CHELATE ASSISTED, PRESSURIZED, LIQUID EXTRACTION FOR THE REMOVAL OF ADSORBED METAL CONTAMINANTS FROM SOILS

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ABSTRACT

Chelate Assisted, Pressurized, Liquid Extraction (CAPLE) has been developed in our laboratory as an efficient, separation-based, extraction methodology for heavy metals in soils. Unlike current extraction methods used in environmental determination of contaminated soils, CAPLE is able to selectively remove adsorbed metals from the soil matrix without requiring the total destruction of the sample. By not fracturing the soil matrix particles, as with hot acid digestion methods, geologically bound metals are not liberated in the CAPLE process. This unique feature of CAPLE allows us to quantify levels of contaminant metals and correlate them to anthropological activity in the area.

CAPLE requires the use of a modified supercritical fluid extractor for operation with water at sub-critical levels. The extraction of the sorbed metals is facilitated by the use of a chelating agent. Metal determinations are performed by atomic absorption (FAAS or GFAAS) or ICP emission spectrometry.

CAPLE has been subjected to a variety of experimental conditions in order to elucidate the strengths and possible weaknesses of the extraction technique. The uses of the chelating agents (type and concentration) have been optimized. Possible release of metals from the resulting ionic strength of the chelating solutions have been shown not to be a factor. Both pressure and temperature effects have

been studied and adjusted for optimal conditions.

The majority of the research lies in the application of CAPLE to a variety of soil conditions. The effect on particle size of the soil and soil coating (humic acid and iron oxides) has been studied. In all soil systems and coatings studied, CAPLE could be optimized to completely remove chemisorbed metals. Tests of CAPLE on Cu-sludge amended soils provided excellent agreement with traditional methods of soil analysis. Not only was good agreement obtained between the recoveries of the methods, but CAPLE was also found to be much faster, more environmentally friendly, and much less prone to sample loss or sample contamination compared to traditional soil extraction methods.

A final portion of this work involves a rigorous statistical analysis of CAPLE to a sequential extraction method. Since a Standard Reference Material (SRM) has not been provided for chemisorbed metals onto soils, a comparative analysis was chosen to validate the technique. Using the Cu-amended soils, CAPLE was found to effectively liberate all chemisorbed metals as compared to the sequential extraction technique. There was no statistical difference in recovery between the two extraction methods.

CAPLE is shown in this work to be a viable extraction method for analyzing contaminant metals in soils. It is a rapid and efficient technique. Unlike traditional digestion methods, it is able to differentiate anthropological metals from geologically occurring metals. Its ease of use, coupled with simplicity of instrumental design and analytical reagents make it an attractive extraction technique for environmental analysis.

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LIST OF ABREVIATIONS

C.I.   Confidence Interval

CAPLE   Chelate Assisted, Pressurized, Liquid Extraction

CDTA   1,2-cyclohexylenedinitrilotetracetic acid

DTPA   Diethylentriamine-pentaacetic acid

EDTA   Ethylenediamine tetra-acetic acid

EGTA   Ethylenebis-(oxyethylenenitrilo) -tetracetic acid

EPA   Environmental Protection Agency

FAAS   Flame Atomic Absorption Spectroscopy

GFAAS Graphite Furnace Atomic Absorption Spectroscopy

HA   Humic Acid

ICP   Inductively Coupled Plasma

PAH   Poly-Aromatic Hydrocarbon

PCB   Poly-Chlorinated Biphenols

SC-CO2 Supercritical Carbon Dioxide

SEM   Scanning Electron Microscope

SFE   Supercritical Fluid Extraction

SOM   Soil Organic Material

SRM   Standard Reference Material

Chapter 1: Removal of Contaminants from Soils

1.1 INTRODUCTION

As municipal, agricultural, and industrial activity throughout the world increase, the risk of metal contamination to municipal supplies, groundwater and aquatic systems becomes more probable. To better understand these risks, chemists, biologists, engineers and soil scientists have been working over the past three decades on better ways of determining metal concentrations in aquatic systems and of describing the interaction of contaminant metals and soils.

On the chemical forefront, analysts have been struggling with improving the accuracy and precision of chemical methods for soil analysis. Whereas metal analysis normally entails quantification by an atomic spectrometric technique, the soil sample must be transformed to an aqueous state. This transformation presents the analyst and the soil scientist with a paradox; analytic methods exist for efficiently rendering the soil to an aqueous state, but this transformation complicates the identification of the source of metal. A total acid digestion will allow the complete identification of all metals contained in the soil, but will report both the geologically occurring metals and the contaminant (anthropological) metals as a single result.

In order to avoid misrepresenting metal concentrations from total soil methods as contaminant levels, soil chemists developed methods to chemically separate the contaminant metals according to the major forms of soil-metal interactions. These

methods, however, are slow, labor intensive, and not suitable for trace metal determinations.[1,2,3]

This dissertation addresses a new analytical method for the rapid and efficient extraction of metals from contaminated soils. The method, Chelate Assisted, Pressurized, Liquid Extraction (CAPLE), utilizes subcritical water and a chelating agent to extract chemisorbed anthropological metals from soils and solubilize them in the aqueous matrix for subsequent determination by atomic spectrometry. In this work, CAPLE is shown to be an effective technique for extraction of only contaminant or adsorbed metals and not geological metals.

This work is best presented by first discussing soil-metal chemistry, absorption processes, and then outlining the current methods used in metal analysis.

1.2 SOIL CHEMISTRY

Like many topics in science, the topic of soil science has different schools of thought. The two branches of soil science are edaphology and pedology. Edaphology is the view of soils as media for plant production. A pedologic definition of a soil is "a body in nature that has a length, width and depth that occurs next to and surrounded by individual soil bodies"[4]. Each soil body is an individual particle that contains its own morphology. The culmination of these individual morphologies is what comprises a soil profile and consequently the studied soil properties. This work views soils from the Pedological point of view.

[1] Rapin, F.; Tessier, A.; Campbell, P.; Carigan, R. *Environmental Science and Engineering.* **1986**, *20*, 836.
[2] Nirrel, P.; Morel, F. *Water Resources.* **1990**, *24*, 1055.
[3] Tessier, A.; Campbell, P. *Water Resources.* **1991**, *25*, 115.

All soils begin with primary minerals. Primary minerals are the original rock and crystal structures that have not been chemically altered from which soils are generated (*i.e.* quartz, feldspars, and micas). Over many hundreds of thousands of years, the primary rock weathers or decomposes and very small pieces are broken or washed off. These pieces of primary mineral are then subjected to a wide range of chemical processes. Under high temperature and pressure, the rock can meld with other minerals and substances and recrystallize as a secondary mineral. A decomposition of primary mineral also results in the formation of a secondary mineral. These soils could have undergone an isomorphous substitution and rearrangement. Isomorphous substitution is the replacement of one atom by another of similar size in a crystal lattice without disrupting or changing the crystal structure of the mineral.[5] The various forms of secondary minerals include, but are not limited to, vermiculite, illite, geothite and kaolinite, known as alumino-silicates.

Many types of soils, especially silicate clays, have permanent charge sites. A permanent charge in soil does not vary with a change in soil pH. These sites are surface positions on the soil particle that chemisorb metal cation contaminants. Heavy metal and transition metal cations, known to the soil community as "trace metals", can be both nutrients and toxic elements to flora and fauna. Some of these trace metals can be contained in the primary or secondary mineral structure and are therefore biologically unavailable. Trace element cations that enter a system as pollutants can be organically complexed or can be a metal salt. As a metal salt, the

[4] McBride, M. Environmental Chemistry of Soils. New York: Oxford University Press. **1994**.
[5] Spark, D. Environmental Soil Chemistry. San Diego: Academic Press. **1995.**

cation is absorbed to the soil surface, either at a mineral or an organic site. The

oxides and hydroxides of iron, aluminum and manganese each present a different

chemical process for chemisorption of trace elements. Reactions on this specific

class of soils tend to have a high degree of specificity for particular trace elements.

The adsorption rate of a trace element on one of these surfaces is orders of

magnitude faster than the desorption rate. There is evidence a change in the

surface charge once trace elements have been chemisorbed to an oxide or

hydroxide mineral, therefore inferring that the chemisorbed element becomes an

integral part of the mineral surface.[4]

The level of reactivity for these soil minerals is directly related to the type and

number of valence-unsatisfied groups at the mineral's surface. The groups at a

mineral's surface can be organic or inorganic in nature. The organic groups tend to

create outer-sphere complexes with contaminants. An outer-sphere complex is a

metal complex that has at least one solvent molecule between the bound molecule

and the surface functional group. The inorganic functional groups tend to form

inner-sphere complexes with bound molecules. These complexes form as result of

either ionic or covalent bonding between the surface functional group and the bound

molecule.[4]

Mutlivalent cations can displace monovalent cations at the surface of a soil.

The purpose of these sites is to non-selectively retain multivalent cations, and to

release the monovalent cations into the environment as trace elements. The
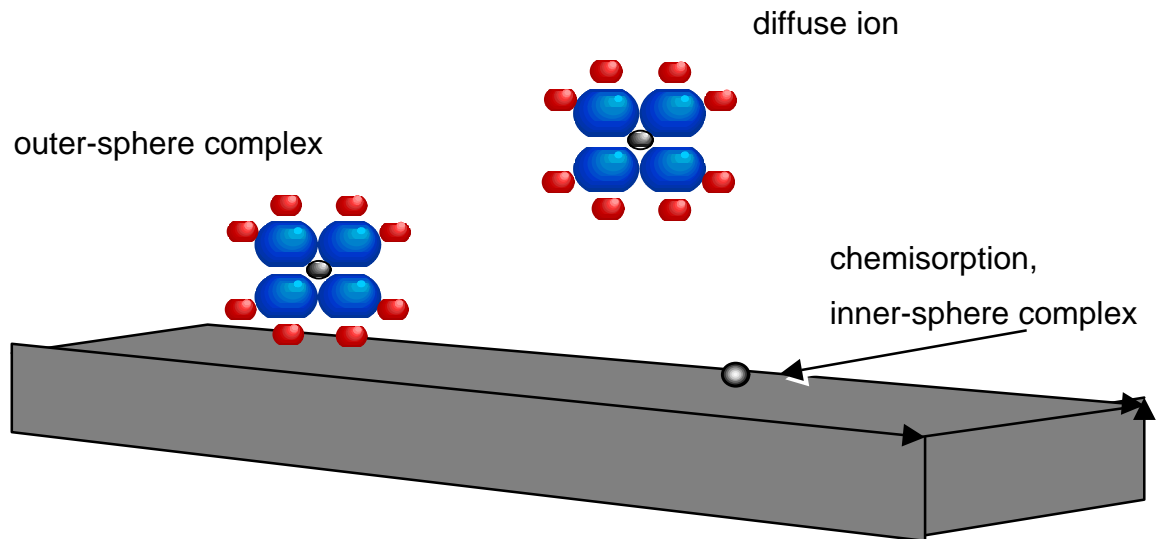
FIGURE 1.1 GRAPHIC REPRESENTATION OF METAL COMPLEXES WITH SOIL SURFACES

This figure shows the difference between the inner-sphere complex, or specifically adsorbed metal and the outer-sphere complex that metals form with a soil's surface.

released monovalent cations can then advance an ion exchange reaction with another soil particle or as part of a biological cycle to benefit a plant's growth.

Organic matter found throughout a soil profile can also react with soil particles.[6] Organic matter can be any sort of plant, animal or insect matter left to decay in the soil matrix. Soil organic matter (SOM) is very important to maintenance of an ecosystem. SOM provides improved soil structure by increasing water retention, nutrient retention via ion exchange with oxygen containing functional groups, release of trace, bio-available elements by mineralization and potentially absorb toxic organic pollutants like pesticides and industrial waste. SOM is commonly referred to as Humic Material. Humic Material or Humic Acid is an all encompassing term for any decomposing plant or animal matter. Humic acid is a polymeric chain of organic moieties that can have a vast number of different terminal functional groups. These functional groups can be phenolic, carboxylic, sulfonic, aminic, just to name a few of the potential reactive sites.[4] Soils typically contain between 0.5 - 5.0% organic matter. Humic acids have the capacity to chelate metals and enhance the fixation of both humus material and cations to the surface of a mineral.[7] The strength of the Humic Acid – M^{n+} complex is dependent of the type of functional group on the surface as well as the type of metal.[8,9]

[6] Spark, K..; Wells, J.; Johnson, B. *Australian Journal of Soil Research.* **1997,** *35*, 103.
[7] Varadachari, C.; Chattopadyay, K. *Soil Science.* **1997,** *28*, 162.
[8] Spark, K..; Wells, J.; Johnson, B. *Australian Journal of Soil Research.* **1997,** *35*, 113.
[9] Spark, K..; Wells, J.; Johnson, B. *Australian Journal of Soil Research.* **1997,** *35*, 89.

1.3 ADSORBED CONTAMINANTS

A contaminant can be defined as any substance in an environment that could pose a detrimental imbalance by its presence. Contaminants can be organic or inorganic in nature and are typically the result of anthropological activity. Land application of synthetic fertilizer or pesticides followed by a moderate to heavy rain can force these organic chemicals and their degradation products through the unsaturated soil zone and eventually to the saturated zone or water table. As discussed earlier in this chapter, a high humic (SOM) content will trap many of these contaminants. But under proper conditions, the soil will also release these compounds, thereby causing a delayed leaching of contaminants into the underground aquifers. Organic contaminants can be from industrial waste as well. Even with increased regulation and monitoring, bio-hazardous materials leach into surrounding fields and streams disturbing the ecological balance.

Inorganic contaminants are largely industrial in origin. Manufacturing plants and industries are required to meet a code specific to their local area as well as federally mandated codes on waste disposal.

1.4 CURRENT METHODS

There are four classes of remediative techniques used today. There are destructive technologies that involve incineration, biological technologies where biodegradation occurs, stabilization technologies which is generally the fixation of the contaminant and matrix in portland cement, and separation-based technologies

where the contaminant is removed from the environmental matrix[10]. Kocher

deemed a number of remediative techniques inadequate for removing contaminants

to an environmentally acceptable level.[11] Included in these undesirable techniques

are: immobilization, de-chlorination, thermal desorption, froth floatation and solvent

extraction. The product from these techniques must be further treated to be

disposed of in an environmentally acceptable manner. All of these methods have

had reported success from other researchers, but admit that they do not remove the

studied contaminant with results suitable to be declared remediative.

The most common way to study organic contaminants in soils today is with

supercritical carbon dioxide. Organic contaminants can be studied with a

combination of supercritical fluid extraction and supercritical fluid chromatography.

Capriel, *et.al*[12]. extracted bound pesticide residues from soil and plant residues

(humic acid). Capriel used supercritical methanol for his work. Papilloud, *et. al.*[13]

removed atrazine by SFE using carbon dioxide as the supercritical fluid. These

researchers did not quantitatively extract the contaminant from sediments, but did

demonstrate a method with good reproducibility. This method is time saving over

the traditional soxhlet extractions typically performed for removal of atrazine and its

metabolites. Hawthorne *et. al*. have published a number of recent literature articles

depicting their work using supercritical carbon dioxide to extract many different

organic compounds from environmental media.[14-17] Hawthorne has described work

on the solubility of PAHs in supercritical CO_2 at various temperatures and

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[10] Akgerman, A. *Waste Management.* **1993,** *13*, 403.
[11] Kocher, B.; Azzam, F.; Cutright, T.; Lee, S. *Energy Sources.* **1994**, *17*, 213.

pressures.[14] Hawthorne and co-workers have also extensively studied the interactions of PCBs and soils. They have published a three part series to discuss the sorption, extraction and re-sorption of PCBs from contaminated soils.[15,16,17] All of this work was performed in supercritical carbon dioxide. It has been proven time and time again that supercritical fluid technology for the extraction of organic contaminants from environmental samples is both effective and efficient.

A method growing in popularity for extracting organic compounds from soils is supercritical water. However, this technique lacks the safety of working with supercritical carbon dioxide. The critical parameters for water are; $T_c=374^oC$ and $P_c=218$ ATM, $\mu = 1.85$ debye[18]. These parameters are in sharp contrast to the critical parameters for carbon dioxide, $T_c=31^oC$ and $P_c=72.9$ ATM, $\mu = 0$ debye.[18] Kocher and researchers used supercritical and subcritical water to extract poly-aromatic hydrocarbons (PAH's) from soils.[19] By enhancing the solvating power of the water at an elevated temperature, the researchers noted an increased miscibility of many organic compounds. Despite the safety issues, supercritical water has many advantages over other supercritical fluids, including a strong polar dipole moment, decreased dielectric constant, and miscibility with many organic compounds. Kocher theorized that these were the reasons that his work achieved near complete extraction of organic contaminants from soil.[19] They concluded that

[12] Capriel, P.; Haisch, A.; Khan, S. *Journal of Agriculture and Food Chemisty.* **1986,** *34*, 70.
[13] Papilloud, S.; Heardi, W.; Chiron, S.; Barcelo, D. *Environmental Science and Technology.* **1996,** *30*, 1822.
[14] Miller, D.; Hawthorne, S.;Clifford, A.; Zhu, S. *Journal of Chemical Engineering Data.* **1996,** *41,* 779.
[15] Bjorklund, E.; Bowadt, S.; Mathiasson, L..; Hawthorne, S. *Environmental Science and Technology.* **1996,** *33,* 2193.
[16] Pilorz, K..;Bjorklund, E.; Bowadt, S.;Mathiasson, L.;Hawthorne, S. *Environmental Science and Technology.* **1999,** *33,* 2204.
[17] Hawthorne, S.; Bjorklund, E.; Bowadt, S.; Mathiasson, L., *Environmental Science and Technology.* **1999,** *33,* 3152.
[18] <u>CRC Handbook for Chemistry and Physics.</u> New York: CRC Press. **1992**.
[19] Kocher, B.; Azzam, F.; Cutright, T.; Lee, S. *Energy Sources.* **1994,** *17*, 213.

when the water's density approached the critical density, the extraction efficiency approached a maximum of 99% for PAH's from soil.

Using water and it's various properties at different temperature and pressure combinations, Hawthorne, *et. al.* demonstrated a sequential extraction procedure.[20] These researchers were able to remove compounds of varying polarity by controlling the temperature of the extraction. Hawthorne and co-workers have also studied the solubilities of PAHs[21,] various organics from gasoline[22] as well as other hydrophobic organics[23].

When the attention turns to metals, supercritical CO_2 posed an interesting challenge to many researchers. Regardless of the metal cation or ligand used, the resulting complex must be neutral to achieve solubility in CO_2. The majority of the focus of current literature concerning metals in SC-CO_2 is the solubility of the metal-chelate complex. Cross and Akgerman studied the solubility of cupric acetylacetonate and diethlydithiocarbomate copper salt.[24] They assembled a series of equations for the determination of metal chelate solubilities and concluded that there should be a standardized method of solubility determination for these studies. Wai, *et. al.* have studied the solubility of several metal-chelate complexes in supercritical carbon dioxide. They have studied the copper, mercury and zinc

[20] Hawthorne, S.; Yang, Y.; Miller, D. *Environmental Science and Technology.* **1997,** *31*,430.
[21] Miller, D.; Hawthorne, S.; Gizir, A.; Clifford, A. *Journal of Chemical and Engineering Data.* **1998**, *43,* 1043.
[22] Yang, Y.; Miller, D.;Hawthorne, S. *Journal of Chemical and Engineering Data.* **1997,** *42*, 908.
[23] Miller, D.;Hawthorne, S. *Analytical Chemistry.* **1998,** *70*, 1618.
[24] Cross, W.; Akgerman, A. *Industrial Engineering and Chemical Research.* **1996,** *35,* 1765.

complexes of seven dithiocarbamate ligands. It was concluded that the fluorinated ligand is more soluble than the non-fluorinated ligand in a CO_2 system.[25]

The first work using supercritical carbon dioxide to remove metals from soils was published by Wai *et. al.*[26] Wai and co-workers studied the use of organophosphorus reagents to remove metals loaded onto filter paper as well as native, contaminated clay. They concluded that the metals were easy to remove from the inert filter paper and much more difficult to remove from the soil system. It would take 3-4 "extraction batches" or runs to remove 10-50% of the metals from the clay.[26] These extractions were considered not quantitative. This equates to 120-160 minutes of extraction time, but the soil matrix was left intact. The fluorine-substituted ligand systems were expensive to buy and at times had to be synthesized in-house through a lengthy procedure. Meguro, *et. al.* studied the solubility of organophosphorus ligands and their metal complexes.[27] Although the article does not state what, if any metal complexes were studied, they theorized that the more soluble the ligand in the extraction media, the more soluble the metal-complex would be.

A widely accepted method for studying the metal content of various soils is total acid digestion. Until 1991, digestion methods utilized a combination of hot nitric acid and hot hydrochloric acid[28]. This method did not completely dissolve the total contents of the soil sample. A method that utilizes hydrogen peroxide to ensure greater mineral digestion was developed and later became EPA method 3050,

[25] Wai, C.; Wang, S.; Yu, J. *Analytical Chemistry*. **1996**, *68,* 3516.
[26] Smart, N.; Carleson, T.; Elshani, S.;Wang, S.; Wai, C. *Industrial Engineering Chemical Research*. **1997,** *36* 1819.

"Acid digestion of sediment, sludge and soils." Although the undissolved fractions do not contain bio-relevant minerals, it was desired to obtain a more complete digestion for a more accurate metal analysis.

Sequential extraction is probably the most popular method to extract metals from soils. A soil sample is subjected to a series of solvents to remove successive layers of contaminants and establish the distribution of metals. Tessier, *et. al.*[29] outlined the original sequential extraction procedure and the types of metals each step would remove. This procedure has been slightly modified by each researcher who has utilized sequential extraction in his or her research. The main types of metals removed have been classified as exchangeable, associated with carbonates, associated with metallic oxyhydroxide, associated with organic matter and trapped in crystalline lattices of silicate minerals.[30] Other researchers have divided these main categories into sub-categories[31,32,33], but Tessier's classifications are universally accepted. Clavet *et. al.*[33] concluded that the sequential and parallel extraction procedures they studied, which were all variations on Tessier's method, did not provide a clear delineation of metals in their respective fractions nor did each soil fraction provide similar results between methods. The main conclusion from this research[33] was that the results concerning a particular fraction of metals in soils were completely procedure dependent.

