Table 2-2). In addition, but with the exception of the oxic peat collected during dry season (D1), Fe(II)-histidine was identified as a potential form of iron in the peat samples accounting for 15-56 % of the total Fe. We must acknowledge an amorphous Fe mineral (likely ferrihydrite or other polymeric Fe species) formed during laboratory synthesis of the Fe(III)-organic complexes so that we cannot differentiate between pure Fe(III)-organic and pure ferrihydrite phases based on LCF analyses.
However, Fe(III) species (organic and/or ferrihydrite) accounted for 80-99 % of the total Fe in four out of the six peat soils (exceptions are D16 and W12). Fe(II)-containing mineral phases, namely siderite (35%) and pyrite (43 %) were selected as likely forms of Fe in the peats D16 and W12, respectively (Table 2-2). The selection of siderite in analyses of sample D16 is consistent with selective chemical extraction results where the residual fraction was significant (Tables 2-1 and 2-2) but the selection of pyrite in LCF analyses of sample W12 is not. However, it might be possible that some amorphous pyrite is present which gets dissolved when treated with ammonium oxalate Interestingly, Fe(II)- or Fe(III)- complexes with cysteine were never selected by the LCF procedure even though these peats contain large amounts of reduced sulfur (60-80 % thiol/sulfide of 10-40 g kg\(^{-1}\) total S).\(^{16}\) It is also worth noting that Fe complexes with histidine (although the Fe(III)-histidine complex was not pure) were consistently selected as a significant component in LCF analyses over complexes with only O-containing functional groups. This suggests that Fe might have a higher affinity to bind with heterocyclic N-containing molecules which have an available pair of electrons on their N atoms for donation to the Fe center, thereby stabilizing the complex, compared to the O-containing functional groups. Visual inspection of the RSFs (Figure 2-1C) suggests the peat samples D16 and W12 (and to a lesser extent W1) contain strong backscatter atoms in their second shell (i.e., Fe-containing mineral phases, for example) which supports the results from LCF analyses. Consistent with LCF analyses and selective chemical extractions, the other peat samples show RSFs characteristic of bonding environments with weakly scattering atoms in the second coordination shell (i.e., Fe bonding with organic molecules) or with short-range poorly ordered minerals (i.e., ferrihydrite and/or polymeric Fe species).
2.3.2 Oxidation states of iron in peats.

0.5N HCl extraction tests were used to estimate the concentration of ferrous (Fe$^{2+}$) iron in the peat soils. The results show that Fe$^{2+}$ and Fe$^{3+}$ co-exist in all peat samples independent of their redox condition or time of sample collection (dry or wet season), albeit in different proportions (Table 2-3). Ferrous iron is present in the oxic (D1 and W1) and suboxic (D16) peats at 9-17% of the total Fe, whereas Fe$^{3+}$ is present in anoxic (D14, W8 and W12) and suboxic (D16) peats at 25-89% of the total Fe. Note the concentration of Fe$^{2+}$ in peats D16 and W12 are probably underestimates due to the presence of the minerals siderite and pyrite, which are unlikely to dissolve (at least completely) using a weak acid digestion. Moreover, peat digestions with 0.5M HCl seem to yield about 20% of the total Fe when compared to concentrations obtained from a more aggressive (HClO$_4$) digestion procedure. This can also result in underestimates of the concentration, and therefore of the percentage, of Fe$^{2+}$ present in the peat samples (Table 2-3). The co-existence of Fe$^{2+}$ and Fe$^{3+}$ in all peats is further verified by the Fe L-edge XANES results.

The total intensity, energy position and spectral shape of Fe L$_{3,2}$-edge XANES spectra are the three characteristics which provide detailed information about the bonding environment and oxidation state of Fe in these peats (Figure 2-2). The Fe L-edge intensity is directly proportional to the Fe d-character in the valence orbitals on the metal, and thus the % metal character (i.e., ability to lose outer valence electrons) in the d-orbitals can be probed by the integrated L-edge intensity. Based on the peak positions of Fe L-edge XANES spectra, we can identify whether Fe is present in 2+ or 3+ oxidation state. Typically a Fe$^{2+}$ peak occurs at ~705.5 eV whereas that for Fe$^{3+}$ occurs at ~707.8 eV (Figure 2-2). Besides the peak energy positions, the relative intensities of the peaks indicate the relative concentrations of Fe$^{2+}$ and Fe$^{3+}$ present in a system. Also, the lateral separation between the peaks is an indicator of the degree of charge transfer between the metal center and ligand. The greater the separation, the higher the degree of charge transfer.
Therefore, inspection of the Fe L-edge XANES spectra indicate the presence of Fe\(^{2+}\) and Fe\(^{3+}\) in all peats regardless of redox condition (Figure 2-2). Considerable Fe\(^{2+}\) contribution to the L-edge spectra is clearly observed in the oxic (D1, W1), and as expected, in the anoxic (D14, W8) peats. Similarly, the L-edge spectra indicate Fe\(^{3+}\) are present in the anoxic (D14, W8, W12) and suboxic (D16) peats.

Furthermore, modeling of the Fe L-edge XANES spectra by CTM4XAS calculations gives a theoretically calculated quantitative estimate of the contribution of Fe\(^{2+}\) and Fe\(^{3+}\) redox species to the spectra, along with their coordination environments. Spectral fits are shown in Figure 2-3 and the percent contributions are presented in Table 2-4. CTM4XAS calculations indicate that Fe\(^{2+}\) and Fe\(^{3+}\) are both present in all peat soils. Specifically, the modeling results show that Fe\(^{2+}\) is present in the oxic peats of both the dry (37.5%) and wet (26.5%) seasons, with more than 50% contribution to anoxic peats D14 and W8. CTM4XAS calculations also indicate that Fe\(^{3+}\) is present in the anoxic peats D14 and W8 at close to 50% contribution, whereas higher Fe\(^{3+}\) contributions are found in the suboxic (66%) and oxic (62-74%) peats. A large discrepancy is found in the CTM4XAS simulations for the anoxic peat W12 that indicate 20% contribution from Fe\(^{2+}\) and 80% contribution from Fe\(^{3+}\) to the overall spectra. These results, however, are in agreement with the Ferrozine test results (Table 2-3), but in sharp contrast with the K-edge EXAFS spectra (Figure 2-1) and LCF results (Table 2-2) that show Fe\(^{2+}\) contribution is significant.

2.3.3 Local coordination environment of iron in peats.

Besides quantifying the contribution of Fe\(^{2+}\) and Fe\(^{3+}\) redox species, modeling of the Fe L-edge XANES spectra of the peats by CTM4XAS is used to obtain detailed information about their coordination environment. Crystal field strengths, given by 10Dq values and spin-orbit
coupling are two parameters which give an estimation of the geometry and the nature of the ligand atoms which stabilizes Fe$^{2+}$ and Fe$^{3+}$ in the peats. CTM4XAS calculations show Fe$^{2+}$ and Fe$^{3+}$ coexist in tetrahedral and octahedral coordinations respectively in all the peats with different parameters for spin-orbit coupling and/or crystal field strengths (Table 2-4 and Figure 2-3). The crystal field strength parameter (10Dq value) gives an estimation of the separation of peaks in the L$_3$ region which arises due to the energy separation between the lower energy t$_{2g}$ and high energy e$_g$ molecular orbitals when a metal atom is placed in a ligand field. The 10Dq value is thus, a measure of the % ionic character of the metal-ligand bond. The higher crystal field strength for octahedral Fe$^{3+}$ in the peats indicates a greater difference in electronegativity between the metal and the donor atoms suggesting the formation of an ionic bond (Fe-O) between the two whereas a lower 10Dq value a covalent one (Fe-N or Fe-S). The 10Dq value also gives an indication of the coordination geometry in which the metal is present. A lower 10Dq value indicates the species to be possibly in tetrahedral coordination whereas a higher 10 Dq value suggests an octahedral coordination. However, to say more definitively whether a metal is present in tetrahedral or octahedral coordination, we need more evidence from Fe K-edge EXAFS (see next section). Thus, variations in 10Dq values reveal differences in the strength of binding of Fe to the donor atoms of the respective functional groups. On comparing the 10Dq values of Fe-peats with the Fe-minerals and Fe-organic from literature, it can be concurred that Fe$^{2+}$ and Fe$^{3+}$ in the peats have the same parameters which have been used for modeling Fe-minerals and Fe-organics. Fe$^{3+}$ in all the peats showed 10Dq values ranging from 1.4-1.6, indicating that Fe$^{3+}$, a hard acid might be present in the octahedral coordination with O or N-containing functional groups (carboxyls or amines), a hard base which has a higher electronegativity. Fe$^{2+}$ in these complexes was, in contrast, found to exhibit much lower 10Dq values (0.6) suggesting that Fe$^{2+}$ might be in tetrahedral coordination with N or S-functionalities (heterocyclic amines or thiols), a soft base which has a lower electronegativity. Only W1 had 4% of the total Fe in octahedral Fe$^{2+}$ form
The spin-orbit coupling parameter, on the other hand, takes into consideration the interactions between the 2p and 3d electrons and the value of this parameter determines the shape of the L\textsubscript{3} and L\textsubscript{2} edges of the spectra. The spectral nature obtained from the spin-orbit coupling parameter indicate that Fe\textsuperscript{2+} and Fe\textsuperscript{3+} (d\textsuperscript{6} and d\textsuperscript{5} electron systems, respectively) in these complexes are present in their high spin states since these states are the ones which have unpaired electrons in their valence d-orbitals and hence can readily participate in electron exchange reactions.\textsuperscript{25,26} The considerably high Fe L-edge peak intensity for oxidized Fe(II) cysteine at t=12 months indicates a much higher Fe-ligand covalence in this particular complex.

Hence, CTM4XAS is a robust modeling tool which predicts the oxidation state of the metal along with their proportions, the geometry in which they are present and also some indication of the functionalities involved in stabilizing the Fe\textsuperscript{2+} in oxic and Fe\textsuperscript{3+} in anoxic and suboxic peats.

First shell analyses of K-edge EXAFS spectra yield information regarding the average coordination number of the atom (i.e., Fe), and about the identity and distance at which ligand atoms occur. Table 2-5 and Figure 2-4 show the fitting results for first coordination shell in q-space for the peats; corresponding Fourier transforms are shown in Figure 2-1. The first coordination shell is composed of O/N atoms at a distance of 1.96-2.06 Å in all the peat samples. The coordination numbers for Fe indicate that Fe tends to be mostly in octahedral coordination with the ligand atoms. Of all the peats, D16 and W1 indicate that most of the Fe is in tetrahedral coordination environment (4.8 and 4.4 respectively). Fe\textsuperscript{2+} in tetrahedral coordination can be present either by binding to N, S containing organic functional groups (histidine or thiol) or O,S containing minerals (siderite or pyrite). However, there are no indications for S associations in any of our peat samples, which is contrary to our LCF analyses where pyrite was found. The coordination numbers for octahedral Fe\textsuperscript{3+} are in accordance with data for Fe-O bonding found in Fe(III) complexes with organics such as tris(oxalato)iron (Fe(III) in octahedral coordination with
three bidentate oxalate ions\textsuperscript{45}, tridentate oxalate\textsuperscript{45} and Fe(III)-DFO-B\textsuperscript{46,47}. Previous EXAFS studies on Fe minerals suggest that octahedral Fe\textsuperscript{2+}-O and tetrahedral Fe\textsuperscript{2+}-O bonds occur at 1.983Å and 2.096Å respectively whereas EXAFS on ferrihydrite suggest that octahedral Fe\textsuperscript{3+}-O occur at 1.93Å.\textsuperscript{22} Comparing these data with the EXAFS of soils, we might make the following observations: Octahedral Fe\textsuperscript{3+} in the anoxic peats gets stabilized by binding to either O, N containing organic functional groups or by being present as amorphous ferrihydrite, as shown by the chemical extractions or LCF analyses. Fe\textsuperscript{2+} in the oxic peats might get stabilized by bonding to N-containing heterocyclic chelating agents. With EXAFS, it is not possible to distinguish between O and N atoms, hence the structure in which Fe is present in these peats should be seen as an average of several possible combinations of O- and N-containing functional groups such as carboxyl, carbonyls and amines. Overall, we can say that the first coordination shell of Fe K-edge EXAFS data yields only limited information (identity and distance of the binding atom) about the coordination environment, which turned out to be the same (O/N at 2Å) for all the peats and most of the information is erased out due to the complex heterogeneity present in these peats.

However, the results from the experimental first shell EXAFS fits when combined with the Fe L-edge XANES modeling results from CTM4XAS simulations yield a more detailed insight about the coordination environment of Fe in these peats. In contrast to the EXAFS first shell fits, CTM4XAS calculations specifically indicate Fe\textsuperscript{2+} in tetrahedral and octahedral coordination in addition to Fe\textsuperscript{3+} being in octahedral coordination. Combining the results from EXAFS (first shell coordination and LCF fits) analyses and CTM4XAS approaches, we can conclude that Fe\textsuperscript{2+} in tetrahedral form was stabilized either by being a part of Fe(II) bearing minerals (siderite and traces of pyrite) or binding to the –COOH or –NH\textsubscript{2} of histidine-like ligands. On the other hand, Fe\textsuperscript{3+} in octahedral coordination was stabilized mostly in association with O/N containing organic functional groups (histidine, citrate, acetate) and/or as amorphous
ferrihydrite in oxic (D1 and W1) and anoxic (D14, W8, and W12) peats. It should be repeated that although pyrite was not picked by LCF nor by first coordination shell fits, the pre-edge Fe K-edge XANES features had marked resemblance to those of pyrite, further indicating the presence of this Fe$^{2+}$ mineral being present in W12.

2.3.4 Co-existence of ferrous and ferric iron in peats of variable redox condition

Ferrozine tests, LCF analyses of the EXAFS data, and CTM4XAS simulations of the L-edge XANES spectra are all in general agreement and indicate the coexistence of Fe$^{2+}$ and Fe$^{3+}$ in the peat soils independent of redox condition or time of sampling (dry or wet season). Results from the above-mentioned methods can also be illustrated by creating a pe-pH diagram of Fe which indicates the potential stable Fe phases as function of redox potential and pH in these peats. Fig 2-6 shows the pe-pH diagram of oxic, anoxic and suboxic peats. pe-pH stability diagrams predict Fe$^{2+}$ (aq) and Fe oxides (i.e., Fe(OH)$_3$) as dominant species in reduced and oxidized environments, respectively, at pH values between 4 and 7. The formation of FeCO$_3$ is expected under highly reduced conditions at pH values greater than 7.8, and FeS$_2$ species are expected to form under anoxic conditions. However, pe-pH diagrams do not take into consideration the presence of organic matter, solid or dissolved, which can shift the stability boundaries for the Fe species present in the peats. Based on the results obtained, the following mechanisms have been suggested to be instrumental in stabilizing Fe$^{2+}$ in oxic and Fe$^{3+}$ in anoxic/suboxic peats.