[27] Meguro, Y.;Iso, S.; Sasaki, T.; Yoshida, Z. *Analytical Chemistry*. 1998, *70,* 774.
[28] Diaz-Barrientos,R.;Madrid,L.; Cabrera,F.;Contreras,M. *Communications in Soil Science and Plant Analysis.* **1991,** *22,* 1559.
[29] Tessier, A.; Campbell, P.; Bisson, M. *Analytical Chemistry.* **1979**, *51,* 844.
[30] Clavet, R.; Bourgeois, S.; Msaky, J., *International Journal of Environmental Analytical Chemistry.* **1990,** *39,* 31.
[31] Kuo, S.; Heilman, P.; Baker, A. *Soil Science.* **1983,** *135,* 101
[32] Miller, W.; Martens, D.; Zelazny, W. *Soil Science Society of America Journal.* **1986,** *50,* 598.
[33] Ure, A. *Fresenius Journal of Analytical Chemistry.* **1990,** *337*, 577.

Subcritical water has been applied to the study of metal contaminants from soils as well. The main focus of these works is to surround the metal ion with a chelate to neutralize the charge and remove the metal-chelate complex with the reduced dielectric constant of heated, pressurized water.

1.5 RESEARCH OBJECTIVE

This study outlines the development of CAPLE as an extraction method for contaminated soils, studies the possible interaction of soil-metal reactions on the method, and validates the method by comparison of recoveries with that of sequential extractions. In this work, Chapter 2 is concerned with the development of CAPLE. A synthetic contaminant system was created to study temperature, pressure and additive effects using CAPLE. The chelate, disodium ethylenediamine tetra-acetic acid, (EDTA), is the sole chelating agent of this chapter. In Chapter 3, the developed method is applied to natural soils. These natural soils are a loam, clay, and sandy soil. Chapter 4 broadens the CAPLE study to three more chelating agents: diethylentriamine-pentaacetic acid (DTPA), 1,2-cyclohexylenedinitrilotetracetic acid (CDTA), and ethylenebis-(oxyethylenenitrilo) -tetracetic acid (EGTA). The results of these extractions are compared with the results of the EDTA extractions as well as extractions performed with no modifying agent. Chapter 5 summarizes the previous four chapters and outlines the proposed chemical mechanism of the extraction of contaminant metals from soils.

Chapter 2: Chelate Assisted, Pressurized, Liquid Extraction for the removal of metals from sea sand.

2.1 INTRODUCTION

A method for removal of adsorbed copper from soil using heated, pressurized water was developed to obtain a more accurate quantitation of potential contaminant metals.   In order to develop a method suitable for the extraction of adsorbed metals from soils, a relatively inert matrix and a known amount of metal are studied under various conditions. Sea sand was chosen for this work for a number of reasons: sea sand has a relatively inert surface, it is a naturally occurring soil, it is readily available and easy to clean. This controlled system facilitates the study of other variables like the interaction of the CAPLE system with soil coatings. The soils used for this method development include: acid washed sea sand, iron oxide coated sea sand, humic acid coated sea sand and a combination iron oxide and humic acid coated sea sand. These soils, except the acid washed sea sand, were equilibrated with copper to a known amount and the adsorbed copper was subsequently extracted.

All extractions in this chapter were performed using a Suprex SFE 50 (Lincoln, NE), supercritical fluid extractor. This system was modified to accept water as the solvent fluid. The modifications are described in the Experimental Section of this chapter.

2.2 EXPERIMENTAL

2.2.1 MODIFICATIONS OF THE SUPREX SFE SYSTEM

For the modifications to the Suprex SFE 50 (Lincoln, NE) system, a sparging tank was fabricated from a 2-L polyethylene storage bottle, Figure 2.1. The top of the storage bottle had two holes drilled into it to permit Tygon tubing to be passed through. One of the tubes extended to and looped around the bottom of the bottle. This tube had a number of holes punched in it to deliver nitrogen to the water in the bottle. The other tube was considerably shorter. The function of this tube was to deliver the water to the pump and was approximately 1.5" long inside the bottle. All of the joints were sealed with epoxy.

The pump head seal was replaced with an all Teflon fitting as opposed to the Teflon and stainless steel seal present for CO_2 work. A preheating loop was added to the system to ensure heated water was introduced to the vessel.[34]

2.2.2 PREPARATION OF SOILS

The soils were based on sea sand obtained from Fisher Scientific (Fairlawn, NJ). The sand was sieved at 200 mesh to eliminate frit clogging with the smallest particles.

For all extractions, 0.5L of 18 MΩ water was purged with nitrogen for 30 minutes prior to filling the pump. The extraction vessel was an empty Keystone (Bellefonte, PA) HPLC vessel, 1.67 mL capacity, that was fitted with 2 μm frits at both ends.
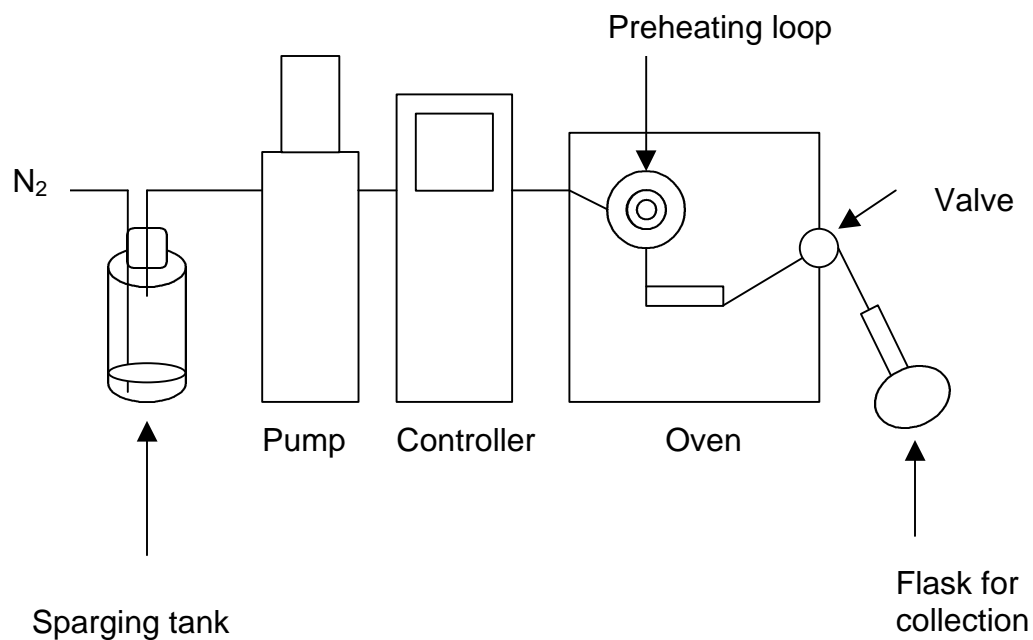
FIGURE 2.1: DIAGRAM OF INSTRUMENT SETUP.
This is a schematic of the modifications to the Suprex SFE 50 system. The sparging tank, on the left, was inverted prior to pump filling to deliver the water to the pump inlet

[34] Yang, Y.; Hawthorne, S.;Miller, D. *Environmental Science and Technology.* **1997,** *31,* 430.

The iron oxide coated sand was prepared with Fe_2O_3 obtained from Fisher Scientific (Fairlawn, NJ) and using a variation of the procedure described by Scheidegger *et. al*[35].

A solution of 30 g Fe_2O_3 and 100 mL dilute HNO_3, at pH=2.5, was placed on an orbital shaker for 24 hours. To this solution, 180 g sea sand was added. This mixture was placed in a 120°C oven for 24 hours and then allowed to cool to room temperature. The sand was then washed several times with 18 MΩ water until the rinse water was clear. This soil was then equilibrated with a 3000 ppm $CuCl_2$ solution. The mixture was shaken periodically and 1.0 mL aliquots were taken at various time intervals for analysis. The equilibration was deemed complete from the steady state achieved in Figure 2.2 after 24 hours. The soil was rinsed with water and dried. The amount of copper adsorbed to the surface of the soil was determined to be 100 µg Cu/g soil.

The humic acid coated soil was prepared[36] by first purifying the technical grade humic acid (HA) obtained from Fisher Scientific (Fairlawn, NJ). Approximately 4 g of HA was dissolved in water and centrifuged at 10,000 rpm for 10 minutes. The supernatatent liquid was then filtered through a Whatman 542 Hardened Ashless filter (Kent, England). The purified HA was then precipitated out of solution by increasing the pH of the liquid with redistilled HCl (Fisher Scientific, Fairlawn, NJ) to a pH ~ 1. At this pH the purified HA could be filtered from the liquid and dried. A solution of 2000 ppm HA (pH=9) was mixed with 100 g sea sand and

[35]Scheidegger, A.; Borkovec, M.; Sticher, H. *Geoderma*. **1993**, *58*, 43.
[36] Chaney, K.;Swift, R. *Journal of Soil Science*. **1986,** *37*, 337.

allowed to equilibrate for 48 hours at a pH=6. The HA coated sand was then equilibrated with a 3000 ppm cupric chloride solution for 24 hours, as described above, with 1.0 mL aliquots taken to determined the equilibrium concentration of copper adsorbed to the prepared soil. The amount of copper adsorbed to the surface of the soil was determined to be 260 µg Cu/g soil, Figure 2.2.

The combination soil, sea sand coated with both iron oxide and HA was prepared by first adsorbing the iron oxide to the sea sand as described above and equilibrating this soil with a copper solution. The soil was then coated with HA as described previously and this soil was equilibrated with a cupric chloride solution to create a layered system. The amount of copper adsorbed to the surface of this soil was determined to be 300 µg Cu/g soil.

2.2.3 EXTRACTIONS WITH SEA SAND
2.2.3.1 VARIED PRESSURE

The first study concerned the effect of pressure on a heated water extraction of metal from sea sand. Approximately 1.25 g of Sea Sand was packed into the Keystone (Bellefonte, PA) vessel and then a spike of 20 µL delivering 2.0 µg cupric chloride was placed on the top of the sand. The extraction vessel was then packed with an additional 1.25 g Sea Sand. Three different conditions were chosen to observe the effect of modifying agents: 20 µg ethylenediamine tetraacetic acid, disodium salt (Aldrich Chemical Co., Milwaukee, WI), 40 µg NaCl (Fisher Scientific, Fairlawn, NJ) and a no modifier condition. If an additive was to be used, it was then applied to the top of the vessel. The vessel was then placed in the extraction

Equilibration of Coated Sea Sands with copper.
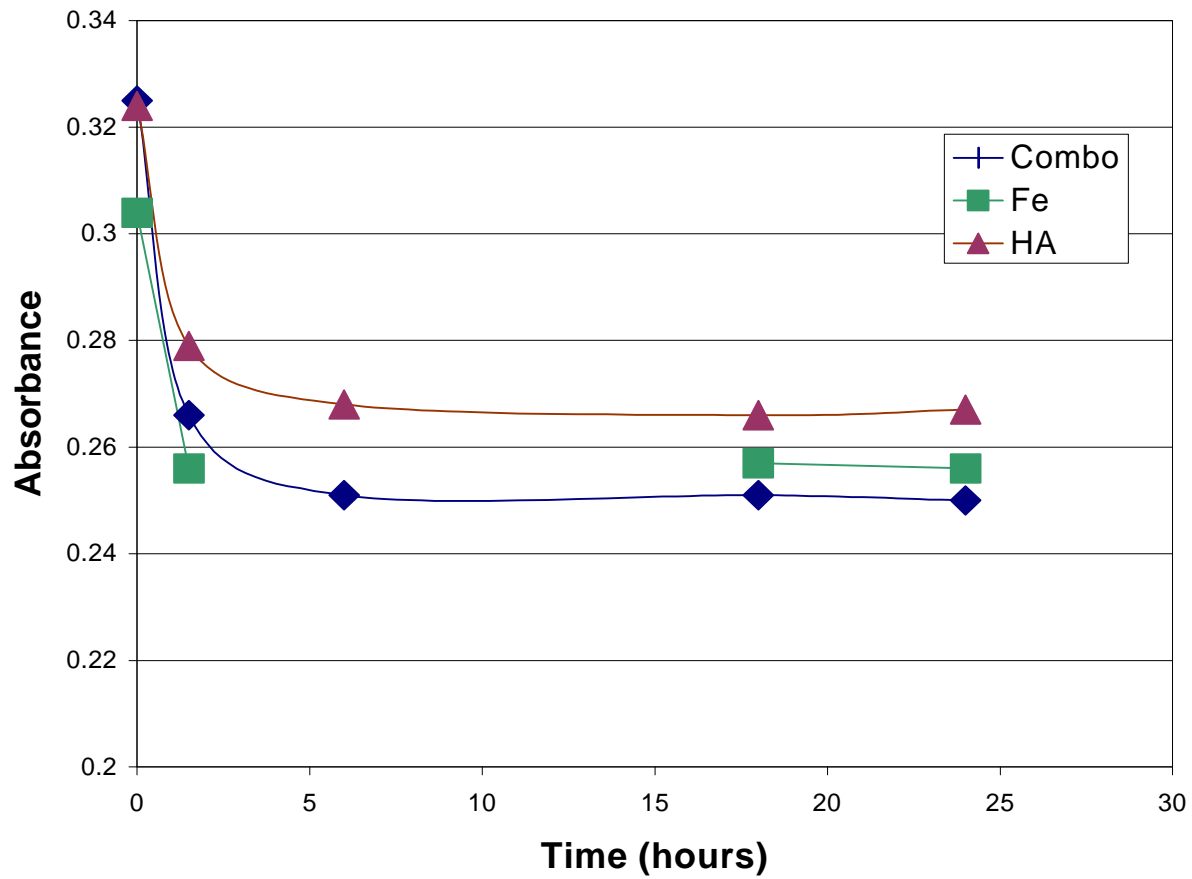
FIGURE 2.2: GRAPH OF COPPER EQUILIBRATION OF ALL COATED, SEA SAND SOILS
This graph displays the steady state achieved with the coated sea sands after six hours of equilibration with a copper solution.

chamber and the system was brought to equilibrium. As packed, the dead volume of the cell was 0.57 mL. A "flush volume" was defined as 10 times the dead volume and therefore a flush volume for these samples is 5.7 mL.[37]   A liquid flow rate of 19 mL per minute was achieved at the lowest pressure to be studied, therefore, it was determined that a dynamic time of one minute would exceed the flush volume. The trap for this work was a 50-mL beaker containing 10 mL 18 MΩ water and covered with Parafilm[TM]. The beaker was cooled in an ice water bath contained in an ultrasonic bath.

Three extraction pressures were chosen: 100 ATM, 150 ATM, and 200 ATM. The extractions were isothermal at 160°C. The trap and analyte were made to 100.0 mL using class A volumetric glassware after a one minute dynamic time. The sand was retained for further analysis.

Percent recoveries were determined by comparing the absorbance of the sample analyzed by GFAAS to a standard prepared by diluting a 20 μL aliquot of the spiking solution, delivering 2 μg cupric chloride, in 100.0 mL of 18 MΩ water and an appropriate modifier, if needed. The linear range for copper using the analytical technique of GFAAS was determined to be from 0-60 ppb. A total recovery, based on the amount applied, would be a concentration of 20 ppb copper. For each sample, a 20 μL aliquot of the sample is introduced to the furnace. Conditions for analysis by GFAAS are listed in Table 2.1.

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[37] Taylor, L.T. Supercritical Fluid Extraction. New York: John Wiley & Sons, **1996**.

Buck Scientific GFAAS

Dry	30 s	150°C
Ash	40 s	700°C
Atomize	6 s	2200°C

Perkin Elmer FAAS

Slit width	0.7 nm
flame	air/acetylene

TABLE 2.1: CONDITIONS FOR GFAAS AND FAAS ANALYSIS
The operating parameters for the Graphite Furnace Atomic Absorption Spectrometer (GFAAS) and the Flame Atomic Absorption Spectrometer (FAAS) are given. The wavelength was fixed at the atomic absorption line for copper, 324.8 nm.

The retained sand was further extracted by placing the wet sand in a previously wet Whatman 542 hardened ashless filter (Kent, England). A 5% nitric acid solution (redistilled, Fisher Scientific, Fairlawn, NJ) was diluted with 18 MΩ water, washed over the sand, and then collected in a class A 100.0-mL volumetric flask. The resulting solution was made to volume using 18 MΩ water. These samples were then analyzed with GFAAS.

It was also determined that there was adsorbed copper on the iron oxide phase of the sand. To attempt to remove the iron oxide phase from the Sea Sand, and therefore reduce the copper interference, the sea sand was washed with nitric acid. The sand (500 g) was placed into a one-liter beaker. The sand was covered with a 5% nitric acid solution. This mixture was shaken for 15 minutes and the liquid was decanted. The sand was covered again with a 5% nitric acid solution and shaken on an orbital shaker overnight. The sand was then rinsed with large volumes of 18 MΩ H_2O until the rinse water was a neutral pH.

2.2.3.2 VARIED TEMPERATURE

The next study with sea sand was to extract both copper and cadmium from the acid washed sea sand. This prepared sand was weighed out into 2.1 g samples. A spike of 100 μL, delivering 0.1 mg $CuCl_2$ and 0.1 mg $Cd(NO_3)_2$ was dried onto the 2.1 g sea sand. The sample weight was reduced from 2.5 g in the earlier studies because of the potential for sample loss in the transfer of the spiked sand from the weighing paper to the extraction vessel. There were six different types of extractions to be performed. There were three different background extractions performed with no metal spikes on the sand to determine background

levels of the appropriate metals. These extractions were with no modifier, a spike of 2.3 mg NaCl dissolved in 100 μL 18 MΩ water, or a spike of 5.4 mg Na$_2$ EDTA dissolved in 100 μL 18 MΩ water. These same modified conditions were performed with the metal spiked, sand samples. The temperatures chosen for study were 98°C, 105°C, and 120°C. The pressure was held constant at 100 ATM and the trap was a 50.0-mL class A volumetric flask. The pressure was held constant to evaluate the effect of temperature on the system. There was approximately 0.5 mL 18 MΩ water in the bottom of the flask to just cover the end of the outlet tube. The dead volume for a vessel packed in the above manner was found to be 0.9 mL, therefore, the flush volume would be 9 mL. Again a dynamic time of one minute exceeds the flush volume.

2.2.4 EXTRACTIONS OF THE COATED SOILS

As described previously, the coated soils were equilibrated with a copper chloride solution. The sample size was decreased to 2 g in order to accurately quantitate the extracted copper and decrease the risk of sample loss in transfer. The modifier was added on the top of the soil in the vessel and the same varied temperature and pressure study was performed.

2.2.5 ANALYTE ANALYSIS

The extractions were diluted volumetrically to 50.0 mL with 18 MΩ water and analyzed with FAAS, see Table 2.1. Samples were stored at room temperature in sealed bottles and analyzed within 24 hours.

The copper levels were quantitated by measuring the elemental copper concentration of each extraction run. This was achieved by comparing the absorbance signal of the extraction run with a calibration curve constructed from cupric chloride standards. The linear range was determined and the extraction samples fell within the linear working curve. The calculations were performed with Microsoft Excel '98.

The recoveries were calculated based on the amount of copper applied to the sea sand. These values were confirmed with total acid digestion.

The confidence interval, Equation 2.1, of the recoveries was determined by calculating the mean, $\bar{x}$, and standard deviation, $s_{\bar{x}}$, of the mean. The t_{crit} value was obtained via statistical tables and the confidence interval was constructed. The error for confidence interval is expressed as two significant figures.

$$CI = \bar{x} \pm s_{\bar{x}} t_{crit}$$ **Equation 2.1**

2.3 RESULTS AND DISCUSSION

2.3.1 EXTRACTIONS WITH SEA SAND

2.3.1.1 VARIED PRESSURE

The isothermal study with Sea Sand, at 160°C, provided more than 100% recoveries of the analyte spiked, with the use of EDTA, Table 2.2. For the sodium modified extractions, $52.8 \pm 2.2\%$, $29.3 \pm 0.4\%$, and $26.1 \pm 0.4\%$ of the spiked copper was recovered with the heated, pressurized system, at 200, 150 and 100

160°C	EDTA %	Na$^+$ %	No %
200 ATM	119 ± 4.6	53 ± 2.2	34 ± 0.5
150 ATM	106 ± 2.9	29 ±0.4	33 ±0.4
100 ATM	120 ± 6.4	26 ± 0.4	35 ± 0.5

TABLE 2.2: ISOTHERMAL STUDY (160°C) WITH SEA SAND, RECOVERIES IN % OF SPIKE
The recoveries listed are in percent of copper removed from the spiked sea sand sample and are displayed as the confidence interval. The recoveries are wt/wt% of copper extracted from the sea sand compared to the copper applied to the sea sand. These extractions were performed prior to the acid washing of the sea sand, *n=3*. The 95% confidence interval of the recoveries was determined by calculating the mean, $\bar{x}$, and standard deviation, $s_{\bar{x}}$, of the mean. The t_{crit} value was obtained via statistical tables and the 95% confidence interval was constructed.

$$CI = \bar{x} \pm s_{\bar{x}} t_{crit}$$

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ATM respectively. The extractions that were not modified yielded 34.4 ± 0.5%,

32.9 ± 0.4%, and 35.2 ± 0.5% of the copper applied.

The anomaly of finding a greater than 100% recovery of copper with EDTA

can be attributed to the fact that the sea sand contains a yellow-orange iron oxide

phase that exchanges with copper readily under natural circumstances.   This would

lead to the sand arriving, as packaged, with adsorbed copper. This greater than

unity recovery was a concern, so the sea sand was washed with dilute nitric acid

(5%), as described in the Experimental section. The washing removed the yellow-

orange particulate matter. A total acid analysis of the sea sand, as received,

confirmed a 27.1 ± 2.7 µg Cu/g sand content, listed as the 95% confidence interval.

Acid washed sea sand yielded no detectable copper confirming that the acid

washing procedure removed the copper-containing fraction.   All extractions except

those performed isothermally at 160°C were performed with sand that had been

cleaned and then spiked with a copper solution.

The retained sand for the isothermal study with no modifier provided an

average of 1.0 µg copper or 50% of the copper applied. The sand used for the

EDTA extractions provided further recoveries below the quantification limit, LOQ.

The sand used for the extractions using a sodium solution provided an average of

1.2 µg copper or 60% of the applied copper.   This further recovery of copper

allowed for a mass balance, within 10%.

Statistical analysis shows that at 160°C varying the pressure from 100-200

ATM does not alter the recoveries when using the chelate, EDTA (see Table 2.3).

Pressures	t_{calc} EDTA	t_{calc} Na$^+$	t_{calc} No Modifier
200 v. 150	0.77	2.21	1.27
150 v. 100	0.67	1.00	1.27
200 v. 100	0.35	2.25	0.33

n=3 for all extractions

TABLE 2.3: STATISTICAL ANALYSIS OF 160°C EXTRACTIONS
The statistical test of a "Two tailed t-test for means" at a 95% confidence was performed to display the statistical difference of the recoveries obtained at 160°C. The t_{crit} value is 2.92 for these tests. The null hypothesis was that the recoveries at different pressures were the same. The alternate hypothesis was that the recoveries at different pressures were different.

2.3.1.2 VARIED TEMPERATURE

Varying the temperature of the extraction system from 98°C to 120°C did not provide a statistical increase in recovery with the EDTA. ANOVA analysis was used to confirm that there was no statistical difference between the recoveries obtained at the different temperatures (F_{crit} = 6.94, F_{calc} = 0.68) and there was a statistical difference between the modifying conditions (F_{crit} = 6.94, F_{calc} = 2490). All copper recoveries for the varied temperature portion of the study are contained in Table 2.4 and Table 2.5. The sodium solution provided an average of 67.7 $\pm$ 5.4% Cu and 75.4 $\pm$ 6.3% Cd, indicating for the non-coated sea sand, the extraction of adsorbed metals could largely be a function of ion exchange with the sodium.

The spiking technique could lead to a deposit of metals as opposed to an adsorption; however, for Cu this is not the case. With no modifier, the Cu provided recoveries less than 10%. If there was a deposition of the copper rather than an absorption, the copper recoveries without any modifier would be considerably higher because of the lack of association of the metal with the soil surface with a deposited metal. More of the cadmium was deposited rather than absorbed. This is shown by the high recovery of cadmium without the aid of a chelate. Cadmium may also form an outer sphere complex with the alumino-silicates, thereby leading to the higher recovery with no modifying agent of 50%.

Soil / Temp	EDTA μg/g	Na⁺ μg/g	No Additive μg/g
Non-Coated			
98	38.8 ± 1.6	31.7 ± 1.3	2.3 ± 0.1
105	37.0 ± 1.0	33.1 ± 1.8	nd
120	50.1 ± 1.4	31.2 ± 1.3	4.4 ± 0.2
Fe			
98	88.5 ± 9.5	63.3 ± 3.4	8.1 ± 0.2
105	91.2 ± 8.6	61.0 ± 1.6	8.2 ± 0.3
120	91.6 ±9.9	63.3 ± 2.6	8.1 ± 0.3
HA			
98	241.6 ± 22	124.2 ± 6.7	30.6 ± 0.8
105	248.7 ± 36	126.0 ± 12	42.5 ± 2.8
120	263.4 ± 28	121.0 ± 9.8	54.4 ± 2.9
Combo			
98	279.4 ± 11	152.8 ± 1.0	127.7 ± 10

TABLE 2.4: CAPLE EXTRACTION RECOVERIES OF CU FROM SYNTHETIC SOILS
The recoveries of the CAPLE extractions from sea sand are listed above. The recoveries are wt/wt% of copper extracted from the sea sand compared to the copper applied to the sea sand. The recoveries are listed as the 95% confidence interval in μg copper / g soil, or ppm of copper.

C.I. = Confidence Interval, α=0.05, t=2.92

Soil / Temp	EDTA %	Na$^+$ %	No Additive %
Reg			
98	81.3 ± 5.1	67.3 ± 4.8	4.6 ± 0.3
105	84.3 ± 2.7	70.1 ± 5.8	nd
120	85.3 ± 3.2	66.2 ± 4.7	8.9 ± 5.7
Fe			
98	91.8 ± 13	62.9 ± 6.4	8.4 ± 3.0
105	94.6 ± 8.3	61.3 ± 3.2	8.5 ± 4.9
120	95.0 ± 12	62.9 ± 4.5	8.4 ± 4.5
HA			
98	92.9 ± 11	47.7 ± 4.8	11.7 ± 2.8
105	95.6 ± 4.0	48.4 ± 3.0	16.4 ± 6.3
120	101.3 ± 8.4	46.5 ± 4.8	20.9 ± 8.4
Combo			
98	93.8 ± 2.6	51.3 ± 0.2	43.0 ± 2.6

TABLE 2.5: CAPLE EXTRACTION RECOVERIES OF CU FROM SYNTHETIC SOILS, WEIGHT %
The recoveries of the CAPLE extractions from sea sand are listed above. The recoveries are wt/wt% of copper extracted from the sea sand compared to the copper applied to the sea sand. The recoveries are the average of 3 replicate extractions and are listed as the confidence interval at 95%. The column labeled "EDTA" displays the highest recoveries, most not different from 100%.

2.3.2 IRON OXIDE COATED SEA SAND

The iron oxide coated sea sand adsorbed Cu at 100 µg Cu/g sand, as indicated by the absorbance graph, Figure 2.2. The EDTA extractions provided $90.4 \pm 5.3\%$ of this value. The Na^+ extractions provided 62.4 ± 1.5 % of this value leading to a conclusion that 62% of the copper obtained with EDTA was actually with ion exchange with the sodium in solution. No modifier only produced $8.1 \pm 0.5\%$ of the total Cu adsorbed per gram of sand.

The retained iron oxide coated sand was analyzed with EPA Method 3050, "Acid digestion of sediment, sludge and soils." The analysis led to a mass balance within 15% of total copper available. Table 2.6 displays the recoveries of copper obtained with EPA Method 3050 from the retained sand.

Non-extracted, iron oxide coated, copper equilibrated sand was also subjected to Method 3050. This resulted in an average of 96.4 ± 9.6 µg Cu/g sand. Using this analysis of copper content, the recoveries of the extractions utilizing EDTA are $93.8 \pm 12.6\%$; Sodium, $62.4 \pm 2.8\%$; and no modifier, $8.4 \pm 2.4\%$. This brings the mass balance to within 10% of total copper adsorbed. ANOVA analysis confirmed that there was no statistical difference between the recoveries obtained at the different temperatures ($F_{crit} = 6.94$, $F_{calc} = 2.68$) and there was a statistical difference between the modifying conditions ($F_{crit} = 6.94$, $F_{calc} = 601$).

A set of ambient extractions was also performed on the Iron Oxide coated sand, results contained in Table 2.7. These extractions provided $68.3 \pm 5.0\%$ of the copper in the soil after 30 minutes and with EDTA. This result is statistically smaller

Displayed as a confidence interval, $\alpha=0.05$, $t_{crit}=2.92$

Soil / Temp	Total EDTA	Acid Na$^+$	Digestion No Additive	*Total Cu* μg/g soil
Fe				
98	7.2 ± 1.9	37.4 ± 7.1	77.7 ± 6.3	*100*
105	8.5 ± 4.9	25.1 ± 6.0	74.8 ± 5.9	*100*
120	7.5 ± 3.6	37.9 ± 6.4	73.2 ± 8.4	*100*
HA				
98	8.7 ± 3.1	51.9 ± 9.2	82.8 ± 6.6	*260*
105	5.4 ± 1.1	50.5 ± 6.8	88.5 ± 5.3	*260*
120	7.3 ± 4.3	52.7 ± 5.3	80.6 ± 4.7	*260*
Combo				
98	4.3 ± 2.1	43.8 ± 5.2	46.4 ± 5.4	*298*

TABLE 2.6: PERCENT RECOVERIES OF TOTAL ACID DIGESTION OF RETAINED SAND
The recoveries of copper extracted with total acid digestion from retained soil after CAPLE extraction are listed above. The recoveries are wt/wt% of copper extracted from the sea sand compared to the copper applied to the sea sand. The recoveries are listed as percent recovery of total copper content and listed as a 95% confidence interval.

than the CAPLE result listed above (t_{crit}=4.30, t_{calc}=6.15). The iron oxide coated

soil requires the heat of the CAPLE system to remove the bound metals from the

surface of the soil. The recoveries of the sodium modified ambient extractions were

13.4 ∀ 3.4%. Ion exchange occurred quickly with this soil, 2 minutes or less, and

did not free up any other exchange sites by removing the ion exchangeable metals,

as shown from the similar recoveries at different extraction times of the sodium

modified ambient extractions. The ambient extractions without any additives did not

produce detectable quantities of copper.

2.3.3 HUMIC ACID COATED SEA SAND

The humic acid coated sea sand adsorbed Cu at 260 µg Cu/g sand, as

shown with the copper equilibrium graph, Figure 2.2. The CAPLE, EDTA modified

extractions provided an average of 96.6 ± 12% of the total copper available. The

sodium modified extractions provided 47.5 ± 7.4% total copper and the

non-modified system provided 16.3 ± 9.8%. ANOVA analysis confirmed that there

was not statistical difference between the recoveries obtained at the different

temperatures (F_{crit} = 6.94, F_{calc} = 0.68) and there was a statistical difference

between the modifying conditions (F_{crit} = 6.94, F_{calc} = 2490). This indicates that

increasing the temperature does not statistically change the copper recovery, and

the additive EDTA does statistically change the copper recovery compared to a

sodium additive and no additive.

The retained sand did not provide a mass balance for all modifying

situations, using Method 3050, for the humic acid coated soil. It was noted that

Displayed as a confidence interval, α=0.05, t_{crit}=2.92

Soil Coating/ Time of extraction	EDTA %	Na$^+$ %	No %
Fe			
2 min	51.4 ± 4.7	13.2 ± 4.7	nd
20 min	53.3 ± 2.6	13.3 ± 5.2	nd
30 min	68.3 ± 5.0	13.6 ± 7.6	nd
HA			
2 min	61.6 ± 7.1	37.1 ± 3.3	31.9 ± 8.0
20 min	88.9 ± 3.1	37.3 ± 6.4	31.6 ± 6.7
30 min	90.0 ± 5.8	39.8 ± 3.3	29.6 ± 6.4
Combo			
2 min	46.5 ± 8.2	30.1 ± 6.3	11.3 ± 4.2
20 min	67.8 ± 5.9	40.2 ± 5.2	13.1 ± 4.2
30 min	75.7 ± 8.3	49.7 ± 8.6	13.3 ± 4.3

TABLE 2.7: RECOVERIES OF AMBIENT EXTRACTIONS, WT/WT%
The recoveries of copper extracted with the Ambient Extraction procedure are listed above. The recoveries are wt/wt% of copper extracted from the sea sand compared to the copper applied to the sea sand. The recoveries are listed as the 95% confidence interval of percent recovery of total copper content and are the average of three replicate extractions. Where "nd" is listed, the recovery of copper was not detected.

some of the humic acid substance did leach into the outlet valve and could have contained some of the copper material for the non-modified extractions. Non-extracted, humic acid coated sea sand was also analyzed with Method 3050. This analysis yielded a total copper content of the soil to be 259.0 ± 3.8 µg Cu/g sand. This value is not statistically different from the estimated copper content from the equilibration of the soil ($t_{calc} = 0.59$, $t_{crit} = 1.95$).

This soil, humic acid coated sea sand, was also subjected to ambient extractions to determine the efficiency of the heated, pressurized system. At a time of 30 minutes, using an EDTA modified solution, the soil yielded $90.0 \pm 5.8\%$ of the total copper available. This recovery is not statistically different from the recovery of the CAPLE system with an EDTA additive ($t_{calc} = 0.18$, $t_{crit} = 4.30$). Humic acid creates outer-sphere bonds with contaminant metals. These outer-sphere contaminants lend themselves to extraction fairly readily as shown by the ambient data. If a soil had a very high organic content, the majority of chelateable metals can be extracted at ambient conditions, after 30 minutes. However, for the inorganic soils, a highly chemisorbed media, after 30 minutes only $68.3 \pm 5.0\%$ of the total copper could be extracted.

2.3.4 COMBINATION COATED SOIL

The results from the three previous soils did not show an effect with varying the temperature from 98 - 120°C, ANOVA results above. Therefore, for the combination soil, only 98°C was studied. A total acid digestion of non-extracted, combination coated soil yielded an average of 297.9 ± 7.4 µg Cu/g soil. The

CAPLE extractions modified with EDTA yielded $93.8 \pm 2.6\%$ of the determined

copper content. The sodium modified system provided $51.3 \pm 0.2\%$ of the total

copper. The non-modified system provided $43.0 \pm 2.6\%$ of the copper in the

system. Total acid digestion of the retained sand afforded a mass balance.

The combination soil at ambient conditions with EDTA, gradually released,

after 30 minutes, 225.6 ± 24 µg Cu/g soil or $75.7 \pm 8.3\%$ of the total copper applied.

This value is statistically smaller than the recovery of copper from the combination

soil with the CAPLE/EDTA system ($t_{crit} = 6.69$, $t_{calc} = 2.92$). The sodium modified

system provided similar results to the CAPLE sodium modified system, $49.7 \pm 8.6\%$

after 30 minutes of static time. The non-modified ambient extractions provided a

recovery of $13.3 \pm 4.3\%$.

2.4 CONCLUSIONS

The density of room temperature and room pressure water is 997.06

kg/m^3.[38] When the water is heated and pressurized to CAPLE conditions, the

density decreases by 3% to a value of 966.46 kg/m^3. The decrease in density of

subcritical water is not as severe as the decrease in density approaching the critical

point, indicating at the sub-boiling temperatures and pressures of the CAPLE

system the solvent, water, maintains the desirable properties of liquid water and the

enhanced extractability of a heated system. This decrease in density is shown not

to hamper the extraction of the copper from the soils. As shown by the ambient

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[38] Haar, L.;Gallagher, J.; Kell, G. <u>Steam Tables</u>. Washington, D.C : Hemisphere Publishing Corporation. **1984.**

extraction data, the higher density of room temperature and room pressure water does not afford a higher extraction recovery for any of the synthetic soils. The dielectric constant of room temperature water also decreases as the temperature is increased. Room temperature water has a dielectric value of 78.5 debye, and water at 98°C has a dielectric value of 57.5 debye.[39] Although this is a 27% drop in dielectric value, the water maintains enough polarizability to solvate the ionic complexes. Following the same argument used with the change in density, the higher dielectric value of room temperature water did not afford higher recoveries than the lower dielectric value of the CAPLE system.

The inorganic soil, Fe coated, creates inner-sphere bonds with the contaminants and requires the more aggressive CAPLE system to extract the copper from the surface of the soil. The metals associated with the HA coated, organic soil does not require the reactive environment the inorganic soil requires because of the weaker outer-sphere bonds created with the HA substance[4] and the contaminant metals. The combination coated system did release a substantial amount of copper with ambient conditions, due to the humic acid outer coating, but with the CAPLE system the extraction can be considered near unity. The combination soil required the more reactive CAPLE system to release the chemisorbed contaminants underneath the loosely bound humic acid system.

The use of EDTA to extract adsorbed metals from synthetic soils has been shown to be an effective remediative technique when coupled with heated, pressurized water. The sodium chloride solution could not produce the same high

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[39] <u>CRC Handbook for Chemistry and Physics</u>. New York: CRC Press. **1992**.

extraction efficiencies as EDTA, therefore ion exchange cannot be the predominant mechanism of metal-soil exchange. The chelate assisted pressurized, liquid extraction of metals from soils does provide higher recoveries of adsorbed metals than ambient conditions as shown by a one-tailed t-test. (t_{crit} = 4.30, t_{calc} = 2.92)

The CAPLE method is to be tested on a natural soil system. The next chapter describes the use of the CAPLE system with Loam, Clay and Sandy soils.

Chapter 3: Chelate Assisted, Pressurized, Liquid Extraction for the removal of metals from natural soils.

3.1 INTRODUCTION

In the previous chapter, CAPLE has been developed using a synthetic soil system. When the sea sand was coated with iron oxide, the recovery of adsorbed copper was slightly diminished when using EDTA. The reduction in recovery may be attributed to the inner-sphere complexes formed between the copper and the surface oxide sites that are more difficult for an extraction method to remove. The oxide coatings promote a chemisorption of the contaminant metal onto the surface of the soil particle. The outer-sphere complexes formed between the copper and the humic acid structure were readily chelated under ambient conditions, $90.0 \pm 5.8\%$, because of the weaker interactions in comparison to the chemisorbed metals, ambient recovery $68.3 \pm 5.0\%$. In this chapter three natural soil systems are utilized for study of a more realistic soil response to the CAPLE system.

Chelation techniques have been studied in both land-applied situations[40,41] and laboratory controlled conditions.[42] Jardine and Taylor[42] suggest that land application of EDTA promotes the leaching of metal chelates into ground water systems. This application can lead to metal contamination of natural aquifers[42].

[40] Li, Z; Shuman, L. *Soil Science*. **1996,** *161*, 226.
[41] Jardine, P.; Taylor, D. *Geoderma*. **1995,** *67,* 125.
[42] Kedziorek, M.; Dupuy, A.; Bourg, A.; Comprere, F. *Environmental Science and Technology*. **1998,** *32,* 1609.

The natural soils for this study included a loam, clay and sandy soil. Each group of soils contained four sub-groupings that were applied to this study. For each group there was a standard soil; a naturally amended soil; iron oxide coated, Cu equilibrated soil and a humic acid coated, Cu equilibrated soil. The natural soils were coated to study the propensity of the soils to create inner and outer sphere complexes with unusually high concentrations of surface coatings. To learn more about the soil/CAPLE interactions, the coating and equilibration procedures were the same as used in Chapter 2. From the conclusions of Chapter 2, varying the temperature from 98-120°C did not result in extraction data that were statistically different. For this study, the temperature was fixed at 98°C and the pressure was maintained at 100 ATM, to eliminate the phase change involved in super-heating water.

In this chapter the CAPLE method was tested with natural soils. The natural soils create a more porous and challenging environment for the chelate to find the surface metals. The natural soils were coated to study the ability of the CAPLE system to remove surface contamination from a porous substrate with abnormally high concentrations of inorganic and organic coatings.

The CAPLE system was then tested against an accepted method of contaminant analysis, the sequential extraction. The first four steps of the extraction remove the surface metals, therefore, the sum of the recoveries of the first four steps are defined as 100% of all available surface metals. The recoveries of the CAPLE system were also tested against extractions at ambient conditions. These extractions are both ambient temperature and pressure. The extractions

then progress to include analysis with ambient temperature, pressurized extractions and ambient pressure, heated extractions. By studying the recovery of the CAPLE system with respect to several non-CAPLE extraction sets, the mechanism of the CAPLE system can be better understood.

3.2 EXPERIMENTAL

All extractions for this work were performed on the same modified Suprex SFE 50 (Lincoln, NE), supercritical fluid extractor used in Chapter 2. The extraction vessel used was a 1.67 mL capacity, empty, HPLC, Keystone (Bellefonte, PA) vessel fitted with 0.5 μm frits at each end.

Three solvent conditions were employed to coincide with the work from the previous chapter. Because of the potential for a higher concentration of metals available for chelation and exchange on the soil, the concentration of the additives was increased to accommodate the potentially large total contaminant concentration. The chelating agent was 0.2 M disodium ethylenediamine tetra-acetic acid (EDTA) (Fisher Scientific, Fairlawn, NJ). A 400 μL spike delivers 37.4 mg of EDTA.   To study ion exchange phenomena, a 0.4 M sodium chloride solution was prepared. For the sodium solution, a 400 μL spike delivers 9.3 mg of sodium chloride. There was also a system that did not involve a chelate or an ion exchange solution, that system relied solely on the heated, pressurized water which will be referred to as (NO).

3.2.1 PREPARATION OF THE SOIL

The natural soils were coated in order to study the effectiveness of CAPLE on a coated, porous media. This phase of the study was to gain a better understanding of the mechanism of the CAPLE system.

The coated soils were based on their appropriate standard soil obtained from Virginia Polytechnic Institute and State University, Department of Crop, Soil and Environmental Science. Three replicate extractions were performed for each soil, as well as all of the non-extracted, total acid digestions.

The standard and amended soils were prepared by Martens *et. al.*[43,44,45] The standard soils were sampled near the amending sites, or prior to the application of the amending material. The Loam soil was amended with an aerobically digested sludge from a wastewater treatment plant. The soil was isolated with a plastic membrane to prevent lateral contamination from other sources.[43] The Clay soil was amended with copper and zinc sulfates over a fifteen-year period.[44] The Sandy Soil was amended with copper enriched, animal sludge over a three year period of time.[45] All of the soils were statistically sampled with an appropriate grid pattern, dried and packaged for analysis.

Sample soil properties are contained in Table 3.1. Soil components are characterized not only by their chemical composition but also by their particle size. The amount of clay, silt and sand comprising each soil is listed in the following

[43] Rappaport, B.; Scott, J.; Martens, D.; Reneau, R.Jr.; Simpson, T. *Availablility and Distribution of Heavy Metals, Nitrogen and Phosphorus from Sewage Sludge in the Plant-Soil-Water Continuum*, Virginia Water Resources Research Center, Virginia Tech, Bulletin 154, **1987**.
[44] Mullins, G.; Martens, D.; Gettier, S.; Miller, W.   *Journal of Environmental Quality.* **1982,** *11*, 573.
[45] Mullins, G.; Martens, D.; Miller, W.; Kornegay, E.; Hallock, D. *Journal of Environmental Quality.* **1982,** *11*, 316.