Fe$^{3+}$ can be incorporated and stabilized into soil organic matter through dissolution and complexation pathways which mostly includes the reductive dissolution of Fe(III) from minerals and reoxidation of the Fe(II) within the OM matrix.$^{48,49}$ Since the peats contain a lot of amorphous Fe, it can be said that reductive dissolution of ferrihydrite might occur and lead to
soluble Fe$^{2+}$ which can get coprecipitated within the OM matrix. Also, according to the Hard and Soft Acid-Base principle, it can be said that Fe$^{3+}$, a hard acid in the anoxic/suboxic peats gets stabilized via bonding to O- and N- containing organic functional groups like carboxyls, carbonyls, phenols and amines which are hard bases to form stable inner sphere complexes. It can be also be suggested that oxidation of Fe$^{2+}$ in the deeper reduced layers might lead to the formation of stable hydrolyzed Fe(III)-organic complexes through dissolution and precipitation reactions, thereby stabilizing Fe$^{3+}$ in these layers. Biological degradation might also lead to the incorporation of Fe(III) into OM. Besides, Fe$^{3+}$, reductive dissolution of minerals can also lead to the incorporation of Fe(II) into OM which explains the presence of this reduced species in the oxic layers. Although both Fe(III) and Fe(II) have the potential to bind with same coordination sites in OM, oxidation-reduction cycles may lead to the preferential binding of Fe$^{2+}$ and Fe$^{3+}$ to the same organic functional group may occur. Fe$^{2+}$, being a soft acid has a tendency to bind more strongly with soft bases (example, thiols) or N-containing heterocyclic chelating agents (histidine), than Fe$^{3+}$. The formation constants obtained from literature attest to this observation.

20% of the total Fe in oxic peat (W1) is present as stable Fe(II)-histidine which has a stability constant of ~6.0, indicating the stabilization of Fe$^{2+}$ with N-containing organic functional groups whereas Fe(III)-histidine has a stability constant of ~4.0. Also, thermodynamic constants for 1:1 complexes of Fe(III) with weak binding sites in humic substances like carboxylic groups and strong binding sites like phenolic groups have been found to be four to 40 orders of magnitude greater than the respective stability constants for Fe(II) complexes.

The rate of metal sorption or desorption on soil organic matter also plays an important role in predicting the prevalence of one species over the other. The ionic radius has been found to be effective in predicting the stability of metal-organic complexes in this regard. Fe$^{3+}$ which has a lower ionic radius than that of Fe$^{2+}$ are more effective to undergo ion exchange with oxides/organic functional groups than the latter. So, it can be said that since the sorption kinetics
of the former are faster than that of the latter, thereby forming stable Fe(III)-c complexes in the anoxic peats. Thus, the Fe oxidation state could affect the relative proportions of Fe bound to carboxylic vs. phenolic functional groups.

The reaction kinetics of the redox reactions also plays an important role in the predominance of Fe$^{2+}$ over Fe$^{3+}$ and vice-versa. Rapid oxidation of Fe(II) by O$_2$ has been found to depend on the state of Fe(II) complexation with OM,$^{52,54}$ which might also affect the distribution or redistribution of Fe(III) among OM functional groups. The persistence of the reduced forms of Fe in the oxic pore waters of the peats have been found inversely related to the variable oxidation kinetics of the system, and can be attributed to the high stability constant of Fe(II)-histidine. However, the presence of Fe$^{2+}$ with depth can also be a result of microbial reduction or due to the presence of inorganic carbonates or divalent amorphous sulfides.

Solubility products given by k$_{sp}$ values also indicate the complexes can also provide an insight of the ongoing precipitation and dissolution reactions in the oxic and anoxic peats. It can be said that the anoxic peats have dissolved organic matter which cause ligand promoted dissolution of the amorphous oxides present in these peats, thereby speeding up their precipitation process. The pathways of Fe reaction in the presence of OM could affect the propensity for Fe(OH)$_3$ to precipitate.$^{55}$

Thus, the dynamic Fe redox environments in the different peat layers influence Fe biogeochemical process through the stoichiometry of oxidation-reduction reactions. These results help us to develop a concise understanding of the Fe speciation in these natural environments, which are important in aquatic and terrestrial ecosystems.
References


### Table 2-1 Properties of Peat Samples and Results from Selective Chemical Extractions

<table>
<thead>
<tr>
<th></th>
<th>D1</th>
<th>D14</th>
<th>D16</th>
<th>W1</th>
<th>W8</th>
<th>W12</th>
</tr>
</thead>
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<tr>
<td><strong>Physico chemical properties(^1)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depth (cm)</td>
<td>0-5</td>
<td>65-70</td>
<td>75-80</td>
<td>0-5</td>
<td>35-40</td>
<td>55-60</td>
</tr>
<tr>
<td>Redox Condition</td>
<td>Oxic</td>
<td>Anoxic</td>
<td>Suboxic</td>
<td>Oxic</td>
<td>Anoxic</td>
<td>Anoxic</td>
</tr>
<tr>
<td>Water content (%)</td>
<td>123.3</td>
<td>342.6</td>
<td>125.5</td>
<td>137.1</td>
<td>269.8</td>
<td>378.3</td>
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<tr>
<td>pH</td>
<td>4.8</td>
<td>6.0</td>
<td>5.2</td>
<td>5.3</td>
<td>5.8</td>
<td>6.9</td>
</tr>
<tr>
<td>% OM (LOI)</td>
<td>83.2</td>
<td>76.0</td>
<td>25.2</td>
<td>81.8</td>
<td>75.3</td>
<td>79.3</td>
</tr>
<tr>
<td>Total Fe (g kg(^{-1}))</td>
<td>8.28</td>
<td>11.80</td>
<td>18.00</td>
<td>10.09</td>
<td>4.46</td>
<td>9.32</td>
</tr>
<tr>
<td>Total Al (g kg(^{-1}))</td>
<td>6.97</td>
<td>3.70</td>
<td>30.77</td>
<td>2.26</td>
<td>0.94</td>
<td>3.86</td>
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<td><strong>Selective Chemical Extractions (expressed as % of Total Fe)</strong></td>
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<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Organic Fe</td>
<td>60.62</td>
<td>55.32</td>
<td>10.86</td>
<td>43.40</td>
<td>52.31</td>
<td>45.15</td>
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<tr>
<td>Amorphous Fe</td>
<td>20.50</td>
<td>18.22</td>
<td>17.02</td>
<td>52.81</td>
<td>56.45</td>
<td>57.93</td>
</tr>
<tr>
<td>Crystalline Fe</td>
<td>(Nd)(^2)</td>
<td>(Nd)</td>
<td>6.00</td>
<td>(Nd)</td>
<td>(Nd)</td>
<td>(Nd)</td>
</tr>
<tr>
<td>Residual Fe</td>
<td>18.88</td>
<td>26.47</td>
<td>66.10</td>
<td>3.79</td>
<td>(Nd)</td>
<td>(Nd)</td>
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</table>

\(^1\) From Martínez et al. (2007)

\(^2\) \(Nd\): not detected
Table 2-2 Fe species identified by Linear Combination Fit (LCF) analyses of k3-weighted Fe K-edge EXAFS (expressed as %)

<table>
<thead>
<tr>
<th>Fe-Standard</th>
<th>D1</th>
<th>D14</th>
<th>D16</th>
<th>W1</th>
<th>W8</th>
<th>W12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe(II)-histidine</td>
<td>Nd$^1$</td>
<td>15.8</td>
<td>55.9</td>
<td>19.5</td>
<td>14.7</td>
<td>49.3</td>
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<tr>
<td>Fe(III)-histidine</td>
<td>60.2</td>
<td>55.3</td>
<td>8.0</td>
<td>13.1</td>
<td>47.9</td>
<td>Nd</td>
</tr>
<tr>
<td>Fe(III)-citrate</td>
<td>17.7</td>
<td>Nd</td>
<td>Nd</td>
<td>12.8</td>
<td>15.8</td>
<td>4.0</td>
</tr>
<tr>
<td>Fe(III)-acetate</td>
<td>21.06</td>
<td>28.9</td>
<td>1.0</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td>Ferrihydrite</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>54.6</td>
<td>21.7</td>
<td>3.2</td>
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<tr>
<td>Siderite</td>
<td>Nd</td>
<td>Nd</td>
<td>35.0</td>
<td>Nd</td>
<td>Nd</td>
<td>42.6</td>
</tr>
<tr>
<td>Pyrite</td>
<td>Nd</td>
<td>Nd</td>
<td>0.3</td>
<td>Nd</td>
<td>Nd</td>
<td>0.9</td>
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$^1$ Nd: not detected
Table 2-3: Ferrous and ferric ion concentrations in peat soils

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<thead>
<tr>
<th></th>
<th>D1</th>
<th>D14</th>
<th>D16</th>
<th>W1</th>
<th>W8</th>
<th>W12</th>
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<tr>
<td><strong>Total Fe</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(HClO₄)</td>
<td>8.28</td>
<td>11.80</td>
<td>18.00</td>
<td>10.09</td>
<td>4.46</td>
<td>9.32</td>
</tr>
<tr>
<td><strong>Total Fe</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(0.5M HCl)</td>
<td>--</td>
<td>--</td>
<td>3.42</td>
<td>1.74</td>
<td>--</td>
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<tr>
<td><strong>Fe²⁺</strong> (0.5M HCl)¹</td>
<td>0.72</td>
<td>5.77</td>
<td>1.90</td>
<td>1.69</td>
<td>3.35</td>
<td>1.52</td>
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<tr>
<td><strong>Fe³⁺ (total</strong></td>
<td>7.56</td>
<td>6.03</td>
<td>16.10</td>
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<td>7.80</td>
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<td>Fe(HClO₄) –</td>
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<tr>
<td>Fe²⁺ (0.5M HCl)¹</td>
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<td><strong>Fe³⁺ (total</strong></td>
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<td>1.52</td>
<td>0.05</td>
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<tr>
<td>Fe(0.5M HCl) –</td>
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<tr>
<td>Fe²⁺ (0.5M HCl)²</td>
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<tr>
<td><strong>Fe²⁺ (Fe²⁺</strong></td>
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<td>--</td>
<td>1.90</td>
<td>1.69</td>
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<tr>
<td>(HClO₄) –</td>
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<tr>
<td>Fe²⁺ (0.5M HCl)²</td>
<td></td>
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<td></td>
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</table>

¹ Values in parenthesis are the percentage of ferrous (Fe²⁺) and ferric (Fe³⁺) iron calculated based on concentrations obtained from 0.5M HCl (for Fe²⁺) and HClO₄ (for total Fe) digests.

² Values in parenthesis represent the percentage of ferric (Fe³⁺) and ferrous (Fe²⁺) iron calculated based on concentrations obtained from 0.5M HCl digests for both Fe²⁺ and total Fe. Values are presented for only two of the soils because peat samples were no longer available.
Table 2-4: Modeling parameters chosen for CTM4XAS calculations

<table>
<thead>
<tr>
<th>Soil</th>
<th>Octahedral or Tetrahedral Fe$^{2+}$</th>
<th></th>
<th>Octahedral Fe$^{3+}$</th>
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<tbody>
<tr>
<td></td>
<td>%</td>
<td>10 Dq value*</td>
<td>SO</td>
<td>%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>coupling$^\dagger$</td>
<td></td>
<td></td>
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<tr>
<td>D1</td>
<td>37.50</td>
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<td>0.95</td>
<td>62.5</td>
</tr>
<tr>
<td>D14</td>
<td>55.5</td>
<td>0.6</td>
<td>0.95</td>
<td>45.5</td>
</tr>
<tr>
<td>D16</td>
<td>33.34</td>
<td>0.6</td>
<td>0.95</td>
<td>66.0</td>
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<tr>
<td>W1</td>
<td>22.5</td>
<td>0.6</td>
<td>0.95</td>
<td>73.5</td>
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<tr>
<td></td>
<td>4.0</td>
<td>1.6</td>
<td>1.00</td>
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<tr>
<td>W8</td>
<td>54.3</td>
<td>0.6</td>
<td>0.95</td>
<td>45.7</td>
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<tr>
<td></td>
<td>4.60</td>
<td>0.6</td>
<td>1.00</td>
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<tr>
<td>W12</td>
<td>20.00</td>
<td>0.6</td>
<td>0.95</td>
<td>80.0</td>
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*10Dq values indicate crystal field strengths for each Fe configuration

$^\dagger$SO coupling indicates spin-orbit interaction parameters used for the simulations
Table 2-5: Least Square fits for the first coordination shell of Fe K-EXAFS

<table>
<thead>
<tr>
<th>Sample</th>
<th>Interaction</th>
<th>CN</th>
<th>R (Å)</th>
<th>$\sigma^2$</th>
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<tr>
<td>D1</td>
<td>Fe-O/N</td>
<td>5.4</td>
<td>2.004</td>
<td>0.0069</td>
</tr>
<tr>
<td>D14</td>
<td>Fe-O/N</td>
<td>6.3</td>
<td>2.014</td>
<td>0.0090</td>
</tr>
<tr>
<td>D16</td>
<td>Fe-O/N</td>
<td>4.8</td>
<td>2.064</td>
<td>0.0109</td>
</tr>
<tr>
<td>W1</td>
<td>Fe-O/N</td>
<td>4.4</td>
<td>1.982</td>
<td>0.0010</td>
</tr>
<tr>
<td>W8</td>
<td>Fe-O/N</td>
<td>5.4</td>
<td>1.969</td>
<td>0.0092</td>
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<tr>
<td>W12</td>
<td>Fe-O/N</td>
<td>5.4</td>
<td>1.966</td>
<td>0.0075</td>
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</tbody>
</table>

*Coordination number (CN), bond distance (R), Debye-Waller factor ($\sigma^2$)*
Figures

**Figure 2-1:** Fe K-edge EXAFS data for peat samples. A. shows the normalized Fe K-edge spectra, B. shows the $k^3$-weighted EXAFS spectra, and C. shows their Fourier transform magnitudes (not corrected for phase shift). Solid lines are experimental data and filled circles represent best model fits of LCF analyses.
Figure 2-2: Fe L-edge XANES of peats. Left panel: Dry Season (D); Right panel: Wet Season (W). Fe$^{2+}$ peaks occur at ~705.5 eV and Fe$^{3+}$ peaks occur at ~707.8 eV.
Figure 2-3: Modeling of Fe L-edge XANES experimental data with CTM4XAS calculations. Blue=experimental; red=calculated; black= Oh Fe$^{3+}$ with 1.6 10Dq and SO1; grey= O$_h$ Fe$^{3+}$ with 1.410Dq and SO1; brown= T$_d$ Fe$^{2+}$ with 0.6 10Dq and 0.95SO; green= T$_d$ Fe$^{2+}$ with 0.610Dq and SO1; orange= O$_h$ Fe$^{2+}$ with 1.6 10Dq and SO1 (O$_h$=octahedral; T$_d$=tetrahedral)
Figure 2-4: Fourier Transforms (solid lines), not corrected for phase shift, and fit results (dotted lines) for the first coordination shell of peats.
Chapter 3