	Loam[44] %	Clay[45] %	Sandy[46] %
Clay	37.6	49.0	5.9
Silt	47.3	40.0	26.0
Sand	15.3	11.0	68.1
Organic matter	1.8	1.3	1.7
pH	6.3	6.7	6.4
CEC – meq/100g	12.5	11.2	5.6

TABLE 3.1: SAMPLE SOIL CHARACTERISTICS OF SOILS TO BE STUDIED
These soil characteristics display the differences in the soil components. The pH's
of the soils are in the natural buffering region for soils. All three of these soils will
buffer their own solutions. The CEC is the cation exchange capacity. This value
measures the soil's ability to replace native cations with contaminant cations.

table. The pH of the soils used in this study are between 6.3 – 6.7.[43,44,45] Soils with pH's in this range are considered natural buffers and with this extraction system, should not need the addition of a buffering chemical.[46] The CEC or cation exchange capacity is also listed. This value is the soil's ability to exchange a surface cation with a cation in solution. The higher the CEC value, the more likely the soil will adsorb contaminant metals.[47]

The Iron Oxide coated soil was prepared as described in Chapter 2, with Fe_2O_3 obtained from Fisher Scientific (Fairlawn, NJ) and using a variation of the procedure described by Scheidegger *et. al.*[47] A solution of 30 g Fe_2O_3 and 100 mL dilute HNO_3, at a pH=2.5, was placed on an orbital shaker for 24 hours. To this solution, 30 g of each standard soil were added in separate beakers. This mixture was placed in a 120°C oven for 24 hours and then allowed to cool to room temperature. The soil was then washed several times with 18 MΩ water until the rinse water was clear. This soil was then equilibrated with a 3000 ppm cupric chloride solution. The mixture was shaken periodically and 1.0 mL aliquots were taken at various time intervals for analysis. The equilibration was deemed complete after 24 hours, as shown in Figure 3.1a.

The humic acid coated soil was also prepared as described in Chapter 2, by first purifying the technical grade humic acid (HA) obtained from Fisher Scientific (Fairlawn, NJ). Approximately 4 g of HA was dissolved in water and centrifuged at

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[46] McBride, M. Environmental Chemistry of Soils. New York: Oxford University Press. **1994.**
[47] Scheidegger, A.; Borkovec, M.; Sticher, H. *Geoderma*. **1993,** *58*, 43-65.

A) Copper Equilibration of Fe coated soils.
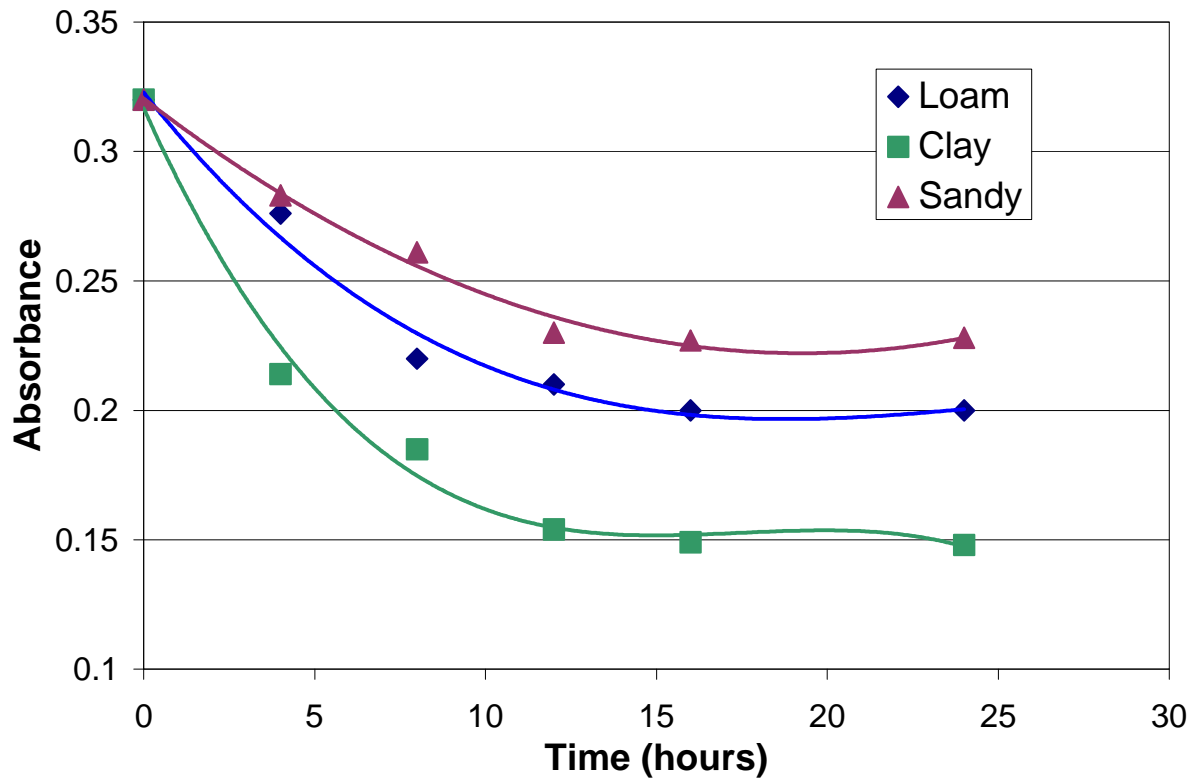
FIGURE 3.1: COPPER EQUILIBRATION OF SOILS. A) IRON COATED SOILS B) HUMIC ACID COATED SOILS

These graphs display the steady state achieved with the coated natural soils after 24 hours of equilibration with a copper solution. The copper solutions were analyzed with FAAS, $\lambda=324.8$nm.

B) Copper Equilibration of HA coated soils.
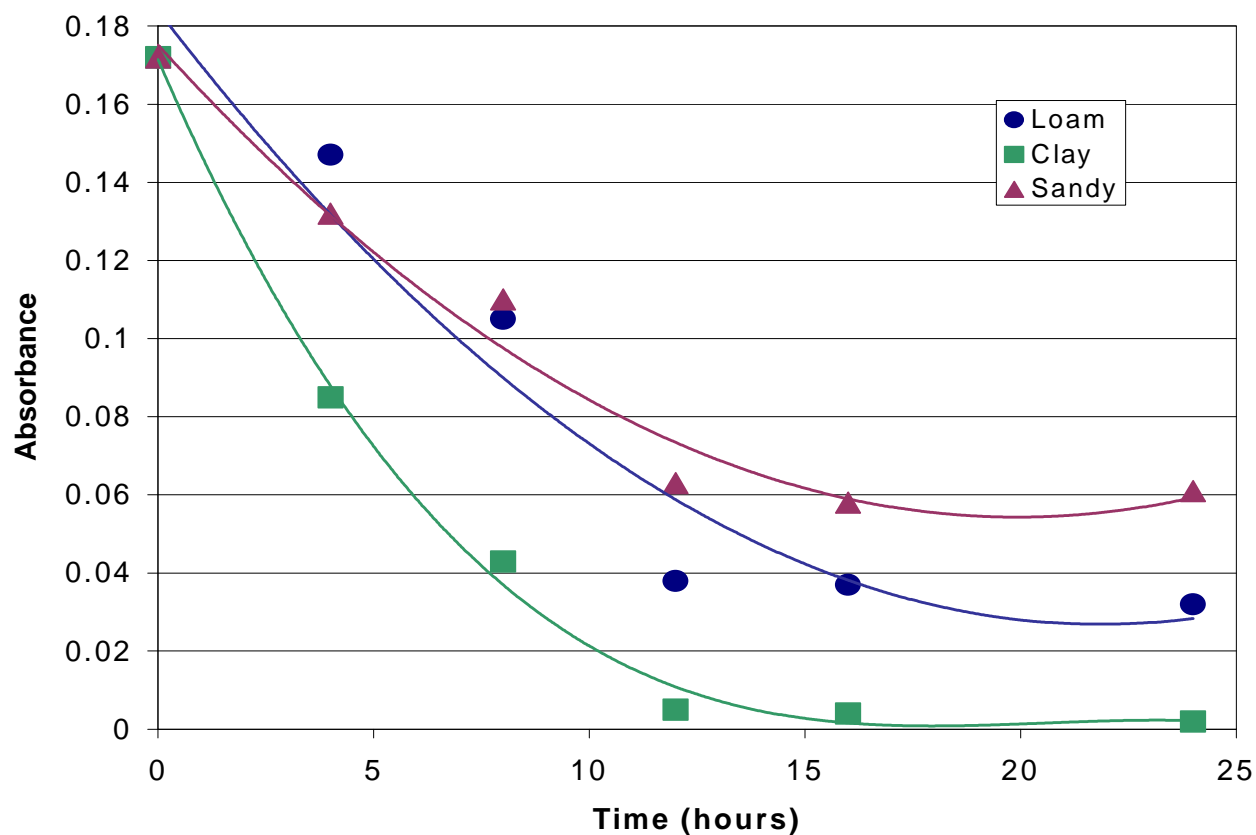
These graphs display the steady state achieved with the coated natural soils after 24 hours of equilibration with a copper solution.

10,000 rpm for 10 minutes. The supernatatent liquid was then filtered through a

Whatman 542 hardened ashless filter (Kent, England). The purified HA was then

precipitated from the solution by decreasing the pH of the liquid with high purity HCl

(Fisher Scientific, Fairlawn, NJ) to a pH ~ 1. At this pH, the purified HA can be

filtered from the liquid and dried. A solution of 2000 ppm HA (pH=9) was mixed with

30g of each standard soil in separate beakers and allowed to equilibrate for 48

hours at a pH=6. The HA coated soil was then equilibrated with a 3000 ppm cupric

chloride solution for 24 hours, as described above, with 1.0 mL aliquots taken to

determine the equilibrium concentration of copper adsorbed to the prepared soil, as

shown in Figure 3.1b.

3.2.2 SEQUENTIAL EXTRACTIONS

Sequential extractions were performed on all of the natively amended soils

studied in this chapter using Tessier's sequential extraction method.[48] These

extractions were performed in triplicate. A flow chart of the steps of the extractions

is located in Figure 3.2. A 1 g sample of amended loam soil was accurately

weighed and placed into a 50-mL polyproplyene centrifuge tube. An 8 mL aliquot of

1 M sodium acetate at a pH of 8.2 was added to the tube. The sample was capped

and placed on a shaker table for continuous agitation for one hour at room

temperature. The tubes were then placed into a centrifuge for 30 minutes at 10,000

rpm. The supernatant liquid was carefully removed and made to volume in a 50.0-

mL volumetric flask. The soil was washed with 8 mL of 18 MΩ water. The tube was

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[48] Tessier, A.; Campbell, P.; Bisson, M. *Analytical Chemistry.* **1979,** *51*, 844.

Surface Metals

Geological Metals

Step 1:

Exchangeable

8 mL 1M sodium acetate pH=8.2, 1 hour, R.T., continuous agitation

Step 2:

Bound to

Carbonates

8 mL 1M sodium acetate pH=5, adjusted with acetic acid, 3 hours, R.T., continuous agitation

Step 3:

Bound to Fe-

Mn Oxides

20 mL 0.04M NH_2OH HCl in 25% (v/v) acetic acid 3 hours, 96°C, occasional agitation

Step 4:

Bound to

Organic Matter

4a) 3 mL 0.02M nitric acid, 5 mL H_2O_2 2 hours, 85°C, occasional agitation

4b) 5 mL 3.2M amino acetate in 20% (v/v) nitric acid, 7 mL 18 MΩ water 0.5 hour, R.T., continuous agitation

Step 5:

Residual

Total Acid

Digestion

shaken by hand and placed back into the centrifuge for 30 minutes as described

 sample, decanting the supernatant liquid and washing the soil is hereafter referred

to as "the centrifuging procedure."

To the remaining soil, 8 mL of 1 M sodium acetate, pH of 5 adjusted with

acetic acid, was added. The samples were then placed on the shaker table for 3

hours for constant agitation at room temperature. The centrifuge procedure was

repeated. The residue from the above extraction was then heated at 96°C for 3

hours with 20 mL of 0.04 M NH_2OH HCl in 25% (v/v) acetic acid, and was shaken

occasionally. The solution was cooled to room temperature and the centrifuging

procedure was repeated.

To the residue from the above extraction, 3 mL of 0.02 M nitric acid and 5 mL

of hydrogen peroxide (30%) were added and heated at 85°C for 2 hours with

occasional shaking. The sample was cooled and 5 mL of 3.2 M amino acetate in

20% (v/v) nitric acid and the sample was further diluted to 20 mL with 18 MΩ water.

The sample was placed on the shaker table for 30 minutes of continuous agitation.

The centrifuging procedure was repeated.

To the remaining residue, a total acid digestion was performed. EPA method

3050 was used in place of Tessier's acid digestion because of the use of HF and to

coordinate the acid digestion step with the other acid digestions performed in these

studies.

3.2.3 CAPLE EXTRACTIONS

A 1 g-soil sample was analytically weighed and placed into the extraction vessel. Any additive that was to be used was first placed on top of the soil, and then the vessel was placed into the extraction system. The system was brought to equilibrium, at conditions of 100 ATM and 98°C, and the outlet valve was opened to collect the extraction fluid. The collection vessel was a 50.0-mL class A volumetric flask. After a flush volume was obtained (8-15 minutes) the system was prepared for another sample.

All of the soils contain a clay fraction and because of the clay content there was vessel plugging. Acid washed sea-sand, from Chapter 2, was mixed, 1:1, to the sample and then placed into the vessel.[49] The addition of the sea sand did not statistically effect recoveries for any of the soils, but did alleviate slow output for the first 4 minutes of extraction. The calculated t_{calc} values for the loam, clay and sandy soils were 0.02, 0.97, 1.03 respectively with t_{crit}= 2.92.

3.2.4 AMBIENT PRESSURE & TEMPERATURE EXTRACTIONS

The ambient temperature and ambient pressure extractions were performed in 50-mL beakers. The soil was weighed analytically into a 1 g sample and placed in a beaker. The additive, if any, was added, with 10 mL of 18 MΩ H_2O. The solution was allowed to sit for the appropriate length of time, 2, 10 or 30 minutes, and was then filtered through a pre-wet Whatman 542 hardened ashless filter (Kent,

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[49] Scokart, P.;Meeus-Verdinne, K.; DeBorger, R. *International Journal of Environmental Analytical Chemistry*. **1987,** *29*, 305.

England) into a 50.0-mL class A volumetric flask. Additional water was added to ensure quantitative transfer of the soil and solution to the filter.

3.2.5 TOTAL ACID DIGESTIONS

The total acid digestion procedure was EPA method 3050, "Acid digestion of sediment, sludge and soils." A 1g sample of soil was placed in a small beaker and 10 mL of 1:1 HNO_3 was added. This solution was swirled and covered with a watch glass to reflux at $95^{o}C$ for 10 minutes. The sample was cooled and 5 mL of concentrated HNO_3 was added and the solution was refluxed again for 30 minutes. The solution was cooled and this step was repeated. The solution was then evaporated to 5 mL. To the cool acidic solution, 2 mL of 18 MΩ H_2O and 3 mL of 30% H_2O_2 was slowly added. The solution was covered and heated until the effervescence subsided. To this cool solution, 1 mL of 30% H_2O_2 was added and the sample was heated again. This was repeated until the effervescence was minimal or a total of 10 mL of H_2O_2 was added. The solution was cooled again and 5 mL of concentrated, redistilled HCl was added with 10 mL 18 MΩ H_2O. The beaker was covered again and refluxed for 15 minutes. The solution was cooled and filtered through a 542 Whatman hardened ashless filter (Kent, England) and made to 100 mL in a class A volumetric flask.

3.2.6 ANALYTE ANALYSIS

The extractions were diluted volumetrically to 50.0-mL with 18 MΩ water and analyzed with FAAS. For the analysis of copper, standard conditions were used.[50] Samples were stored at room temperature in sealed bottles and analyzed within 24 hours.

A portion of 18 MΩ water was used as the blank for the FASS measurements. The elemental levels were quantitated by determining the elemental metal concentration of each extraction run via analytical working curves. Samples were in the analytical working range for copper. The calculations were performed with Microsoft Excel '98.

3.3 RESULTS

3.3.1 SEQUENTIAL EXTRACTION RESULTS

It has been demonstrated that each step of the sequential extraction removes a different type or layer of metals from the surface of a soil particle.[56] The first step removes the "exchangeable" metals or those commonly mobile in a soil environment. The second step removes the metals bound to carbonates. The third step removes the metals bound to the iron and manganese oxides that coat the surface of the soil particle. The fourth step removes the metals bound to organic matter, also covering the surface of the soil particle. The final step is a total acid digestion performed to remove the "residual" or geological metals bound in the

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[50]Perkin Elmer Corp. Analytical Methods for Atomic Absorption Spectrometry. **1994**, 69.

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Soil	Step	μg Cu/g soil	Wt/wt %
Standard Loam	1	7.6 ± 0.3	47.5 ± 8.1
	2	0.7 ± 0.2	4.3 ± 1.2
	3	nd	nd
	4	0.7 ± 0.4	4.3 ± 2.5
	5	7.8 ± 1.2	48.8 ± 7.5
	Sum of 1 - 4	*9.0 ± 1.2*	*56.3 ± 7.7%*
Standard Clay	1	nd	nd
	2	3.8 ± 0.9	4.5 ± 1.1
	3	7.2 ± 3.4	8.5 ± 4.0
	4	nd	nd
	5	74.7 ± 5.1	87.9 ± 6.0
	Sum of 1 - 4	*11.0 ± 3.5*	*12.9 ± 4.1%*
Standard Sandy	1	nd	nd
	2	nd	nd
	3	nd	nd
	4	nd	nd
	5	147.4 ± 4.5	104.5 ± 3.2
	Sum of 1 - 4	*nd*	*nd*

TABLE 3.2: SEQUENTIAL EXTRACTION RECOVERY OF COPPER, STANDARD SOILS

The sequential extractions yielded results displayed above. The recoveries are listed in parts per million or μg copper per g soil, as well as in wt/wt %total copper. The wt/wt % total copper column is calculated as a ratio of the total amount of copper determined in the sequential extraction step (section 3.3.1) vs. the amount of copper determined with total acid digestion (section 3.2.5, Table 3.6). The recoveries are displayed as the 95 % confidence interval.

Soil	Step	μg Cu/g soil	Wt/wt %
Amended Loam	1	6.7 ± 3.3	4.0 ± 1.7
	2	12.1 ± 2.4	7.1 ± 1.2
	3	87.9 ± 8.2	51.6 ± 4.3
	4	17.5 ± 3.4	10.3 ± 1.7
	5	50.3 ± 4.1	29.6 ± 2.1
	Sum of 1 - 4	*124.1 ± 3.4*	*73.0 ± 2.0%*
Amended Clay	1	7.2 ± 2.5	6.6 ± 2.0
	2	9.6 ± 4.0	8.7 ± 3.2
	3	65.8 ± 7.3	59.8 ± 5.8
	4	9.6 ± 0.2	8.7 ± 0.1
	5	23.9 ± 3.9	21.8 ± 3.1
	Sum of 1 - 4	*92.2 ± 0.2*	*83.8 ± 0.1%*
Amended Sandy	1	5.7 ± 0.3	2.5 ± 0.1
	2	9.1 ± 0.5	4.0 ± 0.2
	3	109.4 ± 12	47.5 ± 4.5
	4	80.5 ± 7.6	35.0 ± 2.9
	5	62.5 ± 2.3	27.2 ± 0.9
	Sum of 1 - 4	*204.7 ± 7.6*	*89.0 ± 4.5%*

TABLE 3.3: SEQUENTIAL EXTRACTION RECOVERY OF COPPER, AMENDED SOILS

The sequential extractions yielded results displayed above. The recoveries are listed in parts per million or μg copper per g soil, as well as in wt/wt %total copper. The wt/wt % total copper column is calculated as a ratio of the total amount of copper determined in the sequential extraction step (section 3.3.1) vs. the amount of copper determined with total acid digestion (section 3.2.5, Table 3.6). The recoveries are displayed as the 95 % confidence interval.

Soil	Step	μg Cu/g soil	Wt/wt %
Iron Coated Loam	1	123.3 ± 2.5	8.3 ± 0.2
	2	58.9 ± 3.7	4.0 ± 0.2
	3	868.9 ± 7.4	58.7 ± 5.0
	4	89.6 ± 5.2	6.0 ± 0.4
	5	310.5 ± 5.4	20.9 ± 0.4
	Sum of 1 - 4	*1140.8 ± 10.1*	*77.0 ± 0.7%*
Iron Coated Clay	1	243.9 ± 6.2	11.7 ± 0.3
	2	7.1 ± 2.1	0.5 ± 0.1
	3	1583.2 ± 16	75.7 ± 0.8
	4	3.9 ± 0.6	0.2 ± 0.0
	5	256.8 ± 4.8	12.3 ± 0.2
	Sum of 1 - 4	*1838.1 ± 18.0*	*87.9 ± 0.9%*
Iron Coated Sandy	1	394.4 ± 6.7	19.0 ± 0.3
	2	24.3 ± 3.2	1.1 ± 0.2
	3	1268.0 ± 12	61.0 ± 0.6
	4	27.2 ± 7.3	1.3 ± 0.4
	5	370.7 ± 13	17.8 ± 0.7
	Sum of 1 - 4	*1713.9 ± 16.3*	*82.4 ± 0.8%*

TABLE 3.4: SEQUENTIAL EXTRACTION RECOVERY OF COPPER, FE COATED SOILS

The sequential extractions yielded results displayed above. The recoveries are listed in parts per million or μg copper per g soil, as well as in wt/wt %total copper. The wt/wt % total copper column is calculated as a ratio of the total amount of copper determined in the sequential extraction step (section 3.3.1) vs. the amount of copper determined with total acid digestion (section 3.2.5, Table 3.6). The recoveries are displayed as the 95% confidence interval.