REDOX INTERACTIONS BETWEEN FE AND CYSTEINE: SPECTROSCOPIC STUDIES AND MULTIPLET CALCULATIONS

Abstract

This study monitors the electron exchange interactions between the redox active metal (Fe) center and the different functional groups of cysteine (carboxylic, amine and thiol) in laboratory-synthesized Fe(II)-cysteine and Fe(III)-cysteine complexes as a function of time using synchrotron X-ray absorption spectroscopy (XAS) and multiplet calculations. A study of the oxidation state of Fe indicates preservation of Fe(II) in Fe(II)-cysteine and initial reduction of Fe(III) in Fe(III)-cysteine, the latter caused by an internal electron transfer reaction from S of –SH. Ferrous (Fe$^{2+}$) iron was in tetrahedral coordination with S atoms at a distance of 2.21-2.42Å. However, after 12 months, oxidation of the Fe took place in Fe(II)-cysteine (~60% Fe$^{3+}$) whereas that in Fe(III)-cysteine complexes continued to remain mostly in its reduced state (~33% Fe$^{3+}$). Correspondingly, the oxidized Fe in Fe(II)-cysteine was present in an octahedral coordination with O/N atoms at a distance of 2.05Å, thus revealing intramolecular electron transfer involving the Fe and –COOH/NH$_2$ groups. The reduced Fe in Fe(III)-cysteine, which retained most of its reduced characteristics, was in tetrahedral coordination with O/N/S atoms at a distance of 2.15Å. The metal-ligand covalence was also higher in the oxidized Fe(II)-complex indicating that the hard acid Fe$^{3+}$ gets stabilized through bonding to –O or –N, the hard bases. Besides, the –COOH, -NH$_2$ and –SH of cysteine, reduction of C present in the propyl backbone of cysteine occurred in synchrony reflecting the true potential of cysteine as a redox ligand. Overall, our studies of the Fe(II,III)-cysteine system provide a mechanistic understanding of the electron exchange
mechanism that occurs between a redox active metal and a redox active ligand by providing a detailed depiction of the iron-ligand structure, co-ordination, and electron shuttling capabilities of the complex. Here cysteine represents an organic molecule with functionalities (O-, S-, N-functional groups) and a C backbone that may mimic the functional groups present in organic matter from terrestrial and aquatic environments.

3.1 INTRODUCTION

Amino acids, and in particular, cysteine [HO₂CCH(NH₂)CH₂SH], (Fig 1) contain a variety of functional groups (i.e., carboxyls, thiols and amines) which collectively represent the major functional groups present in natural organic matter. Cysteine is the only naturally occurring thiol-containing amino acid, conferring unique and fundamental properties to the structure and activity of biomolecules. It actively participates in electron transfer reactions taking place in heme proteins, ferredoxins, and rubredoxins. The functional groups in cysteine are capable of interacting strongly with Fe(II) and/or Fe(III), thereby changing iron’s redox state and local bonding environment which ultimately determines the mobility and availability of Fe in natural and biological systems. With three potential binding groups, cysteine is, therefore, a “complex” ligand from a metal ion selectivity point of view. Iron (Fe), on the other hand, is a redox active element which can possibly bind to the three functional groups of cysteine. The valence electrons in the d-orbitals of Fe are capable of exchanging electrons with –N, -O and –S of cysteine leading to the formation of stable inner sphere complexes having Fe-O, Fe-N and Fe-S coordination environments with varying degrees of covalence.

Recent theoretical and molecular modeling studies related to cysteine utilized, conformational and optical characteristics to understand the coordination chemistry of the molecule. In studies concerning binding properties with metals, cysteine is found to mostly
undergo reduction to H\textsubscript{2}S, CH\textsubscript{3}SH or CH\textsubscript{3}CH\textsubscript{2}SH. Both Fe\textsuperscript{2+} and Fe\textsuperscript{3+} bind with cysteine through bidentate chelation via sulfur and nitrogen atoms, forming distorted octahedral clusters, as was proved by spectroscopic and magnetic measurements. Solvated complexes of Fe(II) cysteine, however show tridentate binding via all three O, N and S atoms. Temperature also affects Fe coordination with cysteine; for example, inert Fe(III)-cysteine complexes at low temperature (-78°C) indicate a S, N-coordinated tris complex, as compared to the more labile Fe(III)-cysteine complex at ambient temperatures having S, O coordination sites. Note however that these studies using mostly optical and UV-visible spectroscopic methods assumed that the oxidation state of Fe is preserved throughout the experiments. Since cysteinyI residues are prominent ligands in iron transfer proteins in systems of biological relevance, interactions between cysteine and dissolved Fe(II) and Fe(III) have also been addressed in numerous studies. Some of the studies indicate that pH is the master variable which controls the redox reaction pathway between aqueous Fe(III) and cysteine leading to the different configurations of Fe(II, III)-cysteine in solution. Cysteine has also been reported to abiotically reduce Fe(III) oxides. However, most studies to-date have focused on interpreting the kinetics associated with these reactions, without exploring the details of the binding mechanism and how the oxidation state and coordination environment of not only Fe but also that of the different elements (C, N, O and S) in cysteine change as a function of time. A gap in knowledge therefore exists regarding the electron exchange mechanism between the metal (Fe) center and the different atoms (C, N, O and S) in cysteine; these atoms have different electronegativities and capabilities to donate/accept electrons from Fe(III)/Fe(II), respectively, that might result in varying metal-ligand bond valence character.

In this investigation we develop a molecular level understanding of the electron exchange mechanism taking place between Fe and cysteine and monitor how the oxidation state of Fe as well as that of the light elements in cysteine change with passage of time (0 and 12 months). Cysteine represents an organic molecule with functionalities (O-, S-, N- functional groups) and a
C backbone that may mimic the functional groups present in organic matter from terrestrial and aquatic environments. We hypothesize that cysteine, with three potential metal binding groups, acts as a true redox ligand, capable of oxidizing or reducing Fe with its own elements (C, N, O, S) getting reduced or oxidized simultaneously. Specifically, we hypothesize: a) reduction of Fe(III) in Fe(III)-cysteine is caused by an internal electron transfer reaction either from the N of the amine group or from S of the thiol group, and b) oxidation of Fe(II) and S in Fe(II)-cysteine with passage of time is accompanied by a simultaneous reduction either of the carbon backbone (corresponding to the “propyl” alkyl group) or electron exchange within the unsaturated carboxyl group of cysteine.

To test the hypotheses, we employed Fe L$_{3,2}$-edge X-Ray Absorption Near Edge Structure (XANES) spectroscopy, together with C, N, O and S K-edge XANES studies to monitor the change in oxidation state of Fe and C, N, O and S simultaneously.\textsuperscript{28-30} Iron (Fe) K-edge EXAFS (Extended X-Ray Absorption Fine Structure) experiments were also performed, yielding information about the local coordination environment (coordination number, identity of ligand atom, and distance between Fe and ligand atom) of Fe in complexes with cysteine. Vital to our understanding and interpretation of Fe redox reactions with cysteine was the modeling of the Fe L$_{3,2}$-edge XANES experimental data using the program CTM4XAS (Charge Transfer Multiplet for X-ray Absorption).\textsuperscript{31} CTM4XAS uses a computational approach which employs charge transfer multiplet calculations to obtain a quantitative estimation of the proportions of Fe(II) and Fe(III) present at the different time scales (0 and 12 months) and thus provides a more comprehensive analysis of the metal-ligand bond characters.
3.2 EXPERIMENTAL METHODS

3.2.1. Preparation of Fe(II) and Fe(III)-cysteine complexes.

All solutions were prepared inside an anaerobic glove box to preserve their oxidation state. A cysteine solution was prepared by dissolving cysteine (99% purity, Avocado Research Chemicals) in de-gassed distilled-deionized water (Milli-Q water, 18.2 Ω) and the initial pH of 5.0 was brought to 7.0 by addition of a 0.01N NaOH solution. The Fe(II)- and Fe(III)- complexes with cysteine were formed by addition of Fe(II)-sulfate and Fe(III)-nitrate salts, respectively, to cysteine solutions at a 1:5 Fe:cysteine molar ratio while the pH was maintained at 7. The Fe(II,III)-cysteine solutions were then freeze-dried and kept in sealed vials inside the anaerobic glove box until spectroscopic analyses were performed.

3.2.2. Fe L$_{3,2}$-edge and C, N, O, and S K-edge XANES (X-ray Absorption Near Edge Structure) spectroscopy

Iron L-edge (706.8 eV) and O (543.1 eV), C (284.1eV ), and N (403.9 eV) K-edge XANES experiments were performed at Beamline U4B of the National Synchrotron Light Source (NSLS), Brookhaven National Laboratory under ring operating conditions of 700 mA and 0.808 GeV and under a ultrahigh vacuum (UHV) environment of ~10$^{-7}$ Torr. The Fe(II,III) cysteine-complexes were finely ground and spread across double-sided adhesive conductive graphite tape attached to a copper paddle. For the C K-edge spectra, the graphite tape was removed; instead, a Cu tape was used. The paddle was affixed to a rotary stage and inserted into the experimental chamber aligned at 45° to the incident defocused beam, thus generating isotropic spectra. Spectra at the Fe L-edge were collected over the energy range 680-738 eV with 0.5 eV steps in the pre-edge (680-695 eV) and post-edge (725-738 eV) regions, and with a step size of 0.1 eV between
695-725 eV. A low energy grating (600 lines/mm) monochromator was used to collect the spectra and entrance and exit slits were set to 50/50. The total electron yield (TEY) signal was recorded at room temperature. The Fe L-edge XANES spectral signal was normalized to the incident photon flux ($I_0$), measured simultaneously. The photon energy was calibrated using the spectrum of Fe$_2$O$_3$ as an internal reference, which was acquired simultaneously with each scan of every sample. The second peak of the Fe$_2$O$_3$ spectrum is assigned a value of 706.8 eV and is used for Fe energy calibration. Five scans were collected per sample and averaged. Sample decay was not observed as evidenced by identical spectra initially and at the end of data collection for each sample. Data reduction of the spectra includes baseline removal, energy calibration and normalization. The spectra were processed using the Athena software. The energies for calibration of the reference spectra for C, O and N were 284.1 eV, 543.1 eV and 403.9 eV respectively. The sulfur K-edge (2472 eV) XANES spectra were collected at Beamline X19A of the National Synchrotron Light Source (NSLS), Brookhaven National Laboratory, under standard operating conditions. The monochromator used in these experiments consisted of two parallel Si $\{111\}$ crystals with an entrance slit of 0.5 mm. Each sample was pressed into a 0.5-mm thick acrylic holder with a 2.5 mm thick Mylar film (Chemplex Industries, NY) window. The spectra were recorded in fluorescence mode using a Stern-Heald ionization detector filled with He gas and positioned 90° to the incident beam. The monochromator was detuned 70% at the S K-edge in order to reduce fluorescence induced by high-order harmonics. The elemental S K-edge spectrum (assigned a value of 2472 eV) was used for energy calibration. Scans ranged from 20 eV below to 50 eV above the S absorption edge with 0.2 eV step size. Five scans were collected for each sample to monitor spectrum reproducibility. The spectra were processed using the Athena software.
3.2.3 Fe K-edge EXAFS (Extended X-Ray Absorption Fine Structure) spectroscopy.

The iron K-edge (7112 eV) EXAFS spectra were collected at Beamline X10C at the National Synchrotron Light Source (NSLS), Brookhaven National Laboratory (Upton, NY) under standard operating conditions (2.584 GeV and 100-250 mA). The monochromator used in these experiments consisted of two parallel Si(220) crystals with an entrance slit of 0.5mm. Each sample was pressed into a 0.5-mm-thick acrylic holder with a Mylar film (2.5 μm thick, Chemplex Industries, NY) window. The spectra were recorded in fluorescence mode using a Stern-Heald ionization detector filled with N gas and positioned 90° to the incident beam. The monochromator was detuned 70% at the Fe K-edge in order to reduce fluorescence induced by high-order harmonics. The elemental Fe K-edge spectrum (assigned a value of 7112 eV) was used for energy calibration. Scans ranged from 100 eV below to 500 eV above the Fe absorption edge with 0.2 eV step size. The EXAFS spectra were processed by taking the average of three scans, background removal to isolate the fine-structure scattering curve, and Fourier transformation of the scattering curve to yield a radial structure function (RSF). Background removal involves fitting the pre- and post-edge background and normalization of the edge step. The EXAFS scattering curve was weighted by $k^3$ ($k$ is the electron wave vector) during background removal and prior to Fourier transformation in order to compensate for the diminishing amplitude at high k owing to the decay of the photoelectron wave.

For quantitative analysis, the $k^3$-weighted EXAFS spectra (k space ranges from 2 to 12 Å$^{-1}$ in the Kaiser-Bessel window function) are Fourier transformed using an unsmoothed window to yield a RSF. Peaks in the RSF are individually transformed back into k-space (Fourier-filtered) to isolate the contribution of each to the EXAFS spectrum. Through a curve-fitting process the RSF and Fourier-filtered scattering curves are compared to computed spectra derived from model inorganic crystal compounds to interpret the structure of the binding sites for
Fe in the first coordination shell of the Fe-cysteine complexes. The positions of the peaks in the RSF represent the distances between the absorber and successive shells of neighboring atoms, corrected for phase shift. The amplitudes of the peaks depend on the number and identities of the backscattering atoms. Data reduction and analyses of Fe K-edge EXAFS were performed using the computer program Athena 0.8.041. 32

The EXAFS scattering curves were fitted using FEFF included within the Artemis program for the atomic model to determine the number and identity of atoms bonded to Fe in the first coordination shell of the Fe(II,III)-cysteine complexes. Iron K-edge EXAFS data were processed using the IFEFFIT package. 32,33 Interatomic distances of Fe from representative O, N and S atoms were calculated from crystallographic data of ferrihydrite and pyrite (structures obtained as .cif files from the American Mineralogist Crystal Structure Database) using the ATOMS program within the IFEFFIT package. No inorganic compound containing Fe-N bond was found in the database. Since Fe-O and Fe-N bond distances are similar in the RSF plots, it was decided that if the fits picked up Fe-O bond then it could mean that a Fe-N bond was also present in the complexes. The initial estimate of the Debye-Waller factor (an indication of the thermal and static disorders in the bond lengths) for every atomic shell was 0.004 Å². Backscattering paths and phase corrections were calculated by the FEFF6 program. EXAFS data were Fourier transformed across a $k$ range of $2.0 – 12.0$ Å$^{-1}$ for an $R$-space fitting between 0 and 5.0 Å, which was conducted in the Artemis program. When fitting the data in Artemis, $k = 1$, $k = 2$, and $k = 3$ were chosen to optimize the fits over all three weighting factors. 34 When constructing the model, all paths with a computed amplitude of <20% were discarded because these paths were not needed to fit spectral features across the chosen $k$-space and $R$-space fitting ranges. To simplify the model, paths that are composed of the same atoms and whose difference of $R_{eff}$ (effective bond length from FEFF 6 calculation based on the crystal structure) is within 0.1 Å are grouped together. Typically, for each group of paths, the path whose $R_{eff}$ is the closest to the $R_{eff}$
average is included in the structure model to represent other paths. If all paths in that group have the same difference to the $R_{\text{eff}}$ average, then the path with the higher amplitude is chosen. For each chosen path, the parameter coordination number (CN) is fixed as the combination of the degeneracy of all paths in that group, except for the Fe-O paths in the first coordination shell, due to its most dominant contribution to the overall EXAFS spectrum. An amplitude reduction factor(s) of 0.86 was kept fixed for the fits.

3.2.4 Multiplet Calculations of Fe L$_{3,2}$ –edge XANES spectra.

Quantitative information about bonding, back-bonding, charge-transfer and spin character was obtained by modeling the Fe L-edge XANES spectra using charge-transfer multiplet theory, which has proven its validity in interpreting transition metal L-edge in systems such as organic molecules, condensed matter and proteins. The multiplet program CTM4XAS (Charge Transfer Multiplet for X-Ray Absorption Spectroscopy) compares the Fe L-edge XANES experimental results with multiplet simulations, thereby providing detailed information about the preferred Fe configurations when it binds to cysteine. CTM4XAS is a semi-empirical program that utilizes explicitly the important interactions for the calculation of L edge spectra. These include (1) the core and valence spin-orbit coupling, (2) the core-valence overlap (multiplet effects), and (3) the effects of strong correlations within the charge transfer model. The experimental L-edge spectra will be modeled with the charge transfer multiplet (CTM) approach. The CTM approach is based on the atomic theory, with the addition of crystal field interactions. The CTM many-body Hamiltonian includes the on-site correlation energy of the Fe 3d level, the ligand-to-metal charge transfer energy, the metal-to-ligand charge transfer energy, the 3d3d electron-electron interaction within the 3d shell and the 2p3d multiplet interaction between the 2p and 3d shells, as well as the spin-orbit coupling in each subshell. The CTM4XAS program,
developed by Frank de Groot and Eli Stavitski, was used to calculate the Fe L-edge of Fe(II,III) cysteine complexes.

3.3 RESULTS and DISCUSSION

3.3.1. Oxidation States of Fe, N, C, O and S: X-Ray Absorption Near-Edge Structure Spectroscopy

All XANES spectra for the Fe(II)-cysteine and Fe(III)-cysteine complexes are presented in Figures 2 to 6. The K-edge spectra for cysteine at all absorption edges (C, N, O, S) show identical features to the ones presented by Zubavichus et al. (2005).30

3.3.1.1. Oxidation state of Fe.

Figure 3-2 presents the Fe L_{3,2}-edge spectra for the Fe(II)-cysteine (Fig 3-2a) and Fe(III)-cysteine (Fig 3-2b) complexes at t= 0 and t=12 months, together with the Fe L_{3,2}-edge spectra of the Fe salts used in their synthesis. Fe^{2+} salt has its characteristic high intensity L_{1}-peak at ~705.8 eV and that for the Fe^{3+} salt occurs at ~707.8 eV; reproducibility and accuracy of spectral features indicate vacuum conditions and the beam did not damage the sample. When we compare these spectral fingerprints of Fe^{2+} and Fe^{3+} to the Fe(II,III)-cysteine spectra, it is observed that Fe^{2+} in Fe(II)-cysteine stayed in its reduced form after complexation with cysteine, but after 12 months it got oxidized (Figure 3-2a). On the contrary, Fe^{3+} in Fe(III)-cysteine was reduced immediately after complex formation and remained mostly in its reduced state even after 12 months (Figure 3-2b). Since every oxidation reaction is accompanied by a reduction reaction (and vice-versa) the oxidation of Fe that took place with time in Fe(II)-cysteine must be accompanied by reduction of
any of the elements (C, N, O and S) in cysteine; conversely, the Fe reduction which occurred in Fe(III)-cysteine must be accompanied by a corresponding oxidation reaction. The following spectroscopic results show the changes in speciation which took place simultaneously at the N, C, O and S absorption edges with time in the Fe(II,III) cysteine complexes.

3.3.1.2. Nitrogen K-edge XANES spectra.

The N K-edge of cysteine, where N is in -NH$_2$ form, is dominated by a relatively broad $\sigma^*$ (N-C) peak at $\sim$407 eV (Figure 3-3). When compared to the N K-edge of Fe(II) cysteine, it is observed that the peak for Fe(II) cysteine at this energy position was retained, but it is narrower (Figure 2a). In addition, the spectra at t=0 shows evidence of electron density shift with the emergence of a broad resonance at $\sim$415 eV, indicative of partial N oxidation (Figure 3-3a); this broad resonance is not present at t=12 mo. The N K-edge for Fe(III) cysteine (Figure 3-3b) showed clear spectral features of oxidation. However, we cannot interpret the results since Fe(III)-nitrate was the salt from which Fe(III)-cysteine was synthesized, and even though we used a 1:5 Fe:cysteine ratio the absorptivity of nitrate is greater than that of $-\text{NH}_2$. 39

3.3.1.3. Oxygen K-edge XANES spectra.

In Figure 4 we show the O K-edge XANES for Fe(II)-cysteine (Figure 3-4a) and that of Fe(III) cysteine (Figure 3-4b) at t=0 and t=12 months together with the O K-edge of pure cysteine, where O is in -COOH form. The O K-edge spectrum of cysteine exhibit a dominant $\pi^*$ peak associated with the O of the carboxylate group at $\sim$543 eV and the corresponding broader $\sigma^*$ component at 545-555 eV. The O K-edge spectrum for Fe(II) cysteine at t=0 shows a suppression of the $\pi^*$ peak at 543 eV; instead a sharp peak at $\sim$546 eV occurs indicating a reduction of the
carboxylic O that persisted after t=12 months. The O K-edge in Fe(III)-cysteine did not change with time and resembled the O spectrum of cysteine. Therefore, Fe oxidation in Fe(II) cysteine, which took place from t=0 to t=12 mo, might be accompanied by a simultaneous reduction of O within the carboxyl group.

3.3.1.4. Carbon K-edge XANES spectra.

The C K-edge of cysteine (where C is in -COOH form and in the aliphatic C backbone) is dominated by two primary features, a narrow sharp resonance at ~285 eV attributable to the π*(COO) transition and broader σ* resonances at 286-302 eV. The C K-edge features of Fe(II) cysteine at t=0 (Fig 3-5a) are identical to that of C in cysteine, where the broad σ* resonances are approximately half (1/2) the height of the π* resonance. However, at t=12 months (Fig 3-5b), the sharp feature at ~285 eV appears to be shortened, but this is likely because the broad σ* resonances are now approximately two thirds (2/3) of the height of the sharp π* resonance. These changes in the spectra (t=12 months) suggest that C has suffered a reduction, most likely in the C-backbone (propyl group) of cysteine. Changes in the C K-edge of Fe(III) cysteine at t=0 and t=12 months resemble those observed for the Fe(II) cysteine complex at t=12 months and thus suggest reduction of C occurred initially.

3.3.1.5. Sulfur K-edge XANES spectra.

The S K-edge spectrum of cysteine shows its typical spectral features with the most prominent resonance arising from reduced S (thiol, -SH) occurring at ~2472 eV (Figure 3-6). The S K-edge of Fe(III)-cysteine (Fig 3-6b) shows resonances at higher energy (~2474 eV) initially (t=0) indicating that a portion of the S was oxidized (Figure 3-6b). Furthermore, at t=12 months,
it is clear S exists in reduced (resonances at 2470-2475 eV) as well as in oxidized (resonances at 2480-2484 eV; 5+ and 6+ oxidation) states. The S K-edge spectra of Fe(II) cysteine (Fig 3-6a) is compromised because Fe(II) sulfate was used to synthesize the complex. The spectrum of sulfate is shown in Figure 3-6a, together with the spectra for cysteine and the Fe(II) cysteine complex. The S K-edge spectral features are identical at t=0 and t=12 months with resonances (~2474 eV) indicative of partial S oxidation. We cannot assess, however, whether S oxidation to 5+ and 6+ oxidation states (2480-2484 eV) occurred in the Fe(II) cysteine complex.

3.3.2. Modeling of Fe L_{3,2}-edge XANES spectra by CTM4XAS calculations.

Modeling of the Fe L-edge XANES spectra by theoretical CTM4XAS calculations gives a quantitative estimate of the Fe redox species along with their coordination environments (Figure 3-7 and Table 3-1). CTM4XAS calculations indicate that Fe^{2+} and Fe^{3+} are both present in Fe(II)- and Fe(III)-cysteine at t=0 and t=12 months (Table 3-1). Specifically, the modeling results show that 80% of the total Fe in the Fe(II)-cysteine complex is present as Fe^{2+} initially (t=0) and that 40% remains reduced after 12 months. The fraction of Fe^{2+} present initially in the Fe(III)-cysteine complex is also 80% (note this is newly reduced Fe) but in this case a larger proportion, 66%, remains reduced after 12 months. Note also that with the exception of Fe(II) cysteine at 12 months, the Fe L-edge spectra is modeled using two different components (i.e., populations) of reduced Fe (Figure 3-7 and Table 3-1).

Crystal field strengths (given by 10Dq values) and spin-orbit (SO) coupling are two parameters which give an indication of the geometry and the nature of the ligand atoms which bind and presumably stabilize Fe^{2+} and Fe^{3+} in these Fe(II,III)-cysteine complexes. The crystal field strength parameter gives an estimation of the separation of peaks in the L_{3} region which arises due to the energy separation between the lower energy t_{2g} and high energy e_{g} molecular
orbitals when a metal atom is placed in a ligand field. The 10Dq value is thus a measure of the ionic character of the metal-ligand bond. A high 10Dq value suggests an ionic bond (Fe-O, for example) whereas a low 10Dq value a covalent one (Fe-N or Fe-S, for example). The 10Dq value also gives an indication of the coordination geometry in which the metal is present. A low 10Dq value indicates the species to be possibly in tetrahedral coordination whereas a high 10 Dq value suggests an octahedral coordination. However, more definite information as to whether Fe is present in tetrahedral or octahedral coordination is provided by the Fe K-edge EXAFS results (see next section). Thus, variations in 10Dq values reveal differences in the strength of binding of Fe to the donor atoms of the respective functional groups. Ferric (Fe$^{3+}$) iron in all the Fe-cysteine complexes showed 10Dq values of 1.4, indicating an octahedral geometry with greater difference in electronegativity between the metal and the O (from carboxyl) and N (from amine) functional groups. Ferrous (Fe$^{2+}$) iron in these complexes was, in contrast, found to exhibit much lower 10Dq values (0.6) suggesting that Fe$^{2+}$ was in tetrahedral coordination with S or N, having a lower electronegativity.

The SO parameter, on the other hand, takes into consideration the interactions between the 2p and 3d electrons and the shape of the L$_2$ region (~720 eV) is particularly very sensitive to the values of SO being used for the calculations. For example, a SO value of 0.95 being used for Fe$^{2+}$ species for the calculations causes no splitting of the peaks in the L$_2$ region, whereas a value of 1 causes splitting of the L$_2$ region into two peaks. The splitting is more pronounced for Fe$^{3+}$ species with SO 1, indicating that the redox state, determined by the number of valence electrons is also instrumental in determining the spectral shape. The spectral nature obtained from the SO values indicate that Fe$^{2+}$ and Fe$^{3+}$ (d$^6$ and d$^5$ electron systems, respectively) in these complexes are present in their high spin states since these states are the ones which have unpaired electrons in their valence d-orbitals and hence can readily participate in electron exchange reactions. The two different SO values (0.95 and1.0) chosen to model Fe$^{2+}$ indicates that this parameter is
sensitive to the number of valence electrons and thus influences the stabilization of Fe\(^{2+}\). Fe\(^{3+}\) with six d-electrons is likely present in high spin state with 4 electrons in the low energy \(t_{2g}\) orbital and 2 electrons in the higher energy \(e_g\) orbitals electrons thus making it susceptible to undergo redox reactions readily. However, SO value of 1.0 was consistently used to model Fe\(^{3+}\) in the complexes.

The considerably higher Fe L\(_{3,2}\)-edge peak intensity for the oxidized Fe\(^{3+}\) modeling component within the Fe(II) cysteine complex at \(t=12\) months (compared to all other three complexes) indicates a much higher Fe-ligand covalence in this particular complex due to the shuffling of electrons from S and into the C, N and O atoms of cysteine. This high intensity feature is absent in the Fe(III)-cysteine at \(t=12\) months where O was oxidized indicating the presence of a more ionic Fe-O bond.

### 3.3.4. Local coordination environment: Fe K-edge EXAFS of Fe(II,III)-cysteine complexes.

The Fourier transforms for first coordination shell in k-space for the Fe(II,III)-cysteine complexes at \(t=0\) and \(t=12\) months, and the fitting results are presented in Figure 3-8 and Table 3-2. As with the Fe L\(_{3,2}\)-edge XANES spectra, the results from the Fe K-edge EXAFS spectra represent average values for all of the Fe present in each system. Iron in both the Fe(II) cysteine and Fe(III) cysteine complexes at \(t=0\) (each with 80% Fe\(^{2+}\) and 20% Fe\(^{3+}\)) show the first coordination shell is composed of S atoms at a distance of 2.3Å. The coordination numbers for Fe for these two complexes are 4.0 and 3.64 (respectively) suggesting that Fe (mostly Fe\(^{2+}\)) tends to be in tetrahedral coordination with S atoms of -SH in these complexes. At \(t=12\) months, we see that the first coordination shell of Fe (60% Fe\(^{3+}\)) in Fe(II)-cysteine is composed of O/N atoms with a coordination number of 6 at a distance of 2.05Å. Since EXAFS cannot distinguish between O and N atoms, it can be said that Fe (i.e. Fe\(^{3+}\)) prefers to be in octahedral coordination with
either the O of the -COOH or the N of the -NH₂ group of cysteine. Iron in Fe(III)-cysteine at t=12 months (with ~66% Fe²⁺ and ~33% Fe³⁺) was, however, found in an environment with a coordination number of 4.5 at a distance of 2.15Å; thus suggesting reduced Fe is in tetrahedral coordination, in agreement with the low 10Dq values of Fe²⁺ used to model the Fe L-edge XANES for this complex (Tables 3-1 and 3-2). However, the Fe-ligand bond length is shorter than Fe-S and longer that Fe-O/N, hence it is likely that electron shuttling takes place within the molecule and EXAFS might reflect a combined local environment with Fe-S and Fe-O/N contributions. Therefore, from the EXAFS data, we can conclude that Fe(II)- and Fe(III)-cysteine at t=0 had tetrahedral Fe with Fe-S bonds, Fe(II)-cysteine at t=12 months had an octahedral with Fe-O/N environment while Fe(III)-cysteine at t=12 months show a combined Fe-S/O/N environment in a tetrahedral geometry. The coordination number for octahedral Fe³⁺ is in accordance with data for Fe-O/N bonding found in Fe(III) complexes with organics such as tris(oxalato)iron (Fe(III) in octahedral coordination with three bidentate oxalate ions)⁴⁰, tris(malonato)iron (Fe(III) in octahedral coordination with three -COOH groups)⁴⁰ and Fe(III)-DFO-B (tris hydroxamate chelator of Fe(III))⁴⁴¹,⁴² and those for tetrahedral Fe²⁺ are also in accordance with Fe(II)-S data.⁴³

Thus, modeling of Fe L-edge XANES studies by CTM4XAS calculations and Fe K-edge EXAFS studies provide complementary information of Fe speciation in these complexes. Fe K-edge EXAFS studies can predict the coordination environment in which Fe is likely to be present in the complex whereas CTM4XAS is a robust modeling tool which predicts the oxidation state of the metal along with their proportions, the geometry in which they are present and also some indication of the functionalities involved in retention of Fe²⁺ in Fe(III)cysteine and Fe³⁺ in Fe(II) cysteine.
3.3.5. Electron Exchange Pathways between Fe and cysteine.