Soil	Step	µg Cu/g soil	Wt/wt %
HA Coated Loam	1	247.7 ± 4.3	12.8 ± 0.2
	2	1172.0 ± 14	60.8 ± 7.3
	3	193.2 ± 8.5	10.0 ± 0.4
	4	267.0 ± 5.7	13.8 ± 0.3
	5	55.2 ± 2.3	2.9 ± 0.1
	Sum of 1 - 4	*1879.9 ± 18.0*	*97.5 ± 0.9%*
HA Coated Clay	1	nd	nd
	2	824.3 ± 7.4	27.1 ± 0.2
	3	1168.6 ± 13	38.5 ± 0.5
	4	621.6 ± 12	20.5 ± 0.4
	5	415.7 ± 9.8	13.7 ± 0.3
	Sum of 1 - 4	*2614.5 ± 20.1*	*86.0 ± 0.7%*
HA Coated Sandy	1	166.7 ± 7.5	6.2 ± 0.3
	2	974.2 ± 15	36.2 ± 0.6
	3	699.3 ± 8.7	26.0 ± 0.3
	4	498.9 ± 3.2	18.5 ± 0.1
	5	352.8 ± 6.6	13.1 ± 0.2
	Sum of 1 - 4	*2339.1 ± 19*	*82.4 ± 0.8%*

TABLE 3.5: SEQUENTIAL EXTRACTION RECOVERY OF COPPER, HA COATED SOILS

The sequential extractions yielded results displayed above. The recoveries are listed in parts per million or µg copper per g soil, as well as in wt/wt %total copper. The wt/wt % total copper column is calculated as a ratio of the total amount of copper determined in the sequential extraction step (section 3.3.1) vs. the amount of copper determined with total acid digestion (section 3.2.5, Table 3.6). The recoveries are displayed as the 95% confidence interval.

matrix of the soil particle.[52] The sequential extractions were performed on the naturally amended soils in triplicate, as described previously.

Tables 3.2 - 3.5 contain the average amount of metal obtained from each of the extraction steps for each soil. The sum of the sequential extraction steps 1-4 for the standard loam soil is 9.0 ± 1.2 µg Cu/g soil or $56.3 \pm 7.7\%$ of the total copper contained in the sample. The sum of sequential extraction steps 1-4 of the standard clay soil yielded 11.0 ± 3.5 µg Cu/g soil or $12.9 \pm 4.1\%$ of the total copper in the soil sample. For the standard sandy soil, the sum of the first four extraction steps yielded no detectable copper.

For the amended loam soil, 124.1 ± 3.4 µg Cu/g soil, $73.0 \pm 2.0\%$ total copper was extracted. The amended clay provided 92.2 ± 0.2 µg Cu/g soil or $83.8 \pm 0.1\%$ total copper as the sum of the first four steps of the sequential extraction. The Sandy soil granted 204.7 ± 7.6 µg/g soil or $89.0 \pm 4.5\%$ total copper as the sum of the first four steps of the sequential extraction.

The sum of the sequential extraction steps 1-4 for the iron coated loam soil is 1140.8 ± 10 µg Cu/g soil or $77.0 \pm 0.7\%$ of the total copper contained in the sample. The sum of sequential extraction steps 1-4 of the iron coated clay soil yielded 1838.1 ± 18 µg Cu/g soil or $87.9 \pm 0.9\%$ of the total copper in the soil sample. For the iron coated sandy soil, the sum of the first four extraction steps yielded 1713.9 ± 16 µg Cu/g soil or $82.4 \pm 0.8\%$.

For the humic acid coated loam soil the sum of the copper obtained from the first four steps of the sequential extractions is 1879.9 ± 18 µg Cu/g soil or $97.5 \pm$

0.9% total copper. The humic acid coated clay provided 2614.5 $\pm$ 20 μg Cu/g soil or 86.0 $\pm$ 0.7% total copper. The humic acid coated sandy soil as the sum of the first four steps of the sequential extraction 2339.1 $\pm$ 19 μg/g soil or 82.4 $\pm$ 0.8% total copper.

The ratio of copper extracted to total copper in the sample (wt/wt %) was compared for the CAPLE and Sequential extraction methods. The sum of the first four sequential extraction steps is defined as the total amount of copper available for extraction without removing the geological metals. When compared to the CAPLE results, the aforementioned sum is defined as 100%.

3.3.2 CAPLE E×TRACTION RESULTS

The average results of the CAPLE extractions for each soil are contained in Table 3.6 and Table 3.7. Table 3.6 lists the masses of copper obtained while Table 3.7 shows the calculated recoveries as wt/wt% available copper. The available copper was determined from the sum of the first four steps of the sequential extraction technique. As previously mentioned, EDTA was the only chelate used in this chapter. To rule out the phenomena of ion exchange, a Na^+ solution was used to show that EDTA was extracting the metals from the soils. Extractions without any additive were performed to analyze the amount of metal that could be removed simply with a heated water system.

3.3.2.1 CAPLE RESULTS OF THE LOAM SOILS

The loam standard soil extraction provided a recovery of 110.0 $\pm$ 12%. The natively amended loam soil recovered 107.7 $\pm$ 7.2% of the total available copper.

Soil	EDTA μg/g	Na⁺ μg/g	No Additive μg/g	Total Cu mg/g soil
Loam				
Standard	9.9 ± 1.1	6.0 ± 0.3	nd	16
Amend	134.0 ± 8.9	6.2 ± 0.2	nd	170
Fe	1150.2 ± 95	68.6 ± 2.8	44.2 ± 4.1	1481
HA	1863.3 ± 99	188.7 ± 12	68.4 ± 6.3	1928

Soil	EDTA μg/g	Na⁺ μg/g	No Additive μg/g	Total Cu mg/g soil
Clay				
Standard	12.3 ± 0.3	nd	nd	85
Amend	92.1 ± 4.5	nd	nd	110
Fe	1868.7 ± 74	150.1 ± 6.0	80.8 ± 8.6	2091
HA	2603.1 ± 34	nd	nd	3037

Soil	EDTA μg/g	Na⁺ μg/g	No Additive μg/g	Total Cu mg/g soil
Sandy				
Standard	nd	nd	nd	141
Amend	216.8 ± 14	nd	nd	230
Fe	1820.9 ± 96	214.4 ± 22	163.6 ± 19	2080
HA	2330.3 ± 161	167.6 ± 15	nd	2690

TABLE 3.6: CAPLE EXTRACTION RECOVERIES OF CU FROM NATURAL SOILS
The recoveries of copper from the natural soils extracted with CAPLE are listed above in μg copper / g soil or ppm of copper. These recoveries are calculated with a working curve. The recoveries are the average of three replicates and are listed as the 95% confidence interval.

C.I. = Confidence Interval, α=0.05, t_{crit}=2.92

Soil	EDTA %	Na$^+$ %	NO Additive %
Loam			
Standard	110 ± 12	66.7 ± 3.3	nd
Amend	107.7 ± 7.2	5.0 ± 0.2	nd
Fe	100.8 ± 8.3	6.0 ± 0.4	3.8 ± 0.3
HA	99.1 ± 5.2	10.0 ± 0.7	3.6 ± 0.3

Soil	EDTA %	Na$^+$ %	NO Additive %
Clay			
Standard	111.8 ± 3.7	nd	nd
Amend	99.9 ± 4.9	nd	nd
Fe	101.7 ± 4.1	8.1 ± 0.3	4.4 ± 0.5
HA	99.6 ± 1.3	nd	nd

Soil	EDTA %	Na$^+$ %	NO Additive %
Sandy			
Standard	nd	nd	nd
Amend	105.9 ± 7.0	nd	nd
Fe	106.2 ± 4.5	12.5 ± 1.3	9.5 ± 1.1
HA	99.6 ± 6.9	7.1 ± 0.7	nd

TABLE 3.7: CAPLE EXTRACTION RECOVERIES OF CU FROM NATURAL SOILS, WEIGHT %
The recoveries of copper from the natural soils extracted with CAPLE are listed above as the 95% confidence interval. The percent recoveries are calculated from amount of copper removed with the CAPLE system in comparison to the sum of the total copper removed from steps 1-4 via sequential extraction.

Similar to the natively amended soil, the iron oxide coated loam soil released $100.8 \pm 8.3\%$ of the total available copper. The CAPLE method extracted $99.1 \pm 5.2\%$ of the total copper in the humic acid coated loam soil. The ion exchange extractions with the loam series of soils provided a $66.7 \pm 3.3\%$ of copper from the standard soil, $5.0 \pm 0.2\%$ of copper from the natively amended loam soil, $6.0 \pm 0.4\%$ of copper from the iron oxide coated loam soil and $10.0 \pm 0.7\%$ of copper from the humic acid coated loam soil. The CAPLE extractions performed on the loam series of soils without any additive provided no detectable amount of copper for the standard and the natively amended soils. For the other loam soils the copper was extracted without any additive at amounts of $3.8 \pm 0.3\%$, iron coated and $3.5 \pm 0.3\%$ for the humic acid coated loam soil. This leads to the conclusion that for all of the amended loam soils only a small portion of adsorbed copper is available via ion exchange.

3.3.2.2 CAPLE RESULTS OF THE CLAY SOILS

The standard clay soil yielded $111.8 \pm 3.7\%$ of copper with an EDTA modifier and the CAPLE method. The natively amended soil released $99.9 \pm 4.9\%$ of the total available copper in that soil. With the iron oxide coated clay soil, the surface copper was removed with an efficiency of $101.7 \pm 4.1\%$ of the copper. The humic acid coated clay soil had $99.6 \pm 1.3\%$ of the total copper removed with an EDTA additive and the CAPLE method.

The extractions to study ion exchange with the clay soil series, the sodium modified, CAPLE extractions, did not provide detectable copper except for the iron

coated clay soil. This soil released $8.1 \pm 0.3\%$ of the available copper contained in the soil with the sodium additive. The CAPLE extractions with no modifying agents also did not provide any detectable copper except for the iron coated clay soil. Again, this soil released, $4.4 \pm 0.5\%$ of the available copper contained in the iron coated clay soil.

3.3.2.3 CAPLE RESULTS OF THE SANDY SOILS

The standard sandy soil did not provide any detectable copper from the CAPLE extractions with any of the three conditions studied. The natively amended sandy soil extracted $105.9 \pm 7.0\%$ of the copper available with the EDTA modified CAPLE method. The sodium modified and non-modified CAPLE extractions did not provide detectable copper from the natively amended sandy soils. The iron coated sandy soil provided a recovery of $106.2 \pm 4.5\%$ of the total copper contained in the soil. The humic acid coated sandy soil supplied $99.6 \pm 6.9\%$ of the total copper contained in the soil. The sodium modified CAPLE extraction yielded detectable amounts of copper for the iron coated and humic acid coated sandy soils, $12.3 \pm 1.3\%$ of the total available copper in the iron coated sandy soil and $7.1 \pm 0.7\%$ of the total available copper in the humic acid coated soil. The recoveries for the iron coated sandy soil were also detectable, $9.5 \pm 1.1\%$ without a modifying agent.

The CAPLE and Sequential Extraction results were compared. The sums of steps 1-4, the steps that remove the non-geological or surface metals, were not statistically different than the CAPLE results obtained in this chapter for any of the soils studied. In this comparison the actual mass of copper calculated for the

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extractions were compared. This is due to the fact that the CAPLE results are reported as wt/wt% with respect to the sum of steps 1-4 of the sequential extraction process. Table 3.8 contains the t_{calc} values for each of the soils.

The weight percent geological copper for the amended, Iron Oxide coated and HA coated soils in each soil group are not statistically different (F_{calc}=1.21, F_{crit}=5.14) indicating after an amending process, the surface copper was obtainable via chelate assisted, pressurized, liquid extraction and the use of EDTA.

3.3.3 RESULTS OF EXTRACTIONS AT AMBIENT CONDITIONS

Extractions were carried out at ambient conditions to show the need for the heat and pressure of the CAPLE system. The same additive conditions were used, EDTA or Na^+ or no additive. All of these soils were subjected to ambient extractions, at room temperature and room pressure. The results of the ambient extractions are listed in Table 3.9a-c.

The largest recovery of copper from the loam series of soils was from the iron coated, loam soil and the EDTA condition. There was an 81.3 ± 7.0% recovery after 20 minutes and an 87.4 ± 1.6% recovery of available copper after 30 minutes. The recovery of 87.4 ± 1.2% is statistically different from the 100.8 ± 8.3% recovery using the CAPLE method (t_{calc} = 4.55, t_{crit} = 4.30), indicating that the CAPLE method is still superior to the ambient method of extraction for this soil.

Of the clay-based soils, the standard clay soil provided the highest recoveries with an EDTA and 30 minutes of equilibration time, 122.5 ± 18%. This

Soil	Sum of Sequential Steps 1-4, μgCu/g soil	CAPLE recoveries, μgCu/g soil	t_{calc}	Same or Different
Standard Loam	9.0 ± 1.2	9.9 ± 1.1	1.22	Same
Standard Clay	11.0 ± 3.5	12.3 ± 0.3	0.97	Same
Standard Sandy	nd	nd	1.46	Same
Amended Loam	124.1 ± 3.4	134.0 ± 8.9	0.61	Same
Amended Clay	92.2 ± 0.2	92.1 ± 4.5	0.18	Same
Amended Sandy	204.7 ± 7.6	216.8 ± 14	1.52	Same
Iron Coated Loam*	1140.8 ± 10	1150.2 ± 95	0.15	Same
Iron Coated Clay*	1838.1 ± 18	1868.7 ± 74	0.09	Same
Iron Coated Sandy*	1713.9 ± 16.3	1820.9 ± 96	0.80	Same
HA Coated Loam*	1879.9 ± 18.0	1863.3 ± 99	0.16	Same
HA Coated Clay*	2614.5 ± 20	2603.1 ± 34	0.47	Same
HA Coated Sandy*	2339.1 ± 19	2330.3 ± 160	0.42	Same

TABLE 3.8: COMPARISON OF SEQUENTIAL STEPS 1-4 AND CAPLE RECOVERIES FOR EACH SOIL

This table displays the t_{calc} values for the comparison of the sum of the sequential extraction steps 1-4 and the CAPLE recoveries for each soil. The two-tailed t-test for means, at 95% confidence was used. For all of the soils the t_{crit} is 2.92. The table shows that the CAPLE recoveries for each of the soils studied are statistically similar to the sum of the sequential extraction steps 1-4. This result shows that the CAPLE method is as effective as the sequential method for removing surface metals.   *The soils designated with an asterisk were coated standard soil that was equilibrated with copper after coating. The other soils were not altered in the laboratory.

C.I. = Confidence Interval, α=0.05, t_{crit}=2.92

Soil / Time	EDTA %	Na$^+$ %	No %
Standard			
2 min	nd	nd	nd
20 min	nd	nd	nd
30 min	nd	nd	nd
Amend			
2 min	21.9 ± 0.1	nd	nd
20 min	22.7 ± 0.1	nd	nd
30 min	28.9 ± 3.8	nd	nd
Fe			
2 min	56.3 ± 5.1	nd	nd
20 min	81.3 ± 7.0	16.7 ± 5.1	nd
30 min	87.4 ± 1.6	27.8 ± 1.1	nd
HA			
2 min	48.9 ± 1.8	nd	nd
20 min	54.1 ± 1.8	nd	nd
30 min	55.5 ± 3.5	nd	nd

TABLE 3.9 A: RECOVERIES OF CU VIA AMBIENT EXTRACTION, LOAM SOIL
The recoveries of copper obtained with the ambient extraction procedure are listed above and are the average of three replicate extractions. These percent recoveries are listed as a 95% confidence interval and based on total copper content of the soils.

C.I. = Confidence Interval, α=0.05, t_{crit}=2.92

Soil / Time	EDTA %	Na$^+$ %	No %
Standard			
2 min	95.8 ± 9.2	nd	nd
20 min	105.1 ± 10	nd	nd
30 min	122.5 ± 18	nd	nd
Amend			
2 min	39.8 ± 1.4	nd	nd
20 min	39.7 ± 1.3	nd	nd
30 min	45.0 ± 3.0	nd	nd
Fe			
2 min	44.9 ± 2.0	nd	nd
20 min	47.2 ± 5.3	nd	nd
30 min	56.4 ± 11	nd	nd
HA			
2 min	23.8 ± 0.8	nd	nd
20 min	77.3 ± 0.9	nd	nd
30 min	76.4 ± 0.6	nd	nd

TABLE 3.9 B: RECOVERIES OF CU VIA AMBIENT EXTRACTION, CLAY SOIL
The recoveries of copper obtained with the ambient extraction procedure are listed above and are the average of three replicate extractions. These percent recoveries are listed as a 95% confidence interval and based on total copper content of the soils.

C.I. = Confidence Interval, α=0.05, t_{crit}=2.92

Soil / Time	EDTA %	Na$^+$ %	No %
Standard			
2 min	nd	nd	nd
20 min	nd	nd	nd
30 min	nd	nd	nd

Amend			
2 min	33.0 ± 4.0	nd	nd
20 min	31.2 ± 4.3	nd	nd
30 min	32.8 ± 1.2	nd	nd

Fe			
2 min	52.5 ± 0.5	nd	nd
20 min	52.5 ± 0.5	nd	nd
30 min	63.6 ± 7.9	nd	nd

HA			
2 min	9.8 ± 0.8	nd	nd
20 min	29.1 ± 0.9	nd	nd
30 min	34.7 ± 0.2	nd	nd

TABLE 3.9 c: RECOVERIES OF CU VIA AMBIENT EXTRACTION, SANDY SOIL
The recoveries of copper obtained with the ambient extraction procedure are listed above and are the average of three replicate extractions. These percent recoveries are listed as a 95% confidence interval and based on total copper content of the soils.

Soil	Ambient Recovery, wt/wt%	CAPLE Recovery, wt/wt%	t_{calc}	Same or Different
Loam				
Amend	28.9 ± 3.8	107.7 ± 7.2	53.98	Different
Fe	87.4 ± 1.6	100.8 ± 8.3	4.55	Different
HA	55.5 ± 3.5	99.1 ± 5.2	11.46	Different

Soil	Ambient Recovery, wt/wt%	CAPLE Recovery, wt/wt%	t_{calc}	Same or Different
Clay				
Amend	45.0 ± 3.0	99.9 ± 4.9	20.40	Different
Fe	56.4 ± 11	101.7 ± 4.1	27.07	Different
HA	76.4 ± 0.6	99.6 ± 1.3	16.60	Different

Soil	Ambient Recovery, wt/wt%	CAPLE Recovery, wt/wt%	t_{calc}	Same or Different
Sandy				
Amend	32.8 ± 1.2	105.9 ± 7.0	31.26	Different
Fe	63.6 ± 7.9	106.2 ± 4.5	7.92	Different
HA	34.7 ± 0.2	99.6 ± 6.9	14.70	Different

TABLE 3.10: T-STATISTICS COMPARING AMBIENT EXTRACTIONS AND CAPLE

The statistical test of a "Two tailed t-test for means" at a 95% confidence was performed to display the statistical difference of the recoveries obtained at ambient temperature and pressure with EDTA and the CAPLE extraction with EDTA. The ambient recovery listed was the recovery obtained after 30 minutes using EDTA. The wt/wt% values were the ratio of amount of Cu recovered compared to the sum of sequential extraction steps 1-4. The critical t value is 2.92 for these tests. The null hypothesis was that the recoveries for the different methods were the same. The alternate hypothesis was that the recoveries for the different methods were different.

value is not statistically different from the recovery at 20 minutes, $105.1 \pm 10\%$ (t_{calc} = 0.21, t_{crit} = 4.30). The ambient recovery at 30 minutes, $122.5 \pm 18\%$, is not statistically different from the $111.8 \pm 3.7\%$ recovery using the pressurized, heated method (t_{calc} = 0.77, t_{crit} = 4.30). This shows that for the standard clay soil, the small amount of surface copper available for extraction leads to a high precision and accuracy error in the results.

The highest recovery obtained in an ambient extraction with the sandy soil series is with the iron coated soil, EDTA and 30 minutes equilibration time. The ambient recovery, $63.6 \pm 7.9\%$, is statistically different from the recovery of the CAPLE method, $106.2 \pm 4.5\%$ (t_{calc} = 7.92, t_{crit} = 4.30). As described in the previous chapter, this result indicates the inorganic soil needs the energetic CAPLE system to liberate the chemisorbed surface copper. Table 3.10 contains a statistical comparison of the ambient extraction recoveries and the recoveries obtained with CAPLE.

3.3.4 RESULTS OF EXTRACTIONS WITH AMBIENT PRESSURE/HEATED CONDITIONS AND AMBIENT TEMPERATURE/PRESSURIZED CONDITIONS

The results of the ambient extractions led to a study of pressure and temperature to determine which parameter had the most effect on the extraction efficiency. All of these studies used the amended loam soil. The same Suprex (Lincoln, NE) system was utilized for the pressure study at ambient temperature. Three pressures were chosen; 25, 50, 100 ATM, to study pressure changes from ambient to the pressure being studied in the CAPLE system. A hot plate was used

in the temperature study at ambient pressure.   All of the pressurized extractions were performed with EDTA. There was no statistical difference between the three pressures studied (t_{calc}= 0.04, 0.58, 0.71, t_{crit}= 2.92). The ambient temperature extractions yielded an average of 56.6 ± 4.9 µg Cu/g soil for all of the pressurized extractions. This is a 33.3 ± 2.1% yield of the total copper in the soil. There was also no statistical difference between the different temperatures at ambient pressure (t_{calc}= 1.51, 1.89, 2.60, t_{crit}= 2.92). The heated, ambient pressure extractions yielded an average of 111.5 ± 6.6 µg Cu/g soil or 89.8 ± 5.3% of the total copper in the soil when EDTA is used. This result, utilizing heat and not pressure, is statistically different from the CAPLE result listed above (t_{calc}=4.94, t_{crit}=4.30).   The sodium and non-modified conditions did not yield quantifiable amounts of copper, similar to the results of the CAPLE and ambient analysis.