In previous sections we have detailed the changes in Fe, S, O, C and N speciation of Fe(II,III) cysteine complexes at two time scales. By analyzing the oxidation state and potential coordination environment of the complexes, we are able to provide some aspects of the reactivity of the Fe-cysteine systems. In general, Fe(II)-cysteine complexes suffered oxidation with time, and Fe(III)-cysteine complexes were found to mostly undergo reduction and stay in reduced state. Collectively, the various results of our investigation are consistent with the following mechanistic explanations.

**Fe(II)-cysteine (Fig 3-9).** Ferrous (Fe$^{2+}$) iron remained in its reduced form and a simultaneous oxidation of S took place indicating a possible electron donation from the S of –SH. The coordination environment consisting of tetrahedral Fe$^{2+}$ with 4 S atoms further confirms this electron exchange having a weak Fe-S σ character. CTM4XAS calculations, however, indicate that a partial oxidation of Fe took place, indicating that there may be further electron donation from Fe$^{2+}$ to N of –NH$_2$ and/or O of -COOH group, where N was slightly oxidized and O underwent reduction. Hence, we can conclude that the carboxylate group which stays in resonance form with electron delocalization between C and O (-COO$^-$), is responsible for the oxidation. After 12 months, oxidation of Fe(II) takes place with a simultaneous reduction of C and O and a partial electron shift in N of –NH$_2$. The octahedral Fe-O or Fe-N coordination environment suggests that with time, electron donation takes place from –S of -SH to Fe$^{3+}$, which then gives off the available electrons to the C and N of cysteine which undergoes reduction. Also, retention of Fe$^{2+}$ as shown by CTM4XAS calculations indicate that there may be some internal electron transfer from S of –SH to Fe$^{3+}$ causing a simultaneous oxidation of S.

**Fe(III)-cysteine (Fig 3-10).** An initial reduction of Fe(III) takes place with a simultaneous oxidation of S (and perhaps N), indicating possible electron donation from S of –SH
(and N of –NH₂). EXAFS studies indicate a tetrahedral Fe-S coordination environment. Retention of a part of Fe³⁺ (CTM4XAS calculations) indicates a partial electron transfer pathway from the C backbone (-CH₂-CH-) of cysteine where a reduction of C occurs. About 66% of Fe was still found in its reduced form after 12 months when more of the S (and perhaps N) was in greater oxidation states, thus indicating electron donation from S of –SH (and perhaps from N of –NH₂).

However, tetrahedral Fe-S coordination environment, as revealed by EXAFS, indicates that Fe²⁺ was actually stabilized by electron donation to -S of –SH since N and O-oxidation states did not change. Overall, in these complexes, it is seen that the Hard and Soft Acids and Bases (HSAB) principle holds true where Fe³⁺, a hard acid, has a tendency to bind with the hard base donor atoms like –N (of –NH₂) or –O (of –COOH) whereas Fe²⁺, a soft acid, gets stabilized by bonding to the soft base –S (of –SH). These results suggest the formation of inner sphere complexes of Fe with specific ligand atoms of cysteine. Notably, a -S-S- bond was not observed in the spectroscopic data, indicating that the dimer formation of cysteine did not occur. We can, therefore, conclude that Fe²⁺ and Fe³⁺ in the Fe(II,III)-cysteine complexes get stabilized by internal electron transfer between Fe and the –SH, -NH₂ and -COOH functional groups of cysteine. In addition, the propyl group which constitutes the C-backbone in cysteine also actively participates in the electron exchange reactions with Fe. This investigation highlights the importance of cysteine as a redox active ligand and establishes the electron shuttling capabilities of the Fe-cysteine complex. It also serves as an example of how metal-organic complexation and subsequent stabilization could take place in aquatic and terrestrial ecosystems where such complexes are abundant.
References.


9) Birch, L. and Bachofen, R. Complexing agents from microorganisms. Experientia. 1990, 46, Birkh/iuser Verlag, CH-4010 Basel/Switzerland.


Tables

Table 3-1 Modeling parameters chosen for CTM4XAS calculations

<table>
<thead>
<tr>
<th>Sample</th>
<th>Tetrahedral/Octahedral Fe$^{2+}$</th>
<th>Octahedral Fe$^{3+}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>10 Dq value*</td>
</tr>
<tr>
<td>Fe(II) cysteine, t=0</td>
<td>29.5</td>
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</tr>
<tr>
<td></td>
<td>50.6</td>
<td>0.6</td>
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<tr>
<td>Fe(II) cysteine, t=12 m</td>
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</tr>
<tr>
<td>Fe(III) cysteine, t=0</td>
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<td>0.6</td>
</tr>
<tr>
<td></td>
<td>37.0</td>
<td>0.6</td>
</tr>
<tr>
<td>Fe(III) cysteine, t=12 m</td>
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<td>0.6</td>
</tr>
<tr>
<td></td>
<td>33.33</td>
<td>0.6</td>
</tr>
</tbody>
</table>

*10Dq values indicate crystal field strengths for each Fe configuration

$^\dagger$SO coupling indicates spin-orbit interaction parameters used for the simulation

Table 3-2 Least Square Fits to EXAFS Data in chi-Space for Fe in the Fe-cysteine complexes

<table>
<thead>
<tr>
<th>Sample</th>
<th>Interaction</th>
<th>CN</th>
<th>R(Å)</th>
<th>σ$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe(II)-cysteine; t=0</td>
<td>Fe-S</td>
<td>4.05</td>
<td>2.31</td>
<td>0.0090</td>
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<tr>
<td>Fe(II)-cysteine; t=12 m</td>
<td>Fe-O/N</td>
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<td>2.05</td>
<td>0.0958</td>
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<tr>
<td>Fe(III)-cysteine; t=0</td>
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<td>3.64</td>
<td>2.30</td>
<td>0.0030</td>
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<tr>
<td>Fe(III)-cysteine; t=12 m</td>
<td>Fe-O/N/S</td>
<td>4.5</td>
<td>2.15</td>
<td>0.0030</td>
</tr>
</tbody>
</table>

Coordination number (CN), bond distance (R), Debye-Waller factor (σ$^2$)
Figures

Figure 3-1: Molecular Structure of Cysteine

Figure 3-2 Fe L$_{3,2}$-edge XANES. Fig 2a shows Fe L-edge XANES of Fe(II) cysteine and Fig 2b shows that for Fe(III) cysteine. Short dash indicates t=0 and dotted line indicates t=12 months. L$_3$ region occurs at ~ 704-709 eV and L$_2$ region occurs at ~718-722 eV.
**Figure 3-3** N K-edge XANES of Fe(II)-cysteine (Fig 3a) and Fe(III)-cysteine (Fig 3b) at t=0 (short dash) and t=12 months (dotted). N in cysteine and N in nitrate are also shown. N in Fe(III)-cysteine at t=0 and t=12 months is similar to the nitrate spectra and show signs of oxidation.
Figure 3-4: O K-edge XANES of Fe(II)-cysteine (Fig 4a) and Fe(III) cysteine (Fig 4b) at t=0 (short dash) and t=12 months (dotted). O in –COOH group of Fe(II)-cysteine at t=0 suffered reduction and stayed in reduced state. O in Fe(III)-cysteine did not change with time.
Figure 3-5: C K-edge XANES of Fe(II) cysteine (Fig 5a) and Fe(III)-cysteine (Fig 5b) at t=0 (short dash) and t=12 months (dotted). C in cysteine is also shown. C of the aliphatic C-backbone of cysteine undergoes initial reduction in Fe(II)-cysteine at t=0; gets further reduced at t=12m; C in Fe(III)-cysteine suffers reduction at t=0 and no change of redox state with time.
**Figure 3-6:** S K-edge XANES of Fe(II) cysteine (Fig 6a) and Fe(III) cysteine (Fig b) at t=0 (short dash) and t=12 months (dotted): S in cysteine and S in Zn-sulfate are also shown. S in Fe(II)-cysteine at t=0 and t=12 looks identical to that of S in Zn-sulfate; S in Fe(III)-cysteine undergoes partial oxidation, intermediate between sulfoxide and sulfate oxidation states.
**Figure 3-7:** Modeling of Fe L-edge XANES by CTM4XAS calculations. blue = experimental; red = calculated; black = $O_h$ Fe$^{3+}$ with 1.4 10Dq and SO1; dark green = $T_d$ Fe$^{2+}$ with 0.6 10Dq SO 0.95; brown = $T_d$ Fe$^{2+}$ with 0.6 10Dq and SO1.
Figure 3-8: Fe K-edge EXAFS: Fourier Transform (not corrected for phase shift) and first coordination shell fits in chi-space. Solid lines indicate experimental data and the dotted lines represent the fitting results. Green and red lines indicate potential Fe-S and Fe-O/N coordination environments respectively in the first coordination environment.
**Figure 3-9:** Schematic diagram of electron exchange pathways in Fe(II) cysteine at t=0 and 12 months
Figure 3-10 Schematic diagram of electron exchange pathways in Fe(III) cysteine at t=0 and 12 months
Chapter 4

BINDING OF FE(II) AND FE(III) WITH THE N-CONTAINING BASIC AMINO ACIDS HISTIDINE AND ARGinine: SPECTROSCOPIC STUDIES AND MULTIPLET CALCULATIONS

Abstract

Redox-active Fe(II) and/or Fe(III) have the potential to interact strongly with –O, -N and –S containing functional groups of amino acids through complexation reactions. In this investigation, we detailed changes in the oxidation states and local coordination environment of Fe when it binds with three model amino acids: cysteine, histidine and arginine at pH 7 using synchrotron X-ray Absorption Spectroscopy (XAS) and theoretical CTM4XAS calculations. Fe(II) retained its 2+ oxidation state and Fe(III) suffered reduction upon complexation with cysteine. The first coordination shells of both Fe(II)- and Fe(III)-cysteine were composed of 4 S atoms at a distance of 2.30Å, indicating that Fe$^{2+}$ was stabilized through internal electron transfer by forming a strong covalent bond with the –S of –SH. Fe(II,III)-arginine retained (for the most part) their respective 2+ and 3+ oxidation states. Fe in Fe(II)-histidine underwent partial oxidation whereas that in Fe(III)-histidine stayed in its oxidized form. The coordination environments of Fe(II,III)-arginine and Fe(III)-histidine were stabilized in an octahedral geometry with either the –COOH or -NH$_2$ groups at O/N bond distances ranging from 1.84-2.10Å from the central metal (Fe) atom suggesting varying degrees of the strength of Fe-O/N bond interaction. Fe in Fe(II)-histidine was present in a tetrahedral coordination with O/N atoms at 2.08Å. CTM4XAS calculations indicate the presence of different proportions of Fe$^{2+}$ and Fe$^{3+}$ in all but one of the Fe-amino acid complexes. Only Fe(III)-histidine had Fe$^{3+}$ as the sole component together with the shortest Fe-O/N bond length indicating that Fe in this complex, unlike others, resisted reduction, making us conclude that the Fe$^{3+}$, a hard acid makes a strong
covalent bond with the O/N donor atoms. The radial structure functions of Fe-EXAFS also hint at the formation of ferrihydrite-like Fe-oxide phases. Among the Fe-amino acid complexes, only Fe(II) histidine exhibited prominent features in the second coordination shell, with a Fe-C distance at 2.94Å and Fe-Fe at 3.12Å. This result proves that besides –COOH and –NH₂, the N-containing 5-membered imidazole group in histidine and the basic guanidine chain of arginine also actively participates in electron exchange with Fe. The results presented in this study act as examples to show the potential role of N-containing functional groups in Fe retention and chemical forms.

4.1 INTRODUCTION

Fe is one of the redox-active 3d transition elements which can compete with other metals and readily undergo complexation reactions with naturally occurring organic ligands ranging in size from low molecular weight (e.g., amino acids) to high molecular weight (e.g., humic and fulvic acids) organic molecules that are commonly found in the natural environment, often at significant concentrations.¹⁻³ These organic ligands with oxygen (carboxylic and phenolic), nitrogen (amine, pyridines, phenanthroline) and sulfur (thiol) functional groups can interact strongly with Fe(II) and/or Fe(III) and change iron’s redox state and local bonding environment which ultimately determines Fe mobility and biological availability in natural environments. Some of the OM functional groups may stabilize the Fe(III) in reduced conditions while some others may presumably stabilize the Fe(II) under oxidized environments.⁴⁻¹⁰ The potential exists for the formation of both inner sphere and outer sphere Fe(II, III)-organic complexes containing Fe-O, Fe-N, and Fe-S coordination environments that provide low to high affinity sites. Amino acids are particularly important since they form the basic structural unit of proteins and hence have their biological relevance. However, the specific details of the coordination chemistry and
mechanisms of Fe involved in these complex formations remain unclear. Hence, understanding interactions between Fe and amino acids is a key feature in the comprehension of many biochemical and environmental problems, from naturally occurring metalloprotein chelation to metal-organic matter transport and consequent stabilization in terrestrial and aquatic ecosystems. The most relevant amino acids able to bind metal cations are the side chains of histidine, cysteine, aspartic acid, and glutamic acid. For our studies, we have selected three unique amino acids: cysteine, histidine and arginine (Fig 4-1) to study the binding mode and coordination environment of Fe with these ligands. Cysteine is the only naturally occurring thiol-containing amino acid, giving its unique and fundamental properties in structure and activity of biomolecules. Theoretical and modeling studies related to the conformational properties of Fe-cysteine complexes suggest that Fe has the potential to bind to –O, -N or –S atoms of cysteine via bidentate or tridentate chelation. Histidine, on the other hand has an imidazole functional group with π-electrons which can interact differently with Fe$^{2+}$/Fe$^{3+}$. The distal pocket histidine residue in horse heart myoglobin reportedly directs the O-binding mode of nitrite to the heme Fe$^{3+}$ by binding through the N of nitrite and has a trans configuration. Pulse radiolysis studies for Fe(II)-histidine complexes show that the reactive Fe$^{2+}$-imidazole center undergoes oxidation through one electron intramolecular transfer reactions. Arginine has a strongly basic guanidine side chain which is extremely sensitive to the surrounding environment and renders the proton transfer from the carboxyl group much more facile under the influence of solvent, stabilizing the zwitterionic form of arginine. Fe(III)-Schiff’s base complex of arginine has shown Fe to be in bidentate coordination with N and O. Fe(II)-arginine complexes too, have shown a preference to bind with N or O. Since, N-containing functional groups are the second most abundant functional groups present in soils (O-containing being the most abundant), sediments and waters, it is important to know the mechanism of interaction of metals (Fe, in this case) as it influences the overall mobility and availability of the elements in natural systems. Among the –N containing
functional groups, arginine and histidine are particularly important since both have unique side chains imparting different reactivity. Overall, the chemical bonding between Fe and the different organic functional groups depends on the donor interactions between the occupied orbitals of the ligand and the unoccupied and partially occupied orbitals of the metal, and acceptor interactions between the occupied or partly occupied orbitals of the metal and the unoccupied orbitals of the ligand.

The aforementioned studies, however, assume that the oxidation states of Fe remained unaltered throughout the experiments. In this study, we monitor the oxidation state changes which Fe undergoes while complexing with the amino acids. Our study attempts to link these changes to the presence or absence of the various functional groups by focusing on the molecular level understanding of the electron exchange mechanism between Fe and the different organic functional groups in Fe(II,III)-amino acid complexes. These functional groups would represent, in essence, the complex mixture of natural organic matter (NOM) in soils and waters. Of particular importance would be those Fe(II)-organic complexes presumed to resist oxidation, and those Fe(III)-organic complexes presumed to resist reduction. The question: Is the formation of inner sphere complexes with specific ligand atom(s) responsible for the stabilization of reduced and/or oxidized Fe forms? To address these, we have employed synchrotron X-Ray Absorption Spectroscopy (XAS) studies together with theoretical multiplet calculations to obtain molecular level information about Fe speciation. Rapid advances in XAS techniques now permit structural analysis of organic matter complexes with metal ions. In this work, we have employed both Fe L-edge XANES together with Fe K-edge EXAFS. Fe L-edge XANES monitors the change in oxidation state of Fe upon complexation whereas Fe K-edge EXAFS gives valuable information about the number and average distance of the ligand atoms around Fe. The structural information provided by EXAFS includes average interatomic distances, as well as the number and chemical identity of the atoms within a 5Å radius of the atom absorbing the x-ray photon. Key parameters
in these analyses are the identity, coordination numbers and distances of the atoms present in the first-, and most importantly in the second- coordination shell in Fe-organic complexes. To our knowledge, most of the studies in which EXAFS data for the second shell contributions in Fe-organic complexes have been reported are done with Fe(III)-NOM complexes, without any work being conducted on the redox sensitive Fe(II). Mononuclear Fe-NOM complexes and polymeric Fe(hydr)oxides have been found to stabilize Fe(III) in organic soils and peat humic acid. Herein, we have combined Fe L-edge XANES and Fe K-edge EXAFS together with the charge transfer multiplet program CTM4XAS to model the Fe L-edge experimental data. The main objectives of the study have been to give a quantitative estimate of the proportions of Fe (2+ or 3+) in each of Fe-organic complexes as a result of complexation together with their coordination environment and metal-ligand covalence characters.

4.2 EXPERIMENTAL METHODS

4.2.1 Preparation of Fe(II,III)-amino acid complexes.

All the solutions of Fe(II,III) cysteine, Fe(II,III)-histidine and Fe(II,III)-arginine were prepared inside an anaerobic glove box to preserve their oxidation states. Cysteine, histidine and arginine solutions were prepared by dissolving the respective amino acids in degassed, distilled deionized water (Milli-Q water, 18.2Ω). The cysteine, histidine and arginine solutions had initial pH values of 5.0, 7.78 and 11.0 respectively. The pH of the amino acid solutions were then brought to pH of 7.0 by the addition of 0.01N NaOH or 0.01N HCl, as required. The Fe(II)- and Fe(III)-complexes with cysteine, histidine and arginine were formed by addition of Fe(II)-sulfate and Fe(III)-nitrate salts, respectively, to the ligand solutions at a 1:5 Fe: amino acid molar ratio
while the pH was maintained at 7. The Fe(II,III)-amino acid solutions were then freeze dried and kept in sealed vials inside the anaerobic glove box until spectroscopic analyses were performed.

4.2.2. Fe L\textsubscript{3,2}-edge XANES (X-ray Absorption Near Edge Structure) Spectroscopy.

Iron L\textsubscript{3,2}-edge (706.8 eV) XANES experiments were performed at Beamline U4B of the National Synchrotron Light Source (NSLS), Brookhaven National Laboratory under ring operating conditions of 200 mA and 0.808 GeV and under a ultrahigh vacuum (UHV) environment of \(~10^{-7}\) Torr. The Fe(II,III)-amino acid complexes were finely ground and spread across Fe-free double-sided adhesive conductive graphite tape attached to a copper paddle. The paddle was affixed to a rotary stage and inserted into the experimental chamber aligned at 45° to the incident defocused beam, thus generating isotropic spectra. Spectra at the Fe L-edge were collected over the energy range 680-738 eV with 0.5 eV steps in the pre-edge (680-695-395 eV) and post-edge (725-738 eV) regions, and with a step size of 0.1 eV between 695-725 eV. A low energy grating (600 lines/mm) monochromator was used to collect the spectra and entrance and exit slits were set to 50/50. The total electron yield (TEY) signals were recorded at room temperature. The Fe L-edge XANES spectral signal was normalized to the incident photon flux (I\textsubscript{0}), measured simultaneously. The photon energy was calibrated using the spectrum of Fe\textsubscript{2}O\textsubscript{3} as an internal reference, which was acquired simultaneously with each scan of every sample. The second peak of the Fe\textsubscript{2}O\textsubscript{3} spectrum is assigned a value of 706.8 eV and is used for Fe energy calibration. Data reduction of the spectra includes baseline removal, energy calibration and normalization. Five scans were collected per sample (as needed) at room temperature and averaged. Sample decay was not observed as evidenced by identical spectra initially and at the end of each sample. The spectra were processed using the Athena software.\textsuperscript{30}
4.2.3. Fe K-edge EXAFS Data Collection and Analysis.

The iron K-edge (7112 eV) EXAFS spectra were collected at Beamline X-10C at the National Synchrotron Light Source (NSLS), Brookhaven National Laboratory (Upton, NY) under standard operating conditions (2.584 GeV and 100-250 mA). The monochromator used in these experiments consisted of two parallel Si{220} crystals with an entrance slit of 0.5mm. Each soil sample was pressed into a 0.5-mm-thick acrylic holder with a Mylar film (2.5 μm thick, Chemplex Industries, NY) window. The spectra were recorded in fluorescence mode using a Stern-Heald ionization detector filled with N gas and positioned 90° to the incident beam. The monochromator was detuned 70% at the Fe K-edge in order to reduce fluorescence induced by high-order harmonics. The elemental Fe K-edge spectrum (assigned a value of 7112 eV) was used for energy calibration. Scans ranged from 100 eV below to 500 eV above the Fe absorption edge with 0.2 eV step size. The EXAFS spectra were processed by taking the average of three scans, background removal to isolate the fine-structure scattering curve, and Fourier transformation of the scattering curve to yield a radial structure function (RSF). Background removal involves fitting the pre- and post-edge background and normalization of the edge step. The EXAFS scattering curve was weighted by \(k^3\) (\(k\) is the electron wave vector) during background removal and prior to Fourier transformation in order to compensate for the diminishing amplitude at high \(k\) owing to the decay of the photoelectron wave. For quantitative analysis, the \(k^3\)-weighted EXAFS spectra (\(k\) space ranges from 2 to 12 Å\(^{-1}\) in the Kaisser-Bessel window function) are Fourier transformed using an unsmoothed window to yield a RSF. Peaks in the RSF are individually transformed back into \(k\)-space (Fourier-filtered) to isolate the contribution of each to the EXAFS spectrum. Through a curve-fitting process the RSF and Fourier-filtered scattering curves are compared to computed spectra derived from model inorganic crystal compounds to interpret the structure of the binding sites for Fe in the first coordination shell of the Fe-cysteine complexes. The locations of the peaks
in the RSF represent the distances between the absorber and successive shells of neighboring atoms, corrected for phase shift. The amplitudes of the peaks depend on the number and identities of the backscattering atoms. Data reduction and analyses of Fe K-edge EXAFS were performed using the computer program Athena 0.8.041.\textsuperscript{30}

4.2.4 Fe EXAFS First coordination shell analysis.

The EXAFS scattering curves were simulated using FEFF included within the Artemis program for the atomic model to determine the number/identity of atoms bonded to Fe in the first coordination shell of the Fe(II)- and Fe(III)-amino acid complexes. Iron K-edge EXAFS data were processed using the IFEFFIT package.\textsuperscript{32,33} Interatomic distances of Fe from representative O, N and S atoms were calculated from crystallographic data of ferrihydrite and pyrite (structures obtained as .cif file from American Mineralogist Crystal Structure Database) using the ATOMS program inside the IFEFFIT package. No inorganic compound containing Fe-N bond was found in the database. Since Fe-O and Fe-N bond distances are similar in the RSF plots, it was decided that if the fits picked up Fe-O bond then it could mean that a Fe-N bond also present in the complexes. The initial estimate of the Debye-Waller factor (an indication of the thermal and static disorders in the bond lengths) for every atomic shell was 0.004 Å\textsuperscript{2}. Backscattering paths and phase corrections were calculated by the FEFF6 program. EXAFS data were Fourier transformed across a $q$ range of 0 – 12.0 Å\textsuperscript{-1} for an $R$-space fitting between 0 and 5.0 Å, which was conducted in the Artemis program. When fitting the data in Artemis, $k= 1$, $k = 2$, and $k = 3$ were chosen to optimize the fits over all three weighting factors.\textsuperscript{34} When constructing the model, all paths with a computed amplitude of <20% were discarded because these paths were not needed to fit spectral features across the chosen $k$-space and $R$-space fitting ranges. To simplify the model, paths that are composed of the same atoms and whose difference of $R_{eff}$ (effective bond length from FEFF 6
calculation based on the crystal structure) is within 0.1 Å are grouped together. Normally, for each group of paths, the path whose \( R_{\text{eff}} \) is the closest to the \( R_{\text{eff}} \) average is included in the structure model to represent other paths. If all paths in that group have the same difference to the \( R_{\text{eff}} \) average, then the path with a higher amplitude is chosen. For each chosen path, the parameter coordination number (CN) is fixed as the combination of the degeneracy of all paths in that group, except for the Fe-O paths in the first coordination shell, due to its most dominant contribution to the overall EXAFS spectrum. An amplitude reduction factor (s) of 0.86 was kept fixed for the fits.

4.2.5 Modeling of the Fe L\(_{3,2}\)-edge XANES Spectra

Quantitative information about bonding, back-bonding, charge-transfer and spin character was obtained by modeling the Fe L\(_{3,2}\)-edge XANES spectra using charge-transfer multiplet theory\(^3\) which has proven its validity in interpreting transition metal L-edge in systems such as molecules, condensed matter and proteins.\(^3\) The multiplet program, CTM4XAS (Charge Transfer Multiplet for X-Ray Absorption Spectroscopy) compares the Fe L-edge XANES experimental results with multiplet simulations, thereby providing a detailed information about the preferred Fe configurations when it binds to cysteine. CTM4XAS is a semi-empirical program that utilizes explicitly the important interactions for the calculation of L edge spectra. This includes (1) the core and valence spin-orbit coupling, (2) the core-valence overlap (multiplet effects), (3) the effects of strong correlations within the charge transfer model. The experimental L\(_{3,2}\)-edge spectra will be modeled with the charge transfer multiplet (CTM) approach. The CTM approach is based on the atomic theory, with the addition of crystal field interactions. The CTM many-body Hamiltonian includes the on-site correlation energy of the Fe 3d level, the ligand-metal charge transfer energy, the metal-to-ligand charge transfer energy, the 3d3d electron-
electron interaction within the 3d shell and the 2p3d multiplet interaction between the 2p and 3d shells, as well as the spin-orbit coupling in each subshell. The CTM4XAS program, developed by Frank de Groot and Eli Stavitski, is used to calculate the Fe L-edge of Fe(II,III)-complexes.

4.3 RESULTS

4.3.1 Oxidation State of Fe: Fe L-edge X-Ray Absorption Spectroscopy (XANES).

Typically, the high intensity L₃ peak for Fe³⁺ occurs at ~707.8 eV and those for Fe²⁺ occurs at ~ 705.8 eV. These two energy positions serve as the spectral fingerprints to identify Fe²⁺ or a Fe³⁺ in an unknown sample and also to monitor if Fe has undergone any change in its redox status. Fig 4-2a shows the Fe L-edge of a Fe(II)-cysteine, Fe(II)-histidine and Fe(II)-arginine complexes respectively together with that of a Fe(II) salt [Fe(II) sulfate]. Fe(II)-cysteine and Fe(II)-arginine had their characteristic high intensity L₃-peak at around 705.8 eV and Fe retained its reduced oxidation state. Fe(II)-histidine, however, underwent a partial oxidation with a broad post edge feature at around 707.5 eV. Fig 4-2b depicts the change in Fe oxidation state for Fe(III)-histidine and Fe(III)-arginine complexes together with the spectral features a Fe(III) salt [Fe(III) nitrate] would have. Fe(III)-cysteine underwent reduction (Chapter 3). Fe(III)-histidine and Fe(III)-arginine had their characteristic high intensity L₃ peaks around 707.6 eV, indicating that most of the Fe in these two complexes retained their oxidation features.
4.3.2 Modeling of Fe L\textsubscript{3,2}-edge XANES spectra by CTM4XAS calculations.