3.3.5 PARTICLE SIZE ANALYSIS OF AMENDED SOILS BEFORE AND AFTER CAPLE

The three naturally amended soils used previously in this chapter were analyzed with a Scanning Electron Microscope (SEM) both before and after the CAPLE technique of extraction. The SEM pictures were used to qualitatively compare the soil particle size. The amended loam soil, Figure 3.3-a&b, shows that the CAPLE technique does not alter the particle size of the soil. The amended clay soil, Figure 3.4-a&b, showed that CAPLE does not break up the soil particles and the clay soil flocculates in the CAPLE system. Flocculation occurs with layer silicates in the clay and sand portion of soils when in the presence of Na^+. The EDTA used in this study was the disodium salt and was in high enough

concentration to encourage flocculation in the clay and sandy soils. Unlike the loam, the clay and sandy soils have large percentages of layer silicates. The amended sandy soil, Figure 3.5-a&b, also shows that CAPLE does not break up the soil particles and like the clay soil, flocculates after CAPLE. The SEM of the standard sandy soil before and after total acid digestion, Figure 3.6, showed a distinct difference in particle size. Before the digestion, the soil particles range from large, coarse sand particles to small, fine particles. After digestion the particles are all very fine. The total acid digestion breaks down the particles to allow a greater surface area to be exposed and consequently a greater amount of metals to be solubilized.

3.4 CONCLUSIONS

The combination of temperature and pressure in the CAPLE method of extraction lead to the $107.7 \pm 7.2\%$ recovery of total available copper contained in the amended loam soil. With heat at ambient pressure, a recovery of $89.8 \pm 5.3\%$ total copper was attained. This value is statistically different from the CAPLE value. ($t_{calc}= 3.80$, $t_{crit} = 2.92$)   The heated, ambient pressure extractions removed the deposited, sodium exchangeable and some of the EDTA exchangeable copper. Adding pressure to the heated system adds a solvent replacement step to flush the contaminants out of the system. The pressure facilitates the removal of the contaminants by removing the contaminated solvent from the system. Without the pressure there is not a complete removal of available metal. At ambient temperature with pressure, $33.3 \pm 2.1\%$ of the available copper is removed. The

71
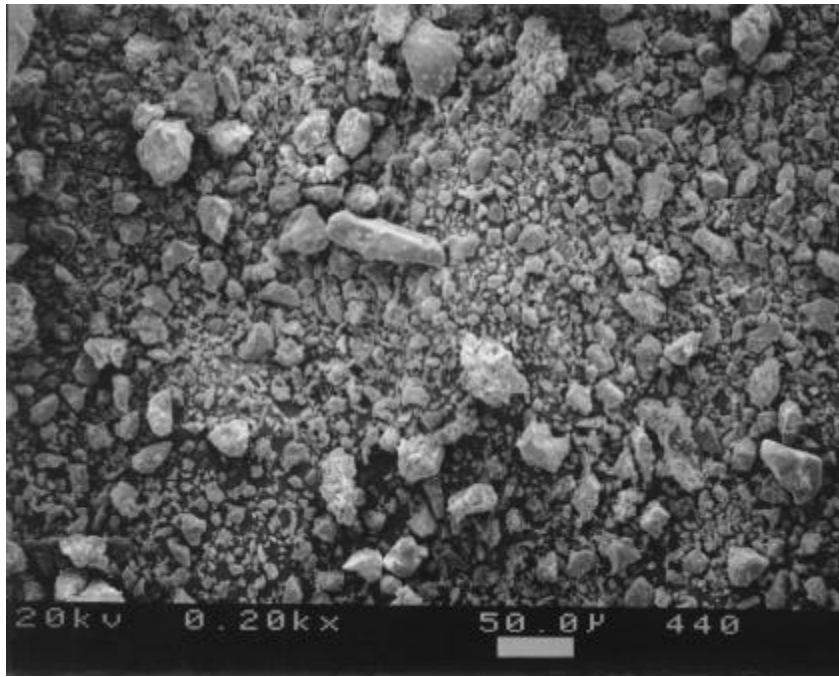
a) Amended Loam, Before CAPLE
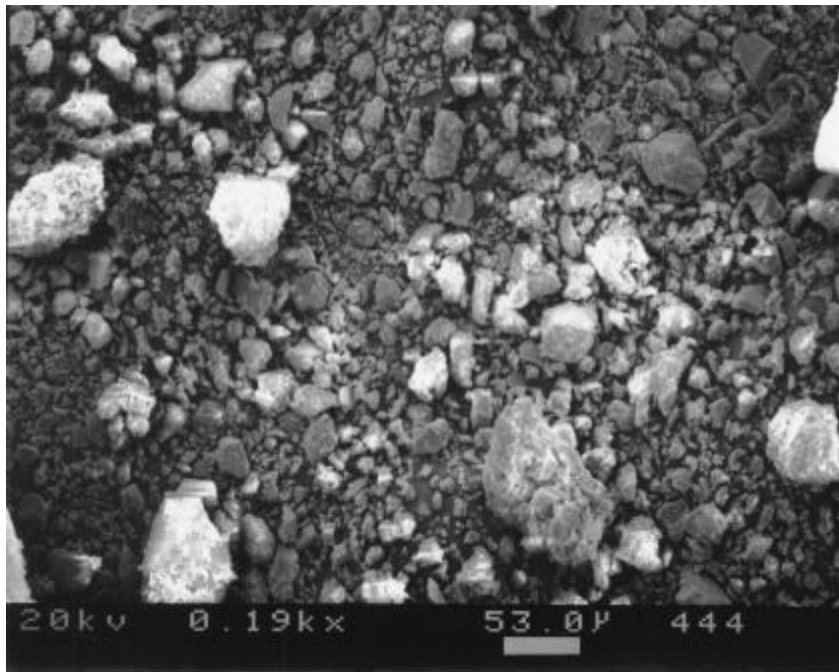
b) Amended Loam, After CAPLE

FIGURE 3.3: SEM OF AMENDED LOAM

a) SEM of the amended loam soil prior to the CAPLE method. b) SEM of the
    amended loam soil after the CAPLE method.
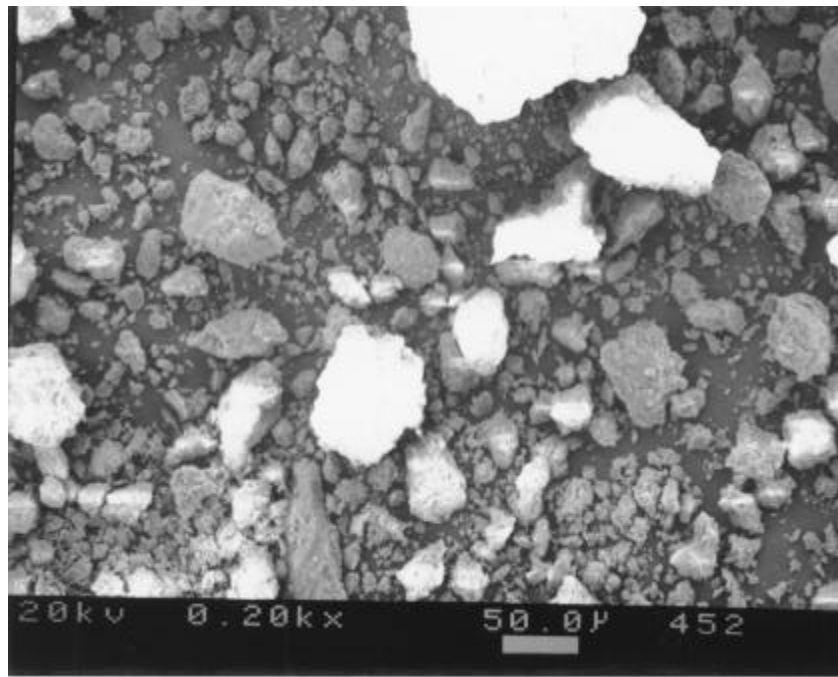
a) Amended Clay, Before CAPLE
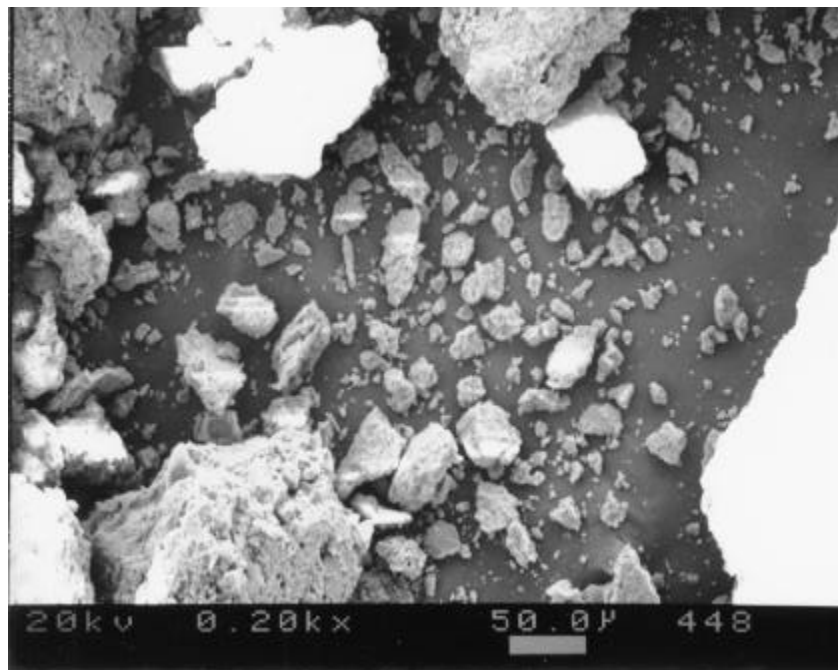
b) Amended Clay After CAPLE

FIGURE 3.4: SEM OF AMENDED CLAY
a) SEM of amended clay before the CAPLE method. b) SEM of amended clay, after the CAPLE method.
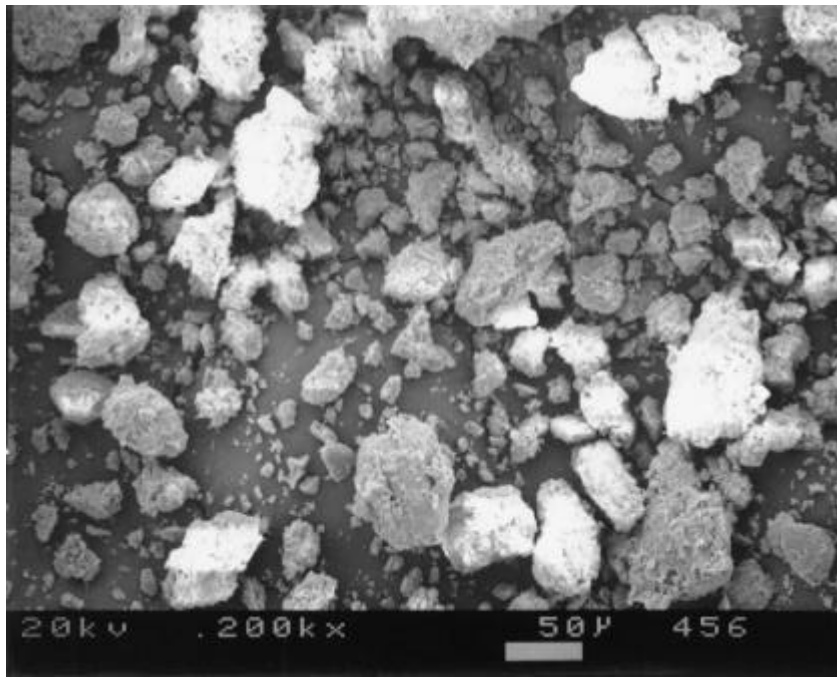
a) Amended Sandy Before CAPLE
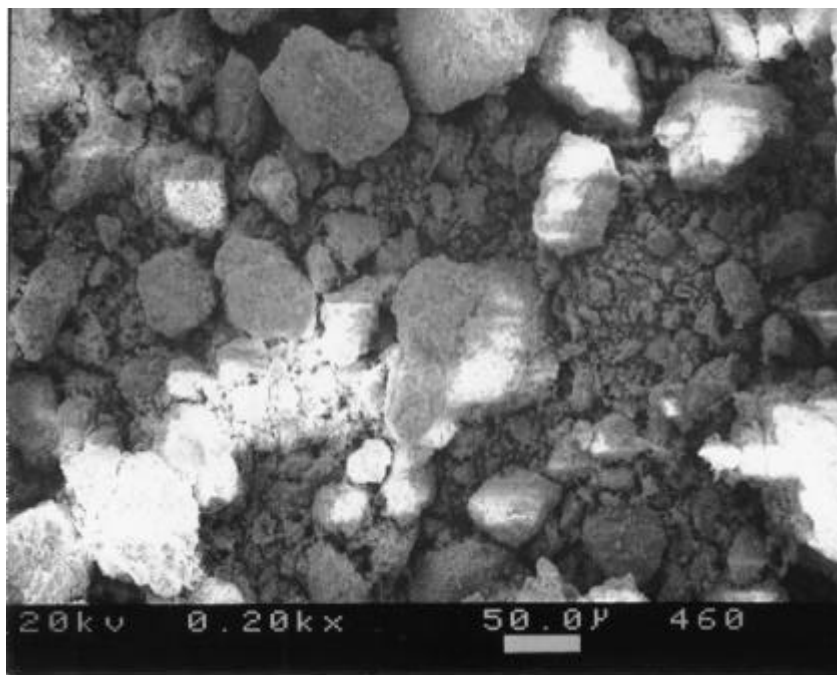
b) Amended Sandy After CAPLE

FIGURE 3.5: SEM OF AMENDED SANDY SOIL
a) SEM of amended sandy soil before CAPLE analysis b)SEM of amended sandy
soil after CAPLE analysis
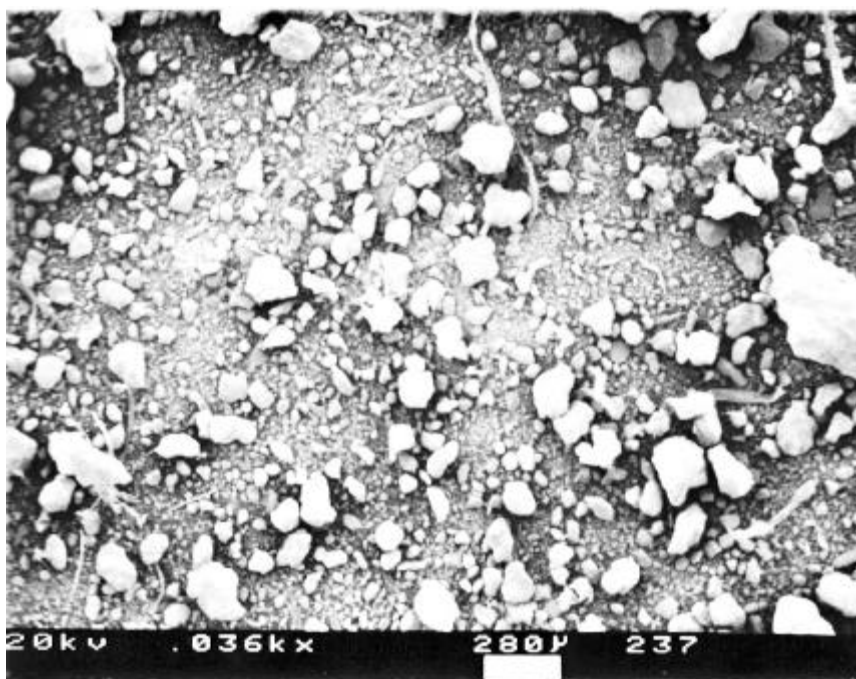
a) Sandy soil before extraction
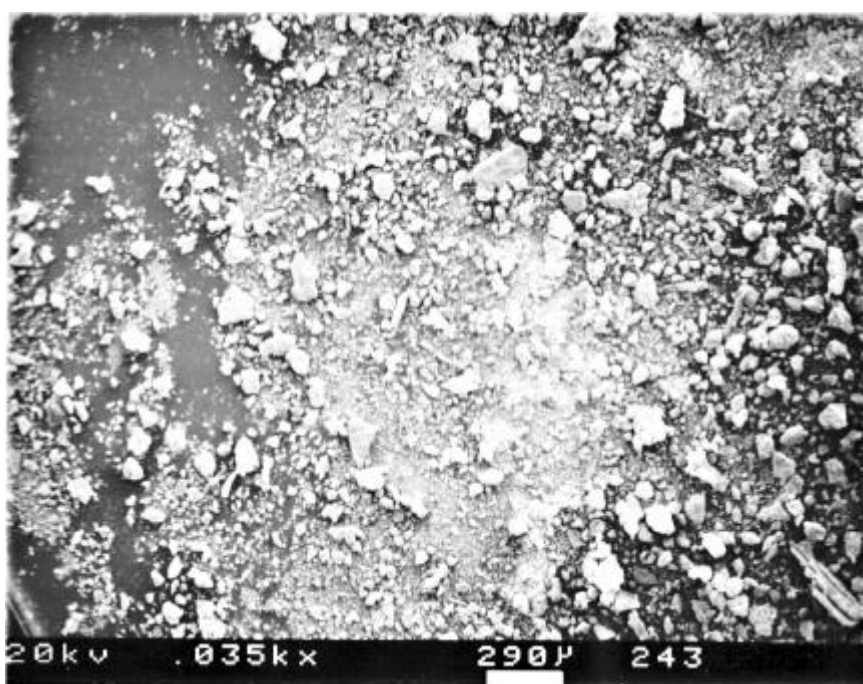
b) Sandy soil after total acid digestion

FIGURE 3.6: SEM OF STANDARD SANDY SOIL

a) SEM of standard sandy soil before extraction b) SEM of standard sandy soil after total acid digestion.[51]

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[51] Burcham, S. M.S. Thesis, Virginia Tech, **1994**.

addition of heat does provide a statistical increase in the removal of adsorbed copper ($t_{calc} = 6.13$, $t_{crit} = 4.30$). The heated system provides an increase in recovery of surface metals over the ambient temperature extractions. The combination of heat and pressure allow for the most complete and time efficient removal of the surface metals, without disturbing the soils geological matrix. As seen from SEM photographs, the size of the soil particles did not decrease after CAPLE, as it does with total acid digestion.

The CAPLE technique does not discriminate between inner-sphere and outer-sphere contaminants. It removes all surface contaminants as shown by the comparison between the CAPLE system and the sequential extraction technique. The CAPLE system removes the metals that are exchangeable, bound to carbonates, bound to iron or manganese oxides, and bound to organic matter.

In the next chapter, the CAPLE technique will be analyzed with different chelating agents.

Chapter 4: Analysis of Chelating agents in the CAPLE method.

4.1 INTRODUCTION

In the previous chapter, CAPLE was examined as an extraction method for adsorbed metal contaminants from soils. CAPLE was tested against the widely accepted, Sequential Extraction technique[50], to determine if CAPLE was successful in removing the surface metals and not disturbing the soil's matrix.

Furrer and Stumm[52] suggest a mechanism involving the release of metal ions from a soil surface. While studying the weathering processes of geothite and hematite, it was found that a ligand-promoted abstraction of metals occurs by creating a surface complex. This surface complex then polarizes the critical metal-oxygen bond to lead to the detachment of the surface metal species. In this chapter the chelate used in the CAPLE method will be varied to study the effect of the conditional formation constant of the metal-chelate complex, K'_{MY}. The three metals in this study are copper, zinc and iron. The four chelates in this study are ethylenediamine-tetra acetic acid, EDTA, diethylentriamine-penta acetic acid, DTPA, 1,2-cyclohexylene-dinitrilo-tetracetic acid, CDTA and ethylene bis(oxyethylene nitrilo)-tetracetic acid, EGTA, Figure 4.1.

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[52] Furrer, G.; Stumm, W. *Geochimica et Cosmoschimica Acta*, **1986,** *50,* 1847.