Modeling of the Fe L\textsubscript{3,2}-edge XANES spectra by CTM4XAS calculations gives a theoretically calculated quantitative estimate of the Fe redox species along with their coordination environments (Figure 4-3 and Table 4-1). Contrary to the experimental Fe L-edge XANES results, CTM4XAS calculations indicate that Fe\textsuperscript{2+} and Fe\textsuperscript{3+} are both present in all Fe(II)- and Fe(III)-organic complexes (Table 4-1), with Fe(III) histidine being the exception reported to bear a single Fe\textsuperscript{3+} species. Specifically, the modeling results show that Fe\textsuperscript{3+} is present in Fe(II)-cysteine, Fe(II)-arginine, Fe(II)-histidine and Fe(III)-cysteine (20–37.5%) where L\textsubscript{3,2}-edge XANES data had shown considerable reduced Fe characteristics. Similarly, ~14% of the total Fe got reduced in Fe(III)-arginine where primarily oxidized iron was observed from Fe L-edge XANES data. As explained in the previous chapters, crystal field strengths, given by 10Dq values and spin-orbit (SO) coupling are two parameters which give an indication of the geometry and the ionic nature of the ligand atoms which stabilizes Fe\textsuperscript{2+} and Fe\textsuperscript{3+} in these Fe-amino acid complexes (Table 4-2). The crystal field strength parameter (10Dq value) gives an estimation of the separation of peaks in the L\textsubscript{3} region which arises due to the energy separation between the lower energy t\textsubscript{2g} and high energy e\textsubscript{g} molecular orbitals when a metal atom is placed in a ligand field. The 10Dq value is thus, a measure of the % ionic character of the metal-ligand bond. A high 10Dq value suggests the formation of an ionic bond (Fe-O) between the two whereas a lower 10Dq value a covalent one (Fe-N or Fe-S). 10Dq values are also indicators of coordination numbers. A high 10Dq value indicates an octahedral coordination whereas a lower 10Dq value indicates a tetrahedral coordination. However, to say more definitively whether a metal is present in tetrahedral or octahedral coordination, we need more evidence from Fe K-edge EXAFS, presented in the next section. Fe\textsuperscript{3+} in all the Fe(III)-amino acid complexes showed 10Dq values of 1.4, indicating an octahedral geometry with greater difference in electronegativity between the
metal and the ionic O and N carboxyl and amine functional groups. Only Fe$^{3+}$ in Fe(III)-histidine had 10 Dq value of 1.2. Fe$^{2+}$ in these complexes, in contrast, exhibited much lower 10Dq values (0.6) suggesting that Fe$^{2+}$ was in tetrahedral coordination. The SO parameter, on the other hand, takes into consideration the interactions between the 2p and 3d electrons and the shape of the L$_2$ region (~720 eV) is particularly very sensitive to the values of SO being used for the calculations. For example, a SO value of 0.95 being used for Fe$^{2+}$ species for the calculations causes no splitting of the peaks in the L$_2$ region, whereas a value of 1 causes splitting of the L$_2$ region into two peaks. The splitting is more pronounced for Fe$^{3+}$ species with SO 1, indicating that the redox state, determined by the number of valence electrons is also instrumental in determining the spectral shape. The spectral nature obtained from the SO values indicate that Fe$^{2+}$ and Fe$^{3+}$ (d$^6$ and d$^5$ electron systems, respectively) in these complexes are present in their high spin states since these states are the ones which have unpaired electrons in their valence d-orbitals and hence can readily participate in electron exchange reactions.\textsuperscript{28,30}

4.3.3 Local coordination environment of Fe K-Edge EAXFS for Fe(II,III)-amino acids complexes.

**First Coordination Shell.** Table 4-2 and Fig 4-4-4-5 show the final fitting results and their corresponding Fourier transforms in chi-space for all the Fe(II,III)-amino acid complexes. The first coordination shells of Fe(II) and the reduced Fe(III)-cysteine (evidence from Fe L-edge XANES) are composed of 4 and 3.64 S atoms respectively at a distance of 2.31 Å. These four S atoms are most likely positioned in the equatorial plane of an octahedron. (as suggested by a octahedral Fe$^{2+}$/Fe$^{3+}$ metal center by CTM4XAS). The S distance in Fe(II,III)-cysteine complex is in accordance with data for inorganic Fe(II)-S containing pyrite. All the other four Fe-amino acid complexes have O/N coordination environment with varying coordination numbers and bond
distances. Fe(II)-histidine had a first shell coordination environment consisting of 4.38 O/N atoms at a distance of 2.07 Å. Fe(III)-histidine had a Fe-O/N interaction at a distance of 1.84 Å with CN of 5.4. The shorter bond length together with the CTM4XAS results which indicate that the Fe in this complex, unlike others, resisted reduction makes us conclude that the Fe$^{3+}$, a hard acid makes a strong covalent bond with the O/N donor atoms in an octahedral geometry, thereby forming an inner-sphere complex. Fe(II,III) arginine had octahedral coordination environments with O/N atoms at a distance of 1.91-2.1 Å. With EXAFS, it is not possible to distinguish between O and N atoms, thus the possible structure could have Fe bonding to either O of –COOH or N of –NH$_2$ groups.

**Second Coordination Shell.** Among the Fe-amino acid complexes, only Fe(II)-histidine exhibited prominent features in the second coordination shell (Fig 4-6). Second shell single scattering from O and Fe gives rise to a shoulder in k-space, which appears in the Fourier transforms appear as peaks between 2.4 and 2.9 Å (not corrected for phase shift). Fe-C distances occur at 2.94 Å and Fe-Fe occurs at 3.12 Å (Table 4-3). These values are in accordance with the reported values for the structure of poorly polymerized Fe-NOM complexes found at freshwater sediments at pH 5.5-7.5 for second shell Fe-C distances and Fe-Fe distances.$^{35}$

The radial structure functions (RSFs) of Fe(III)-histidine and Fe(III)-arginine, when compared to that of F(III)- mineral standards (Fig 4-7) indicate to the formation of ferrihydrite-like Fe-oxide phases in the second coordination shell with Fe-Fe distances. This might lead to the conclusion that nanoparticles of Fe-oxide were formed and stabilized by the organic complex. This is, however, a qualitative explanation and has not been quantified. Similarly, the second coordination shell of Fe(II)-histidine shows the presence of Fe-C bond, a feature seen in the Fe(III)-catechol and Fe(III)-citrate RSFs. This indicates that Fe gets bonded to –O and –C in the first and second coordination shells respectively.
Hence, Fe L-edge XANES studies, on one hand, give an estimation of the proportion of Fe present (+2 or +3), whereas the Fe K-edge EXAFS studies can predict the coordination environment in which Fe is likely to be present in the complex. Thus, together, they provide a complete picture of Fe speciation in these complexes.

4.3.4 Electron Exchange Pathways between Fe and Amino Acids

The results presented above clearly indicate that Fe gets stabilized through internal electron transfer reactions by binding to the different functional groups of the amino acids. Fig 4-8 shows the possible binding sites of Fe to -O, -N, -S in the first coordination shell and to -C in the second coordination shell of Fe-amino acid complexes. As explained in the last chapter, in Fe(II,III)-cysteine, Fe$^{2+}$ stayed in its reduced form and Fe$^{3+}$ suffered reduction accompanied by a simultaneous oxidation of S, indicating a possible electron donation from the S of –SH. The coordination environment consisting of tetrahedral Fe$^{2+}$ with 4S atoms by EXAFS further confirms this electron exchange having a weak Fe-S π character. CTM4XAS calculations, however, indicate that a partial oxidation of Fe took place, indicating that there may be further electron donation from Fe$^{2+}$ to N of –NH$_2$ and O of -COOH group, where N and O suffered reduction. Since, N showed no change in oxidation in our XANES analysis, we can conclude that the carboxylate group which stays in resonance form with electron delocalization between C and O (-COO$^-$), is responsible for the oxidation; This result agrees with Pearson’s Hard and Soft Acid Base principle where Fe$^{2+}$, a soft acid prefers to bind with S$^{2-}$ of –SH. Similarly, Fe$^{2+}$ in histidine is suggested to be stabilized in a tetrahedral coordination with the soft base of –N of –NH$_2$ or N in the imidazole side chain of histidine by forming a chelate structure with the heterocyclic 5-membered ring through intramolecular electron transfer reaction. The lone pair of electrons on N present in the side chain imidazole are extremely effective as nucleophiles and are readily
available for the formation of a Fe-N ionic bond. The second shell of Fe(II)-histidine shows scattering from O and Fe atoms forming a second Fe-O bond. This might be due to bonding of Fe$^{3+}$ with the –O of the –COOH group in the aliphatic C-backbone of histidine. Fe$^{3+}$ in Fe(III)-histidine, too, showed a similar O/N environment, but in an octahedral environment with a shorter O/N bond distance indicating that the electronegativity difference between the Fe and the donor atom was less compared to that in Fe(II)-histidine resulting in a greater stabilization of the Fe-O/N bond. Fe$^{3+}$, being a hard acid has a greater tendency to bind with the carboxylate anion (resonance stabilized carboxylic acid group), a hard base to form an inner sphere complex. Comparing the results of the coordination environments of Fe(II)- and Fe(III)-histidine, we can somewhat suggest that there exists a difference in the binding sites of Fe$^{2+}$ and Fe$^{3+}$ with histidine. A similar explanation can be given to explain the shorter bond length of Fe(III)-arginine compared to Fe(II)-arginine. However, the coordination environment of Fe was octahedral for both Fe(II)- and Fe(III)-arginine complexes. The basic guanidine side chain of arginine presumably binds with the Fe$^{2+}$ while the –COOH group stabilizes the Fe$^{3+}$. It might also be that the unsaturated guanidine has electron delocalization which stabilizes the Fe$^{3+}$ in Fe(III)-arginine. Overall, the strength of Fe-ligand interaction depends on the redox state of the metal atom and the electron accepting/donating capabilities of the ligand atom to form a stable complex through chemisorption. The results obtained from this study aid in a better understanding of the coordination chemistry of Fe-organic complexes by highlighting the factors which are responsible for stabilizing Fe$^{2+}$ and Fe$^{3+}$ in these organic complexes, thereby influencing their availability and biological uptake by the plants.
References


(17) Tomita, A., Hirai., H. and Makishima, S. Ferric Complexes with L-Cysteine at Low Temperature. Inorganic Chemistry. 1968, 7, 760-764.


Table 4-1 Modeling parameters chosen for CTM4XAS calculations

<table>
<thead>
<tr>
<th>Sample</th>
<th>Octahedral/Tetrahedral Fe(^{2+})</th>
<th></th>
<th>Octahedral Fe(^{3+})</th>
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<tr>
<td></td>
<td>%</td>
<td>10 Dq value*</td>
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</tr>
<tr>
<td></td>
<td>coupling§</td>
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<td></td>
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<td>Fe(II) cysteine</td>
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<tr>
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<td>50.6</td>
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<td>1.00</td>
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<tr>
<td></td>
<td>31.25</td>
<td>0.6</td>
<td>0.95</td>
</tr>
<tr>
<td>Fe(II) arginine</td>
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<td>38.46</td>
<td>0.6</td>
<td>1.00</td>
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<tr>
<td></td>
<td>37.0</td>
<td>0.6</td>
<td>1.00</td>
</tr>
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<td>Fe(III) histidine</td>
<td>.................. not</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>detected</td>
<td></td>
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</tr>
<tr>
<td>Fe(III) arginine</td>
<td>13.8</td>
<td>0.6</td>
<td>0.95</td>
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</table>

*10Dq values indicate crystal field strengths for each Fe configuration

§SO coupling indicates spin-orbit interaction parameters used for the simulations
Table 4-2: Least Square Fits for the First Coordination Shell of Fe-EXAFS data in chi-space for Fe in the Fe-Amino acid Complexes

<table>
<thead>
<tr>
<th>Sample</th>
<th>Interaction</th>
<th>CN</th>
<th>R(Å)</th>
<th>$\sigma^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe(II)-cysteine</td>
<td>Fe-S</td>
<td>4.05</td>
<td>2.31</td>
<td>0.0090</td>
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<tr>
<td>Fe(II)-histidine</td>
<td>Fe-O/N</td>
<td>4.38</td>
<td>2.08</td>
<td>0.0116</td>
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<tr>
<td>Fe(II)-arginine</td>
<td>Fe-O/N</td>
<td>5.50</td>
<td>2.10</td>
<td>0.0090</td>
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<tr>
<td>Fe(III)-cysteine</td>
<td>Fe-S</td>
<td>3.64</td>
<td>2.30</td>
<td>0.0030</td>
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<tr>
<td>Fe(III)-histidine</td>
<td>Fe-O/N</td>
<td>5.41</td>
<td>1.84</td>
<td>0.0097</td>
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<tr>
<td></td>
<td>Fe(II)-arginine</td>
<td>6.20</td>
<td>1.91</td>
<td>0.0100</td>
</tr>
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</table>

Coordination number (CN), bond distance (R), Debye-Waller factor ($\sigma^2$)

Table 4-3: Least Square Fits for the Second Coordination Shell of Fe-EXAFS data in chi-space for Fe in the Fe-Amino acid Complexes

<table>
<thead>
<tr>
<th>Sample</th>
<th>Interaction</th>
<th>CN</th>
<th>R(Å)</th>
<th>$\sigma^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe(II)-histidine</td>
<td>Fe-C</td>
<td>0.50</td>
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<td></td>
<td>Fe-Fe</td>
<td>0.59</td>
<td>3.12</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Coordination number (CN), bond distance (R), Debye-Waller factor ($\sigma^2$)
Figures

**Figure 4-1:** Molecular Structures of a. cysteine; b. histidine; c. arginine
Figure 4-2: Fe $L_{3,2}$-edge XANES spectra of Fe(II)-amino acids (2a): Fe in Fe(II)-histidine underwent partial oxidation and in Fe(III)-amino acids (2b), Fe in Fe(III) cysteine suffers reduction. The other four complexes do not show much change in oxidation state.
Figure 4-3: Modeling of Fe $L_{1,2}$-edge XANES by CTM4XAS calculations. blue dotted line = experimental; red solid line = calculated; black dashed line = $O_h$ Fe$^{3+}$ with 1.4 SO and 0.95SO; dark green dashed line = $T_d$ Fe$^{2+}$ with SO 0.95 and 0.6 10Dq; brown dash-dot line = $T_d$ Fe$^{2+}$ with 0.6 10Dq and SO=1.
**Figure 4-4:** Least Square Fits of first coordination shells of Fe EXAFS in chi space for Fe(II)-amino acid complexes. Solid lines indicate experimental data and dotted lines indicate fits.
Figure 4-5: Least Square Fits of first coordination shells of Fe EXAFS in chi space for Fe(III)-amino acid complexes. Solid lines indicate experimental data and dotted lines indicate fits.
Figure 4-6: Second shell Fe K-edge EXAFS fits of Fe(II)-histidine in chi-space. Solid lines indicate experimental data and dotted lines indicate fits.
Figure 4-7: Radial Structure Functions of Fe(III)-organic and mineral standards. Fe-(III)-catechol and Fe(III)-citrate have Fe-C in their second shell at 2.0 Å whereas Fe(III)-minerals have broad Fe-Fe peaks in their second coordination shells at ~3.0 Å (distances are not corrected for phase shift)
Figure 4-8: Possible binding sites of Fe to -O, -N, -S in the first coordination shell and to -C in the second coordination shell of Fe-amino acid complexes.
Chapter 5

CONCLUSIONS

The projects described here highlight the remarkable potential of redox-active functional groups in NOM which control the solid phase speciation of Fe through oxidation-reduction reactions. Ranging from laboratory-synthesized complexes to field-collected samples, there has always been an attempt to prove the overall hypothesis: “Stabilization of Fe$^{2+}$ and Fe$^{3+}$ via bonding to organic functional groups”, be it in natural peatlands or synthesized Fe(II,III)-organic complexes. In all the cases, we linked laboratory investigations to processes that occur in natural systems. We found that: (1) Fe$^{2+}$ and Fe$^{3+}$ coexist in the oxic and anoxic layers of peat soils mostly through complexation with soil organic matter or by the dissolution of Fe-minerals (Chapter 2); (2) coordination environment of Fe depends on its ability to form ionic/covalent bonds with the functionalities present (Chapter 2); (3) cysteine is one such representative amino acid where internal electron transfer reactions cause reduction of Fe$^{3+}$ and stabilization of Fe$^{2+}$ through the formation of stable inner-sphere complexes involving the active participation of the functional groups with Fe (Chapter 3); (4) The mode and strength of binding (or retention) of Fe$^{2+}$ and Fe$^{3+}$ to the functional groups of N-containing histidine and arginine differs between themselves and differs to that of cysteine. Further, since cysteine, histidine, and arginine collectively contain the most abundant functional groups (O > N > S) present in organic matter, I used the results from these model organic complexes to understand and explain what is observed in the organic-rich peat soils (Chapter 4). Overall, the results from these investigations enhanced our knowledge and understanding of Fe interactions with organic matter and help predict the redox state and coordination of Fe and, consequently, its retention and transport in soil systems.