EDTA, ethylene diamine tetra acetic acid

Completely dissociated

DTPA, diethylentriamine-penta acetic acid

Completely dissociated

EGTA, ethylene bis(oxyethylene nitrilo)-tetracetic acid

Completely dissociated

CDTA, 1,2-cyclohexylene-dinitrilo-tetracetic acid

Completely dissociated
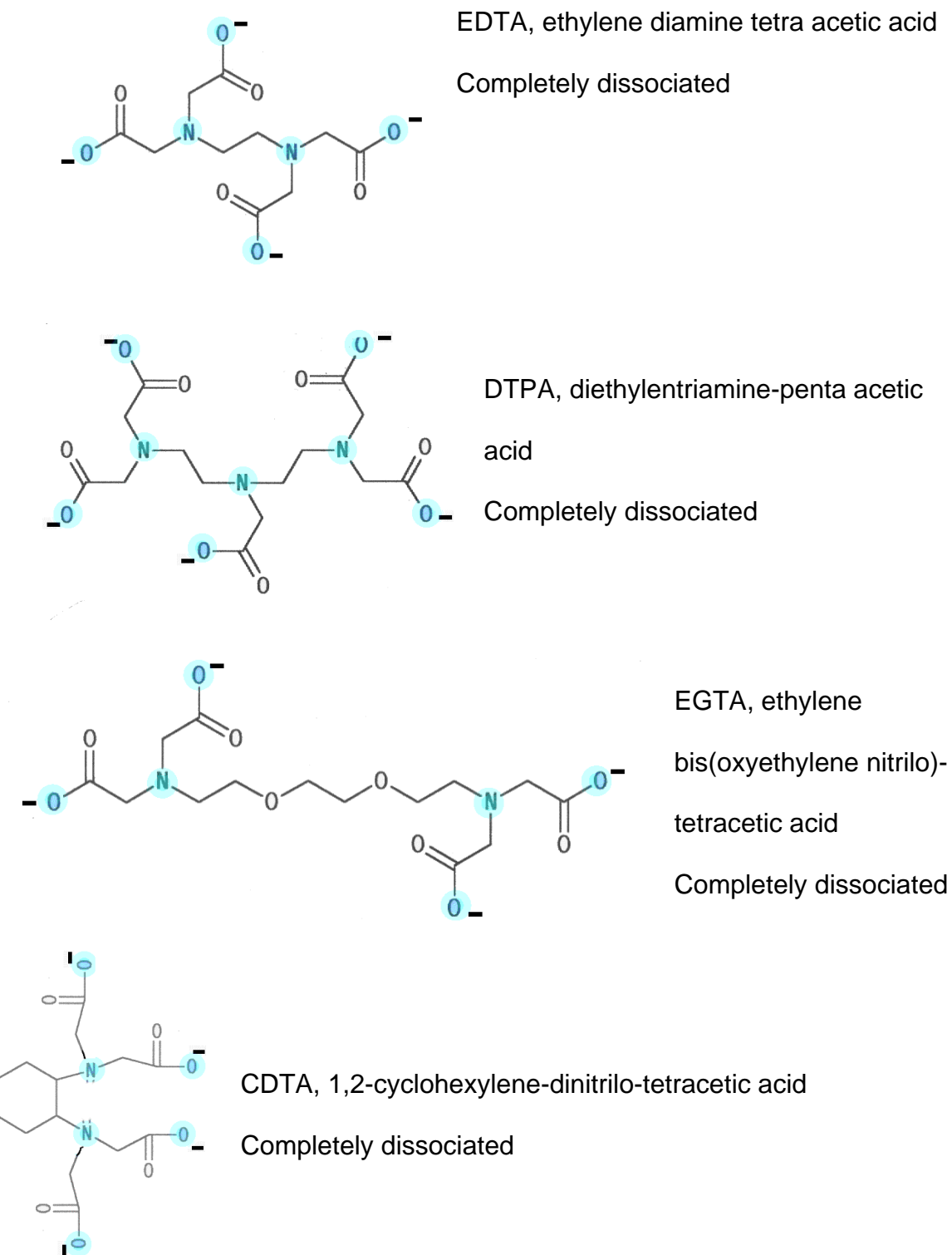
FIGURE 4.1: STRUCTURES OF THE CHELATES USED

The structures of the four chelating agents are shown. All of the chelates are shown in their completely dissociated form. The binding atoms of each chelate are highlighted in blue.

There is a long-standing precedent in the use of chelating agents in soil analysis.[53] There is a standardized EDTA extraction procedure to remove metals from the water soluble, easily exchangeable and strongly adsorbed ion fractions.[55,56] These extraction methods only remove 30-60% of the total surface metals, but they do allow the researcher to study some of the ions associated with the different fractions. Another limitation of this procedure is the time of one to two hours involved per extraction. EDTA has been studied in both land applied and laboratory controlled situations. The land applications of EDTA have been shown to greatly increase the mobility of adsorbed metals from soils and move them directly into ground water systems.[57,54,55] This procedure is a shifting of the polluted matrix, not a remediative technique.

DTPA, diethylentriamine-penta acetic acid, has also been used in soil analysis. It has been shown that DTPA analysis does not extract metals as efficiently as EDTA in fine-textured soils.[53] The formation constants for a number of metals are very similar for EDTA and DTPA. In many studies utilizing soils with larger particles these two chelates have performed very similarly because of their formation constants, see Table 4.1.

Two other chelating agents will be explored in this chapter along with EDTA and DTPA. They are 1,2-cyclohexylenedinitrilo tetracetic acid, CDTA and ethylene

[53] Li, Z.; Shuman, L. *Soil Science*, **1996,** *161*, 226.
[54] Jardine, P.;Taylor, D., *Geoderma*. **1995**, *67*, 125.
[55] Kedziorek,M.;Dupuy, A.; Bourg,A.;Compere,F.. *Environmental Science and Technology*. **1998**, *32*,1609.

Property	EDTA	DTPA	EGTA	CDTA
pKa_1	1.99	2.08	2.00	2.43
pKa_2	2.67	2.41	2.65	3.52
pKa_3	6.16	4.26	8.85	6.12
pKa_4	10.26	8.60	9.46	11.70
pKa_5	---	10.55	---	---

$Log\ K_{ML}^d$				
Ca	10.96	10.74	11.00	12.50
Cu (II)	18.80	21.53	17.80	21.30
Fe (II)	14.33	16.50	11.92	16.27
Fe (III)	25.1	28.60	20.50	28.05
Zn	16.50	18.75	14.50	18.67

TABLE 4.1: CHELATE PROPERTIES AND SELECTED FORMATION CONSTANTS[56]
This table of chelate properties displays the similarities and differences in the selected $log(K_{ML})$ constants for the chelates in this study.

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[56] Cheng, K.; Ueno, K.; Imaura, T. Handbook of Organic Analytical Reagents. Florida: CRC Press. **1982**.

bis(oxyethylene nitrilo)-tetracetic acid, EGTA. These chelates were chosen

because they are polydentate ligands and they all have an affinity for Cu, Zn, and

Fe, and their solubility in water.

4.2 EXPERIMENTAL

The Chelate Assisted, Pressurized, Liquid Extraction technique, developed in

Chapter 2, will be applied to three different classes of soils. These natural soils

included a loam, clay and sandy soil. For each group there was a standard soil and

an amended soil. As in Chapter 3, to eliminate the phase change of decompressing

superheated water, the temperature was fixed at 98°C, the pressure was

maintained at 100 ATM.

All extractions for this work were performed on the Suprex SFE 50 (Lincoln,

NE), supercritical fluid extractor described in Chapter 2. The extraction vessel was a

1.67 mL capacity, empty HPLC, Keystone (Bellefonte, PA) vessel fitted with 0.5 μm

frits at each end of the vessel.

4.2.1 PREPARATION OF THE SOIL

The soils were based on their appropriate standard soil obtained from

Virginia Polytechnic Institute and State University, Department of Crop, Soil and

Environmental Science. Three replicate extractions were performed for each soil at

each condition.

The standard and amended soils were prepared by Martens *et. al.*[48-50] as

described in Chapter 3. The standard soils were sampled near the amending sites,

or prior to the application of the amending material. The Loam soil was amended with an aerobically digested sludge from a wastewater treatment plant. The soil was isolated with a plastic membrane to prevent lateral contamination from other sources.[43] The Clay soil was amended with copper and zinc sulfates over a fifteen-year period.[44] The Sandy Soil was amended with copper enriched animal sludge over a three year period of time.[45] All of the soils were statistically sampled with an appropriate grid pattern, dried and packaged for analysis.

4.2.2 PREPARATION OF CHELATE SOLUTIONS

All of the chelate solutions were made to a concentration of 0.2 M with 18 MΩ water, pH = 8. A volume of 400 μL of chelate solution was used as a spike in the CAPLE extractions. The chelates used for this study were: disodium ethylenediamine tetra acetic acid, EDTA, (Fisher Scientific, Fairlawn, NJ), diethylentriamine-penta acetic acid, DTPA, (Aldrich Chemical, Milwaukee, WI), 1,2-cyclohexylenedinitrilotetracetic acid, CDTA (Fisher Scientific, Fairlawn, NJ), and ethylene bis(oxyethylene nitrilo)-tetra acetic acid, EGTA (Aldrich Chemical, Milwaukee, WI).

4.2.3 CAPLE EXTRACTIONS

A 1 g-soil sample was weighed and placed into the extraction vessel. The chelate to be used, described above, was placed on top of the soil and the vessel was placed into the extraction system. The system was brought to equilibrium, 100 ATM and 98°C, and the outlet valve was opened to collect the extraction fluid. The

collection vessel was a 50.0-mL class A volumetric flask. After a flush volume was obtained, time varied due to soil differences, the system was prepared for another sample. There was a noticeable difference in output volume with the clay and sandy soils. Mixing 1 g soil with 1 g pre-washed sea sand alleviated the slow output, as mentioned in Chapter 3.

4.2.4 TOTAL ACID DIGESTIONS

The total acid digestion procedure was EPA Method 3050, "Acid digestion of sediment, sludge and soils." A 1 g sample of soil was placed in a small beaker and 10 mL of 1:1 HNO_3 was added. This solution was swirled and covered with a watch glass to reflux at $95^{\circ}C$ for 10 minutes. The sample was cooled and 5 mL of concentrated HNO_3 was added and the solution was refluxed again for 30 minutes. The solution was cooled and this step was repeated. The solution was then evaporated to 5 mL. To the cool acidic solution, 2 mL of 18 MΩ water and 3 mL of 30% H_2O_2 was slowly added. The solution was covered and heated until the effervescence subsided. To this cool solution, 1 mL of 30% H_2O_2 was added and the sample was heated again. This was repeated until the effervescence was minimal or a total of 10 mL of H_2O_2 was added. The solution was cooled again and 5 mL of concentrated, redistilled HCl was added with 10 mL 18 MΩ water. The beaker was covered again and refluxed for 15 minutes. The solution was cooled and filtered through a 542 Whatman Hardened Ashless Filter (Kent, England) and made to 100 mL in a class A volumetric flask.

4.2.5 ANALYTE ANALYSIS

The extractions were diluted volumetrically to 50.0 mL with 18 MΩ water and analyzed with FAAS. Samples were stored at room temperature in sealed bottles and analyzed within 24 hours.

The elemental levels were quantitated by determining the elemental metal concentration of each extraction run. This was achieved by comparing the absorbance signal of the extraction run with a calibration curve constructed from standards of the appropriate metal. The linear range was determined and the extraction samples fell within the linear working curve.

The CAPLE results are analyzed with an ANOVA (Analysis of Variance: One Way). The ANOVA tool tests the variability of data sets to determine if an external variable is the cause for the perturbation of the results. The t-test is also used as a method of determining if the means of two sets of data are statistically similar or different. The calculations were performed with Microsoft Excel '98.

All extractions were assayed with a Perkin Elmer Flame Atomic Absorption Spectrometer using standard conditions.[53] There were three elements studied, copper, zinc and iron. The conditions of the FAAS for these three elements are contained in Table 4.2.

	Cu	Zn	Fe
λ	324.8 nm	214 nm	248.3 nm
Slit width	0.7 nm	0.2 nm	0.7 nm

TABLE 4.2: CONDITIONS FOR FAAS ANALYSIS
This table displays the wavelength and slit width used for analysis of the appropriate metals with the Perkin Elmer Flame Atomic Absorption Spectrometer. A multi element lamp was used for all of the analysis. An acetylene / air flame was also utilized.

4.3 RESULTS

4.3.1 CHELATE ANALYSIS

A study of the ligand dissociation constants shows that at a pH of 8, the pH of the chelate solutions, none of the chelates are in their most reactive form. The α_4 values were calculated, α_5 for DTPA, for all of the chelates. The α_4 value is the ratio of chelate in its completely dissociated state, Y^{4-}, to the chelate in all its other forms. An α_4 value of 1.00 would indicate 100% Y^{4-} in solution available to chelate metals. At a pH = 8, the α_4 of EDTA is $5.40 * 10^{-3}$. The ranking order of largest α_4 values are EDTA ($5.40 * 10^{-3}$), EGTA ($4.27 * 10^{-3}$), DTPA ($5.65 * 10^{-4}$), and CDTA ($1.97 * 10^{-4}$).

However, the CAPLE system is not at a pH of 8. The auto-ionization or ion-product constant of water changes with increased temperature. At room temperature, the K_w is known to be $1 * 10^{-14}$. This value indicates a hydrogen concentration of $1 * 10^{-7}$ or a pH of 7.00. When the temperature of water is increased to 100°C, the K_w is increased to $4.9 * 10^{-13}$.[57] This value indicates a hydrogen concentration of $7 * 10^{-7}$ or a pH of 6.15 for water at 100°C. Since the CAPLE system remains below 100°C, the pH of the CAPLE system can be estimated to be between 6.15 and 7.00. This range also happens to be the buffering range of the natural soils studied. As previously stated, all of the chelate solutions were made to a pH of 8. The small amount, 400 μL, of pH=8 solution used

[57] Skoog,D.;West,D.;Holler,J.Fundamentals of Analytical Chemistry.New York:Saunders College Publishing, **1996**.

in the system does not significantly alter the pH of the system because of the natural buffering conditions of the significantly larger volume of water and the pH of the soil. In light of these results, the α_4 and conditional formation constant, logK'_{MY}, values were calculated for the pH range of 6.15 to 7.00. These results can be found in Table 4.3.

The conditional formation constant of the metal-chelate complex is the formation constant at a particular pH value. The conditional formation constant, K'_{MY}, is calculated by multiplying the α_4 and the literature formation constant together. The ranking order of largest to smallest logK_{MY} values changed when the logK'_{MY} was calculated. For copper, the ranking order of largest to smallest logK'_{MY} values is CDTA, DTPA, EDTA, and EGTA. This order is followed for zinc as well. This indicates the theoretical order of the extraction efficiencies of the four chelates from greatest to least. The ranges for these values are found in Table 4.3.

4.3.2 CAPLE Extraction Results

The CAPLE extractions using the four chelates were analyzed for Cu, Zn, and Fe. Iron was only contained in the geological portion of most of the soils studied: therefore, the iron content was studied as a tag for the disruption of the soil's matrix. If a significant iron recovery was obtained, then it would be determined that the CAPLE system was disturbing the soil's matrix. The wt/wt% of metal extracted compared to the total available metal for the standard soils are contained in Table 4.4. The wt/wt% of metal extracted compared to the total

| | α_4 | | logK'$_{MY}$ Cu | | logK'$_{MY}$ Zn | |
	pH =6.15	pH=7.00	pH =6.15	pH=7.00	pH =6.15	pH=7.00
EDTA	3.90×10^{-5}	4.81×10^{-4}	14.39	15.48	12.09	13.18
DTPA*	1.48×10^{-6}	1.76×10^{-5}	15.47	16.55	12.84	13.92
EGTA	9.97×10^{-7}	4.82×10^{-5}	11.80	13.48	8.50	10.18
CDTA	1.42×10^{-7}	6.89×10^{-6}	14.68	16.37	11.90	13.59

* The α_5 value was calculated for DTPA.

TABLE 4.3: RANGE OF CHELATE CONSTANTS

This table lists the minimum and maximum values for the α_4 and logK'_{MY} values for copper and zinc. Although the exact pH of the CAPLE system cannot be calculated, the trend of the constants can be examined. Although the ranking order of α_4 values does change with the change in pH, the ranking order of logK'_{MY} values does not. Therefore, at the minimum and maximum pH of the system, the relative efficacy of the chelate does not change.

Results in % metal recovered compared to the total available metal content in the sample.

Standard Loam	Cu	Zn	Fe
EDTA	112.1 ± 11	29.9 ± 2.4	6.9 ± 0.2
CDTA	nd	89.8 ± 16	nd
DTPA	nd	10.5 ± 1.3	nd
EGTA	nd	22.4 ± 5.2	nd
No	nd	12.6 ± 9.7	nd

Standard Clay	Cu	Zn	Fe
EDTA	105.1 ± 3.1	22.1 ± 2.5	nd
CDTA	nd	47.4 ± 1.2	nd
DTPA	nd	25.3 ± 3.0	nd
EGTA	nd	13.8 ± 1.6	nd
No	nd	11.8 ± 2.6	nd

Standard Sandy	Cu	Zn	Fe
EDTA	nd	58.9 ± 1.9	nd
CDTA	nd	50.1 ± 5.7	nd
DTPA	nd	21.9 ± 5.2	nd
EGTA	nd	15.5 ± 3.6	nd
No	nd	10.9 ± 1.2	nd

TABLE 4.4: RESULTS OF CAPLE EXTRACTIONS FROM NATURAL STANDARD SOILS, WT./WT.%

This table presents the recoveries of three metals extracted from the standard and natively amended natural soils in a 95% confidence interval. These recoveries are calculated by comparing amount of metal extracted with the CAPLE system and the amount of metal removed in steps 1-4 of the sequential extraction procedure. Iron recovery is consistently low because of the high concentration of iron in the geological portion of the soil's matrix. As was shown in the previous chapter, the CAPLE technique does not disturb the geological portion of the soil matrix.

Results in % metal recovered compared to the total available metal content in the sample.

Amend Loam	Cu	Zn	Fe
EDTA	106.3 ± 8.6	16.7 ± 2.3	nd
CDTA	32.0 ± 14	41.6 ± 2.3	nd
DTPA	nd	15.7 ± 1.3	nd
EGTA	20.9 ± 6.2	12.8 ± 4.8	nd
No	nd	11.2 ± 1.5	nd

Amend Clay	Cu	Zn	Fe
EDTA	96.4 ± 4.7	40.2 ± 6.1	nd
CDTA	26.3 ± 4.0	16.9 ± 7.4	nd
DTPA	5.9 ± 3.8	20.0 ± 1.3	nd
EGTA	8.9 ± 0.8	20.8 ± 8.5	nd
No	nd	17.2 ± 6.5	nd

Amend Sandy	Cu	Zn	Fe
EDTA	108.1 ± 5.5	20.8 ± 3.6	nd
CDTA	23.1 ± 1.7	21.2 ± 3.3	nd
DTPA	nd	5.5 ± 0.4	nd
EGTA	59.4 ± 6.5	5.2 ± 2.9	nd
No	1.9 ± 0.1	3.3 ± 0.6	nd

TABLE 4.5: RESULTS OF CAPLE EXTRACTIONS FROM NATURAL AMENDED SOILS, WT./WT.%

This table presents the recoveries of three metals extracted from the natively amended natural soils. These recoveries are calculated by comparing amount of metal extracted with the CAPLE system and the amount of metal removed in steps 1-4 of the sequential extraction procedure. Iron recovery is consistently low because of the high concentration of iron in the geological portion of the soil's matrix. As was shown in the previous chapter, the CAPLE technique does not disturb the geological portion of the soil matrix.

available metal for the amended soils are tabulated in Table 4.5. The total available

metal was determined by sequential extraction in Chapter 3. The sum of the first

four steps of the sequential extraction is defined as the available metal. The first

four sequential extraction steps remove all of the surface metals.

4.3.2.1 CAPLE RESULTS OF THE STANDARD LOAM SOIL

For the standard loam soil, EDTA removed 112.1 $\pm$ 11% of the total available

copper contained in the soil. This value is not statistically different from the results

obtained with EDTA in Chapter 3 (t_{calc} = 0.79, t_{crit} = 4.30), displaying the

reproducibility of the method. The other chelating agents studied did not remove

detectable amounts of copper. The result without any additive was not statistically

different from the result obtained in Chapter 3 with no modifier (t_{calc} = 0.05, t_{crit} =

4.30), also indicating the reproducibility of the CAPLE method. When analyzed for

zinc, EDTA removed 29.9 $\pm$ 2.4% of the total available zinc content. The chelate

CDTA removed 89.8 $\pm$ 16% of available zinc in the soil.   The DTPA removed 10.5 $\pm$

1.3% of zinc and the chelate EGTA removed 22.4 $\pm$ 5.2% of available zinc in the

soil. Without any chelate, 12.6 $\pm$ 9.7% of the zinc was removed. The zinc recovery

with DTPA and no chelate are statistically similar (t_{calc} = 3.74, t_{crit} = 4.30) implying

that the DTPA did not remove the zinc despite the high logK_{MY} of DTPA, 18.75. The

recovery of zinc for EDTA and EGTA are statistically similar (t_{calc} = 1.43, t_{crit} = 4.30)

indicating that the two orders of magnitude difference between the logK_{MY}s is not

significant for these chelates. When these extractions were analyzed for iron,

EDTA recovered 6.9 $\pm$ 0.2% of the total available iron content of the soil. This value

is statistically different from the recoveries of the other chelates ($F_{calc} = 56.0$, $F_{crit} = 4.07$). The extractions performed with CDTA, DTPA, EGTA, and no chelate resulted in iron recoveries that were not statistically different ($F_{calc} = 3.49$, $F_{crit} = 4.07$) from no detectable recovery.

4.3.2.2 CAPLE RESULTS OF THE STANDARD CLAY SOIL

Extractions with the standard clay soil and EDTA resulted in a recovery of 105.1 ± 3.1% available copper in the soil. This result was not statistically different from the result obtained in Chapter 3 with EDTA ($t_{calc} = 0.60$, $t_{crit} = 4.30$), again indicating the reproducibility of the CAPLE method. The other chelates did not recover detectable amounts of copper from the standard clay soil although the logK'_{MY} range for DTPA (14.68-16.37) and CDTA (15.47-16.55) are greater than that of EDTA (14.39-15.48) for copper. The extractions performed without a chelating agent did not recover detectable amounts of copper. This result was not statistically different from the result obtained in Chapter 3 with no modifier ($t_{calc} = 0.60$, $t_{crit} = 4.30$), also indicating the reproducibility of the method. The recoveries of copper from the extractions performed with CDTA, DPTA, EGTA, and without a chelate are statistically similar ($F_{calc} = 2.78$, $F_{crit} = 4.07$), showing that these chelates are as in effective at removing the surface metals as unmodified water. The recoveries of copper from the standard clay soil with EDTA are statistically different to the recoveries obtained without a chelating agent ($t_{calc} = 5.17$, $t_{crit} = 4.30$). The EDTA recoveries of copper are statistically different from the other recoveries of copper obtained for the standard clay soil ($F_{calc} = 13.30$, $F_{crit} = 3.48$), showing that EDTA is better suited than the other chelates studied for the extraction of copper

from standard clay soil. When the extractions were analyzed for zinc content, EDTA recovered 22.1 ± 2.5% of the available zinc in the soil. The chelate CDTA recovered 47.4 ± 1.2% available zinc, DTPA recovered 25.3 ± 3.0% zinc, and EGTA recovered 13.8 ± 1.6% available zinc contained in the standard clay soil. The extractions performed without a chelating agent resulted in a recovery of 11.8 ± 2.6% total zinc. The logK'_{MY} ranges of EDTA and DTPA are very similar and consequently, the zinc recoveries for EDTA and DTPA are statistically similar $(t_{calc} =$ 2.78, $t_{crit} = 4.30)$ indicating that these two chelates react similarly to zinc on the surface of the standard clay soil. The mean results from the use of EGTA and no chelate were statistically the same $(t_{calc} = 2.78, t_{crit} = 4.30)$ indicating that EGTA, with the weakest logK'_{MY} range for zinc of the chelates studied did not preferentially extract the metal. The chelate CDTA extracted the greatest amount of zinc, 47.4 ± 1.2%, from the standard clay. This result follows the logK'_{MY} value trend. The logK'_{MY} values of CDTA are greater than the other chelates studied. The extractions of the standard clay soil did not produce any detectable iron with any of the chelates. This result would indicate that the iron contained in the standard clay soil is in the geological matrix only available for analysis through a total acid digestion.