Possible venues for future research may include linking this molecular level understanding of Fe-organic matter interactions to macro-scale processes where Fe redox cycling
could be intricately coupled to C cycle. The importance of Fe redox cycling has been overlooked in unsaturated upland soils of humid environments. Humid tropical forest soils which fluctuate between oxic and anoxic environments can serve as useful model ecosystems to study C-cycles influenced by Fe redox processes. Changes in the frequency and intensity of precipitation events due to climate change would alter the periodicity of redox fluctuations. This may lead in turn to changes in C cycling directly by altering the terminal electron acceptor of heterotrophic respiration. During more anoxic periods of time (e.g. following precipitation events) or in anoxic microsites, dissimilatory iron reducing bacteria reduce solid Fe(III) oxides to soluble Fe(II), oxidizing organic C compounds to CO$_2$. On a global scale, Fe redox characterization coupled to C-cycling could serve as a foundation for linking to permafrost thawing thereby impacting global climate change patterns.

Knowledge gained from Fe redox chemistry can also be applied to identify new approaches and strategies to help resolve questions about the Fe-catalyzed movement of toxic metals and harmful radionuclides such as uranium in subsurface zones of contaminated sites. Amorphous ferric oxyhydroxides serve as important controls on uranium mobility. Fe oxidizing-reducing microorganisms may also be directly involved in redox reactions of multivalent radionuclides such as uranium as well as secondary mineral distribution (e.g., contents of Fe oxyhydroxides) and thus directly affect radionuclide speciation. Hence, understanding the associated Fe redox chemistry is critical in understanding the mobilization, transport, and fate of harmful pollutants in the shallow surface and subsurface environments of aquatic and terrestrial ecosystems.
APPENDIX

Iron Speciation in Shale Hills Critical Zone Observatory

A1. Soil Sampling: Soil samples were collected as a function of depth along a toposequence on the southern transect of the Shale Hills Watershed in the Ridge and Valley Physiographic province of Central Pennsylvania. The toposequence includes samples from the ridge top, the middle slope and the valley floor as a function of depth. The soil depth in the watershed averages 1.4m with a pH of ~4. Depth to bedrock ranges from <0.25m on the ridge tops and upper side slopes to >2-3m in the valley bottom and swales. The 6 m section of Rose Hill shale is composed predominantly of illite (58 wt.%), quartz (30 wt.%), “chlorite” (either chlorite itself or a mineral exhibiting the same XRD peaks as chlorite; 11 wt.%), and trace amounts of potassium feldspar, anatase (TiO₂) and Fe-oxides (magnetite and hematite). “Chlorite”, as determined from quantitative XRD, is a term that here encompasses chlorite, vermiculite, hydroxyl interlayered vermiculite, and/or mixtures of these phases.

A2. Experimental Methods

A2.1 Soil pH and Fe forms by selective extractions

A 75 ml aliquot of purified water was added to 25 g of soil (3:1 water/soil ratio) and equilibrated for 10 minutes. The pH of the sample was then measured using an alpha pH 200 pH-meter by placing the electrode in the soil suspension with constant stirring. Soil from each depth and landscape position from the Shale Hills was treated using a selective dissolution procedure to estimate the amount of Fe in various solid-phase pools.
or fractions. These are operationally defined fractions and include treatment of the soil with Na-pyrophosphate (for organically-bound Fe), ammonium oxalate in the dark (for Fe in poorly-crystalline or amorphous inorganic materials), and dithionate-citrate (for free, non-silicate, crystalline Fe). For the dithionite-citrate (DC) extraction (Soil Conservation Service, US Department of Agriculture, 1972), homogenized soil samples (< 2mm) were ground to pass a 35-mesh sieve, weighed (0.5 g) into 50-ml centrifuge tubes, and 25 ml of 0.68 M sodium citrate solution and 0.4 g of dithionite powder were added to the tubes. The tubes were shaken overnight, and then centrifuged for 20 min at 10,000 RPM. The filtered (0.45 μm) supernatants are to be analyzed by Inductively Coupled Atomic Emission Spectroscopy (ICPAES). For the acid ammonium oxalate (AO) extraction, soil samples (0.250 g) were weighed into foil-covered centrifuge tubes, 10 ml of the oxalate solution (700 ml of 0.2 M oxalate solution and 535 ml of 0.2 M oxalic acid solution, adjusted to pH 3) were added to the tubes, and the suspensions were shaken for 4 hours. The suspensions were then centrifuged at 10,000 RMP for 20 min, and the filtered (0.45 μm) supernatants are ready to be analyzed for Fe by ICPAES. For the sodium pyrophosphate (SP) extraction, soil samples were ground to pass a 100 mesh sieve, weighed (0.3 g) into 50 ml centrifuge tubes, and 30 ml of 0.1 M Na-pyrophosphate solution added. The tubes were shaken overnight, centrifuged at 20,000 RPM for 10 min, and the filtered (0.45 μm) supernatants are to be analyzed for Fe by ICPAES. It should be noted that pyrophosphate extracts organically-bound Fe by peptization, however, it may also extract some inorganic amorphous Fe. All extraction procedures were done in triplicate. The extracts are kept for analysis for Fe by Inductively Coupled Plasma
Atomic Emission Spectroscopy (ICP-AES). The content of crystalline Fe was estimated as the difference between CD-extractable Fe and AO-extractable Fe (Fed - Feo). The difference between AO-extractable Fe (Feo) and SP-extractable Fe (Fep) will be used to estimate the contents of poorly-crystalline (amorphous) inorganic Fe respectively.

A2.2 Total Organic Carbon (TOC) and Total Nitrogen

Soil samples (< 2 mm) were finely ground (80 mesh sieve) to ensure sample homogeneity and oven-dried overnight at 50 °C. An appropriate amount of sample (7-10 mg) was introduced in a soil solid module (SSM-5000) coupled to a Shimadzu TOC87 5000A Total Organic Carbon Analyzer. It was assumed that all carbon in the soils was organic carbon, and that no mineral carbon (calcite, dolomite) was present. This assumption is adequate due to the low pH of the soils.

A2.3 Fe L-edge XANES (X-ray Absorption Near Edge Structure) spectroscopy

The Fe L$_{3,2}$-edge XANES spectra were collected at beamline U4B at the National Synchrotron Light Source, Brookhaven National Laboratory. Soils and standards were finely ground and spread across N- and Fe- free double-sided adhesive conductive graphite tape attached to a copper paddle. The paddle was affixed to a rotary stage and inserted into the experimental chamber aligned at 45° to the incident (defocused) beam. Fe$_2$O$_3$ was used as the reference for energy calibration. The Fe edge for each sample spectrum was calibrated to 706.8eV. The energy calibration was done in deriv(E). These conditions resulted in isotropic spectra. A low energy grating monochromater was used to
collect the spectra and entrance and exit slits were set to 50/50. The total electron yield (TEY) was recorded at room temperature. The spectra were processed using the Athena software. After energy calibration, the normalized spectra were used to interpret the results.

A3. Results:

A3.1 pH and total concentrations of Fe, C, and N in the soils from Shale Hills

The soils from the Shale Hills are acidic and the pH (Fig. A-2) increases with depth. The organic C content (Fig A-3) was observed to be decreasing with depth with the litter samples in the top layers having the highest C content. Also, the C content in the ridge top samples was the highest compared to the mid slope and valley floor samples. The N content (Fig A-3), however, was appreciably small in all the samples. Total Fe content did not follow a distinct trend. The Fe/C and Fe/N ratios are presented in Fig. A-4. The Fe/C and Fe/N ratios are found to increase with depth. However, this distribution represents the speciation of total Fe, including both dissolved and soluble species. The solution pH and redox potential determines the Fe(II)/Fe(III) speciation and the molar ratios of metal to organic carbon, which, in turn, influences the complexation of Fe with organic matter in acidic forest soils. Polyvalent metal like Fe is a hard Lewis acid and according to the Hard and Soft Acid Base principle is capable of strong and specific bonding to hard Lewis base functional groups. However, Fe can bind to the same functional groups which are also involved in adsorbing to organic matter of the solid components. When this results in a reduced negative charge, it increases the mobilities of
the dissolved organic matter as well as that of the Fe bound to the organic matter. Consequently, we see an increase of Fe/C and Fe/N ratio down the profile. Jansen et al. in 2003 showed that the immobilization of Fe(II) increases strongly with increasing Fe/C at lower Fe/C ratios but remains almost constant at higher Fe/C ratios. For Fe(III) they showed that for an increase in pH at any Fe/C ratio would result in the immobilization of Fe.

A3.2 Selective Chemical Extractions:

Results of selective chemical extraction tests for organic, amorphous and crystalline Fe, Al and Si are shown in Fig A-5. a. Partitioning of Fe: Crystalline Fe had the highest concentration and the percentage increased with depth. Amorphous Fe followed next and did not follow a distinct trend through the profile and organic Fe had the least concentration and decreased slightly with depth. This was true for all the landscape positions: ridge top, middle slope and valley floor. b. Partitioning of Al: Organic Al had the highest concentration in the ridge top and was similar to that of amorphous Al at depth. Crystalline Al had the least concentration, contrary to Fe and decreased with depth and was not observed in the valley floor samples. c. Partitioning of Si: Organic Si was the major fraction and decreased with depth. Crystalline was the next important fraction and did not follow a distinct trend across depths. Amorphous Si was negligible in all ridge top, mid slope and valley floor positions.
A3.3 Fe L-edge Near Edge X-ray Absorption Spectroscopy Data of Soils

Peaks in the 704-710 eV region represents Fe L$_3$-edge transitions while peaks in the 716-724 eV region represents Fe L$_2$-edge transitions. The relative differences in Fe$^{2+}$ and Fe$^{3+}$ concentrations are reflected in the relative amplitude of the peaks at around 707.6 and 709.5 eV, respectively. Irrespective of the landscape position or the depth, the normalized spectra of the Shale Hill soil samples (Fig. A-6) did not show much variation in their peak positions. However, the intensity of the peaks matched with those of illite indicating that most of the soil Fe present is possibly present in their mineral forms (Fig. A-7).
Table A-1: Physicochemical properties of Shale Hill soils

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<tr>
<th>Sample</th>
<th>Landscape Position</th>
<th>Depth (cm)</th>
<th>pH</th>
<th>C (g/kg)</th>
<th>N (g/kg)</th>
<th>Fe (g/kg)</th>
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Table A-2: Organic, amorphous, crystalline and residual fractions of Fe

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<th>Sample</th>
<th>Organic Fe (g/kg)</th>
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<th>Crystalline Fe (g/kg)</th>
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Figure A-1: Schematic of the sampling points as a function of depth and landscape position (not to scale)
Figure A-2: pH and electrical conductivity of the soils of Shale Hills as a function of depth (pH and EC could not be recorded for ridge top samples due to lack of soils)
Figure A-3: C, N and Fe contents in the soils of Shale Hills as a function of landscape position and depth (blue circles = ridge top; green square = mid slope; red triangle = valley floor)
Figure A-4: Fe/C and Fe/N ratios in the soils of Shale Hills as a function of depth and landscape position.
Figure A-5. Selective Chemical Extractions of Fe, Al and Si at ridgetop, midslope and valley floor of Shale Hills: Solid line represents the organic fraction, short dashed line indicates the amorphous fraction and dotted line represents the crystalline fractions of Fe, Al and Si present. (solid=organic; dotted=crystalline; dashed=amorphous)
Figure A-6: Fe L$_{3,2}$-edge XANES of Shale Hill Soils as a function of landscape position and depth (only a few representative soil spectra are shown)
Figure A-7: Fe L$_{3,2}$-edge XANES of Fe(II) and Fe(III) bearing minerals

Fe(III) minerals

Fe(II) minerals
Fig A-8: Fe L$_{3,2}$-edge XANES of Fe(II,III)-organic complexes

Energy (eV)

Fe(II)-NH$_4$ sulfate
Fe(III)-porphyrin
Fe(III)EDTA
Fe(III) acetate
Fe(III)nitrate
Fe(III)citrate

700 705 710 715 720 725

Energy (eV)
CURRICULUM VITAE

Amrita Bhattacharyya

Education
Ph.D. in Environmental Soil Chemistry, PSU, 2012
M.S. in Soil Chemistry, University of Calcutta, India, 2007
B.S. in Chemistry, University of Calcutta, India, 2004

Professional skills
Extensive analytical instrument experience:
  • UV-VIS Spectroscopy, ICP-AES, Elemental Analyzer (EA) for C and N.
Synchrotron work:
  • EXAFS and XANES at Brookhaven National Laboratory
Lab experience:
  • Synthesis of Fe-organic complexes, glovebox experiments, basic soil analysis

Work experience
  • 2008-present: Research Assistant, Department of Crop and Soil Sciences, PSU

Teaching Experience
  • Teaching Assistant, Spring 2009, 2010; Introduction to soils, SOILS 101

Awards
  • Graduate Research Assistantship- NSF, CEKA, The Pennsylvania State University, August, 2008-July 2010
  • Graduate Research Assistantship- The Pennsylvania State University, August, 2010- present
  • Best speaker award in MS oral presentations, University of Calcutta, India, 2007

Community Activities and Public Services
  • Publicity Chair, ECSS, Penn State University, 2012
  • Workshop Instructor in a Math Options STEM workshop for 7th and 8th grade girls, 2010, 2011, 2012