4.3.2.3 CAPLE RESULTS OF THE STANDARD SANDY SOIL

The standard sandy soil extractions with EDTA produced no detectable copper. This result was not statistically different from the result obtained in Chapter 3 with EDTA ($t_{calc} = 3.78, t_{crit} = 4.30$), indicating the reproducibility of the method.

The other chelates studied also did not provide detectable amounts of copper. The

extractions with no chelate also resulted in no detectable copper. This result was

not statistically different from the result obtained in Chapter 3 with no modifier (t_{calc} =

3.78, t_{crit} = 4.30). The results of the copper analysis for the standard sandy soil with

the chelate EDTA and the extraction without chelate were statistically the same (t_{calc}

= 0.79, t_{crit} = 4.30). When the extractions were analyzed for zinc, EDTA resulted in

a recovery of 58.9 $\pm$ 1.9%. The chelate CDTA recovered 50.1 $\pm$ 5.2% which is not

significantly different from the recovery of EDTA, 58.9 $\pm$ 1.9% (t_{calc} = 2.67, t_{crit} =

4.30). The recovery of zinc using DTPA was 21.9 $\pm$ 5.2% available zinc. The

recovery of zinc for the chelate EGTA from the standard sandy soil was 15.5 $\pm$ 3.6%

which is not statistically different without using any chelate, 10.9 $\pm$ 1.2% (t_{calc} = 3.01,

t_{crit} = 4.30). The recovery of zinc using the chelates DTPA and EGTA are

statistically similar (t_{calc} = 3.88, t_{crit} = 4.30). The CAPLE extractions with the

standard sandy soil did not provide any detectable iron. This indicates, as with the

clay soil, all of the iron content of the standard sandy soil is contained within the

soil's geological matrix.

4.3.2.4 CAPLE RESULTS OF THE AMENDED LOAM SOIL
        The extractions with the natively amended loam soil provided 106.3 $\pm$ 8.6%

available copper content when the CAPLE method was performed with EDTA. This

result was not statistically different from the result obtained in Chapter 3 with EDTA

(t_{calc} = 0.47, t_{crit} = 4.30). This result is statistically different (t_{calc} = 6.35, t_{crit} = 4.30)

from the result obtained with CDTA, 32.0 ± 14%. The chelate EGTA resulted in a recovery of 20.9 ± 6.2% of the available copper content of the amended loam soil. The chelate DTPA and the non-chelated extractions did not provide detectable copper for analysis. This result was not statistically different from the result obtained in Chapter 3 with no modifier (t_{calc} = 4.09, t_{crit} = 4.30). When the extractions were analyzed for zinc, the chelate EDTA recovered 16.7 ± 2.3% of the total available zinc contained in the amended loam soil. The chelate CDTA resulted in a recovery of 41.6 ± 2.3% of the available zinc, DTPA resulted in 15.7 ± 1.3% zinc, and EGTA recovered 12.8 ± 4.8% available zinc. The non-chelated extractions removed 11.2 ± 1.5% of the available zinc contained in the amended loam soil. The recoveries using EDTA, DTPA, EGTA, and no chelate are statistically the same (F_{calc} = 2.79, F_{crit} = 4.07). The zinc recovery of CDTA and EDTA are statistically different (t_{calc} = 17.89, t_{crit} = 4.30). The CAPLE extractions did not produce detectable iron.

4.3.2.5 CAPLE RESULTS OF THE AMENDED CLAY SOIL

The natively amended clay soil produced 96.4 ± 4.7% of the available copper in the soil with the CAPLE method and the EDTA chelate. This result was not statistically different from the result obtained in Chapter 3 with EDTA (t_{calc} = 0.60, t_{crit} = 4.30). With the chelate CDTA a recovery of 26.3 ± 4.0% copper was obtained. With DTPA, a recovery of 5.9 ± 3.8% copper was obtained from the amended clay soil and this recovery is statistically similar to the recovery with EGTA, 8.9 ± 0.8% available copper (t_{calc} = 1.90, t_{crit} = 4.30). The extractions performed without any

chelate did not result in any detectable copper. This result was not statistically different from the result obtained in Chapter 3 with no modifier (t_{calc} = 1.00, t_{crit} = 4.30). When the extractions were analyzed for zinc, the extractions with EDTA recovered 40.2 ± 6.1% of the total zinc content of the amended clay soil. The extractions with CDTA, DTPA, EGTA, and no chelating agent were statistically similar (F_{calc} = 0.25, F_{crit} = 5.14) and were 16.9 ± 7.4%, 20.0 ± 1.3%, 20.8 ± 8.5%, and 17.2 ± 6.5% respectively, available zinc contained in the amended clay soil. None of the extractions lead to a detectable amount of iron. Similar to the standard clay soil, this is due to the iron of the clay soils being involved only in the geological matrix.

4.3.2.6 CAPLE RESULTS OF THE AMENDED SANDY SOIL

The amended sandy soil recovered 108.1 ± 5.5% of the available copper in the soil sample using EDTA. This result was not statistically different from the result obtained in Chapter 3 with EDTA (t_{calc} = 0.21, t_{crit} = 4.30). With CDTA 23.1 ± 1.7% of the copper was recovered which is statistically different to the recovery of copper using EGTA, 59.4 ± 6.5% (t_{calc} = 5.87, t_{crit} = 4.30). The chelate DTPA recovered no detectable quantity of copper and is statistically similar to the recovery of copper with no chelating agent, 1.9 ± 0.1% available copper (t_{calc} = 2.19, t_{crit} = 4.30). This result was not statistically different from the result obtained in Chapter 3 with no modifier (t_{calc} = 2.54, t_{crit} = 4.30). When the extractions were analyzed for zinc, the recovery of zinc using EDTA, 20.8 ± 3.6% available zinc, was statistically similar to

the recovery of zinc using CDTA, $21.2 \pm 3.3\%$ zinc ($t_{calc} = 0.14$, $t_{crit} = 4.30$). The

recovery using the chelate DTPA, $5.5 \pm 0.4\%$ available zinc, was statistically similar

to the recovery of zinc from amended sandy soil using EGTA, $5.2 \pm 2.9\%$ zinc ($t_{calc} =$

1.69, $t_{crit} = 4.30$). The recovery of zinc using no chelating agent was $3.3 \pm 0.6\%$ zinc

content and was statistically similar from the recoveries of zinc using EGTA and

DTPA ($F_{calc} = 2.12$, $F_{crit} = 5.14$). None of the extractions lead to a detectable amount

of iron removed with any of the chelating agents. The iron in the amended sandy

soil sample is contained in the geological matrix of the soil, and correlates with the

standard sandy soil samples.

All soils were retained after extraction with the CAPLE system to obtain a

mass balance of metals. The metals not obtained via the CAPLE method were

recovered with a total acid digestion, within 10%.

4.3.3 CHELATE ANALYSIS: II

CDTA should extract the most copper and zinc from the natural soils,

according to the $\log K'_{MY}$ values previously listed. Experimentally, EDTA removes

the surface copper with the greatest efficiency. Although DTPA and CDTA have

$\log K'_{ML}$ values for copper three orders of magnitude greater than EDTA, they did not

extract copper at the efficiencies that EDTA did. The EDTA molecule scavenges

the surface copper ions and removes them from the system. EDTA does remove

the most copper in this study but does not remove the greatest amount of zinc from

the system. Experimentally, CDTA extracts the most zinc from the natural soils of

the chelates studied. The low $\log K'_{MY}$ values for EGTA, 15.43 for copper, 12.13 for

zinc, prevent it from being an efficient chelate for this system. For the removal of zinc, the recoveries follow the logK'_{ML} of the chelates; the greater the logK'_{ML}, the higher the recovery, except for DTPA.

4.4 CONCLUSIONS

The limiting factor in the creation of all of these metal chelate complexes is the liberation of the metal from the soil particle to allow the chelate to complex the now available metal.

It was anticipated that EDTA would be the best chelating agent for the CAPLE system, compared to the other chelates studied. This was due to the use of EDTA in previous studies of this work and the studies of other researchers concerning other water-soluble chelates for the removal of metals from soils.

Experimentally, no one chelate clearly extracted the majority of both copper and zinc. For the six soils studied, EDTA extracted copper with greater efficiency than any of the other chelates. The CAPLE method was shown to be reproducible from the results from all six of the soils in this chapter, both with the EDTA additive and no additive. These results were all statistically similar to the results obtained in Chapter 3.

EDTA extracted more copper from all of the soils studied, than any of the other chelated or non-chelated systems studied. EDTA possesses mid-range logK'_{MY} values in comparison to the other chelates, but it extracted the copper and zinc at consistently quantifiable levels. EDTA and the resulting metal complex are strong enough to be carried away in the extraction fluid once it released the metal

from the soil. This would allow another molecule of ligand to chelate yet another

chemisorbed cation and be carried off in the extraction fluid. DTPA was not favored

for copper extraction despite the more favorable $\log K'_{MY}$ values, 18.25 for copper

and 15.50 for zinc, compared to the other chelates studied. This was attributed to

the very low $\forall_5$ value of the chelate solution.

CDTA was able to extract zinc more consistently than the other chelates.

The $\log K'_{MY}$ value for CDTA for zinc is 14.90. This value is less than that of DTPA

but greater than the other chelates. The standard sandy soil provided statistically

similar results for zinc when extracted with CDTA and DTPA (t_{calc} = 2.67, t_{crit} = 4.30),

these results were statistically higher than the results of the other chelates studied

(F_{calc} = 17.63, F_{crit} = 5.14). The recoveries of zinc for CDTA and EGTA when

extracting from the amended sandy soil were statistically similar (t_{calc} = 0.14, t_{crit} =

4.30) and these recoveries are statistically different from the results of the other

chelates (F_{calc} = 21.52, F_{crit} = 5.14). The only soil that CDTA did not remove a

statistically larger amount of zinc from was the amended clay soil. EDTA extracted

more zinc for the amended clay soil (t_{calc} = 5.97, t_{crit} = 4.30). This is due to the size

of the soil particles. The clay soil contains the most clay particles of the soils

studied (see Table 3.1). The clay fraction is the smallest fraction of soil particles

defined as any particle less than 2 µm in diameter.[54] CDTA is the most consistent

chelate for the removal of surface zinc from natural soils. An analytical recovery of

zinc was not achieved. This is due to zinc isomorphically substituting for aluminum

and iron in a soil system. The isomorphic substitution into the structure of the soil

particle would inhibit the CAPLE system from removing zinc at 100% levels. The 100% level was determined by sequential extraction. The sequential extraction technique promotes weathering of a soil's surface with it's acidic solutions. The CAPLE technique cannot extract zinc near unity because the CAPLE system does not promote the weathering of the soil's surface.

Chelate Assisted, Pressurized, Liquid Extraction (CAPLE) can be used in conjunction with different chelates to selectively extract certain adsorbed contaminants. EDTA has been used as a universal reagent for the removal of all adsorbed metals. Overall, EDTA did not extract as much zinc as CDTA did, but this could be attributed to the high recoveries of copper. If there were not an excess of EDTA in the system, all of the available EDTA ions would be preferentially quenched by the copper on the soil surface, for copper $\log K'_{MY}$=16.63, for zinc $\log K'_{MY}$=14.24. CDTA would preferentially remove the zinc rather than copper because copper is chemisorbed to the surface of these soils and therefore remains closer to the surface. The zinc that is not isomorphically substituted into the soil particle does not form the tightly bound complex that copper does,[54] allowing the zinc to be taken up by CDTA.

CAPLE does not discriminate between the chemisorbed and the outer sphere contaminants. The choice of chelate does effect the recoveries of surface metals from soils. EDTA is still the best overall chelate for removal of contaminant metals from soils.

Chapter 5: Conclusions

5.1 INTRODUCTION

As described in this work, Chelate Assisted, Pressurized, Liquid Extraction (CAPLE) has been shown to be a fast, efficient technique for the removal of adsorbed metal contaminants from soils. The CAPLE method utilizes sub-boiling temperature to enhance the desorption of the chemisorbed metals, a chelating agent to solubilize the liberated contaminant metal, and high pressure to remove the metal complex from the system. In contrast to other methods using elevated temperatures or acidic pH ranges, the chelate is necessary in order to prevent the metal from readsorbing to the surface of the soil.

The most probable mechanisms for the desorption of metal contaminants from soils in the CAPLE system and their subsequent removal will be discussed. This work has shown that by affecting the rate of desorption of a metal from a soil, $K_{desorption}$, the efficiency of removal of that metal can be increased.

5.2 PROPOSED MECHANISMS

The CAPLE system contains two processes in the extraction procedure and therefore requires a proposed mechanism for each process of the CAPLE system. The first process is defined as static, the fluid does not flow through the cell. The second process is a more turbulent, dynamic process. The dynamic process consists of the subcritical fluid passing over the soil particles and through the vessel. The following sections will illustrate these two mechanisms in greater detail.

The first of the two processes describes the system in a static state during the equilibration of oven temperature and vessel pressure. During the static state of the CAPLE system, the vessel containing sample is heated from room temperature and room pressure to 98^oC and 100 ATM. This step could take up to 10 minutes to achieve. During this time, a double layer would form around the negatively charged soil particles. The heat and pressure of the system could possibly create convection within the vessel, but it is considered minimal. At this step in the extraction, the equilibrium mechanism is believed to be dominant. The equilibrium-based mechanism is dependent on the metal-soil solution equilibrium and the resulting aqueous metals.

A soil in solution has a natural equilibrium between the solution and the metals adsorbed to the surface of the soil. The sodium cations, available in the chelate soution, would quench the surface oxygen sites as the contaminant metal is released.

The rate of desorption of a metal from a soil has been described as "orders of magnitude slower than the rate of adsorption."[51] The desorption rate is not measured directly for soils but can be deduced to be less than $4.07 * 10^{-18}$. This is determined by setting the rate of the method, K_{method}, equal to the product of the rate of desorption, $K_{desorption}$, and the conditional formation constant for the metal chelate complex, in this case, Cu-EDTA, K'_{my}.

$$K_{method} = (K_{desorption})(K'_{my}) \qquad \text{Equation 5.1}$$

This dissertation has shown the K_{method} to be much greater than 1, and for calculation purposes, for an analytical recovery, K_{method} will equal 1000. The K'_{my} for the Cu-EDTA complex was calculated in Chapter 4 to be $2.45 * 10^{14}$, leaving the only unknown variable to be the rate of desorption. Since $K_{desorption}$ can be affected by temperature, temperature is one of the important factors surrounding the mechanism of the CAPLE system. By increasing the temperature in the CAPLE system, the equilibrium of desorption is shifting towards the formation of products.

$$SM_{(s)} \overset{K_{desorption}}{\rightleftharpoons} S^{d-}_{(s)} + M^{n+}_{(aq)} \qquad \text{Equation 5.2}$$
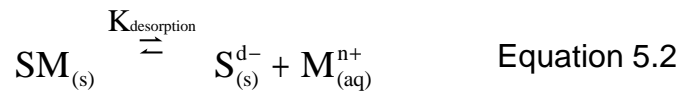
Now that the M^{n+} has been liberated from the surface of the soil lattice, it is available for extraction. Now the process to be studied becomes:

$$M^{n+}_{(aq)} + Y^{4-}_{(aq)} \overset{K'_{my}}{\rightarrow} MY^{n-4}_{(aq)} \qquad \text{Equation 5.3}$$
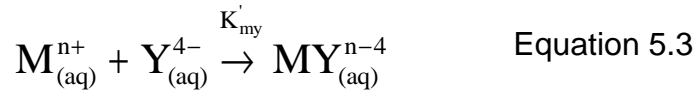
In Chapter 4 the effect of K'_{my} on K_{method} was studied. Theoretically the chelate with the greatest K'_{my} would extract the most of the metal M^{n+}, and consequently the chelate with the smallest K'_{my} would be the least effective at removing the surface metals.

5.2.2 SURFACE COMPLEX MECHANISM

The second step involves a dynamic extraction and lasts from 5 minutes to 15 minutes depending on the soil differences. The system is in a dynamic state when the outlet valve is opened to vent the subcritical fluid. During this time,

convection in the vessel increases dramatically, disturbing if not virtually eliminating, the double layer. Because of this disruption, surface complexation can occur more readily.

The surface complex mechanism is based on the principle that the metal will not release from the soil without a driving force provided by the chelate and an increase in temperature.

Several researchers have proposed a ternary complex of the soil-metal-ligand in solution.[51,56, 58] Typically the surface complexes form following Equation 5.4.

$$>S\text{-}OH + M^{n+} + A^{p-} \implies S\text{-}O\text{-}M\text{-}A^{(n-p)} + H^+ \quad \text{Equation 5.4}$$
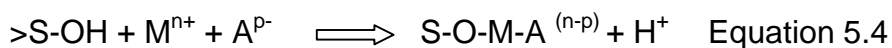
These complexes link the anion to the surface of the soil. The ternary complex forms as a result of the metal ion sorbed to the metal surface only being partially occupied by the surface oxygens. This leaves the opportunity for other ligands to complex the metal at the soil's surface.

The abstraction of the copper from the soil's surface has been suggested to occur by first replacing water at the axial positions with two of the available ligand groups. Previous studies suggest that increasing the temperature of a soil solution increases the bond length of the metal-soil bond.[51,56] This lengthening of the metal-soil bond would allow the metal-ligand complex to complete complexation at the weakened bond site and solvate the metal into solution. The replacement of water for ligand groups, polarizes the already weakened metal-oxygen bond on the soil

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[58] Stumm, W. Chemistry of the Solid-Water Interface. New York: John Wiley and Sons. **1992**.

and abstracts the metal from the surface. The available Na^+ in solution would quench the now negative surface site.

5.3 SUMMARY

The CAPLE method has been shown to affect the rate of desorption of metals from soils, $K_{desorption}$, with increased temperature. The desorption of the metal from the soil is the controlling factor in adsorbed metal removal. The pressure of the system then removes the complexed M^{n+} from the soil.

These two mechanisms are competitive and if conditions were appropriate, surface complexation could occur at the static step and equilibrium would continue at the dynamic step. The actual mechanism of CAPLE could be a combination of mechanisms at each step. It is likely that the equilibrium mechanism and the surface complex mechanism were in competition in the static phase of the extraction. The static phase was the portion of the extraction when the vessel containing the soil was brought to the pressure and temperature of the CAPLE system. During the dynamic phase, the flushing of the system with heated, pressurized water, it is likely that the dominant mechanism was the equilibrium mechanism. As mentioned earlier in this chapter, when a soil is in solution, there is a natural exchange of metals in solution and metals on the soil. During the dynamic phase of extraction, the metals in solution would be chelated and removed from the soil's limit of electrostatic influence further shifting the equilibrium to the production of metals in solution. This shift allows for the complete removal of metals from the soil system.

It was originally considered that the extraction did not occur at appreciable amounts until the temperature and pressure of the CAPLE system were reached, $98^{o}C$ and 100 ATM. It is now suggested that each soil may have its own extracting temperature and pressure. If the extracting temperature and pressure are defined as the lowest temperature and lowest pressure or lowest temperature and pressure combination that unity recovery can be achieved, then this phenomena warrants further investigation.

The Chelate Assisted, Pressurized, Liquid Extraction method has been shown to remove all available surface metals without disturbing the geological matrix of the soil, with the same efficiency as the accepted method, sequential extraction. CAPLE is an efficient, rapid, one step extraction performed in a closed system. This work shows CAPLE to be a viable replacement for sequential extraction in the determination of anthropological contaminants.

Appendix A1: The use of supercritical carbon dioxide to remove metals from sea sand.

A.1 INTRODUCTION

Supercritical fluid extraction (SFE) has received considerable attention as an analytical extraction technique over the past ten years. On the topics of analyte collection and extraction efficiency, many works have addressed the two common trapping methods for SFE: solid phase trapping and liquid trapping. The most common liquid trapping technique involves immersing the restrictor into a volume of liquid and allowing the extraction fluid to decompress into the liquid. Normally, small volumes of solvent are used to minimize analyte dilution. The temperature of the solvent can be reduced to enhance the partitioning of the analyte into the solvent phase. Good recoveries with such techniques range from 45-80%[59,60,61,62]. Another way to enhance trapping of the analyte into the solvent is by pressurizing the trap. Pressurization decreases loss due to volatility of the trapping solvent, or due to a high output flow rate of the resulting gas.

In an attempt to increase the trapping efficiency, Langenfeld[63] *et. al.* studied the effect of cell geometries and collection solvent parameters. The authors examined the height of the collection solvent, the volume of the solvent and the

[59] Akgerman, A. *Waste Management.* **1993**, *13*, 403.
[60] Lancaster, E. Ph.D. Dissertation, Virginia Polytechnic Institute and State University. **1997**.
[61] Papilloud, S; Haerd, W; Chiron, S; Barcelo, D. *Environmental Science and Technology.* **1996,** *13*, 1822.

effect of the collection temperature. It was determined that the boiling point of the collection solvent did not determine the effectiveness of the solvent to trap the analytes studied. The research did provide some insight into the temperature of the collection solvent; the temperature of 5^oC was found to produce the highest yields.

Other authors have examined the effect of the trapping procedure on analyte recovery. Wenclawiak[65] and researchers state that trapping is analyte dependent. Maio[64] *et. al.* describe the trapping step to be difficult to predict. It was found that for each set of analytes, the collection method should be investigated before the extraction conditions are optimized.

The most common problem with liquid trapping is poor analyte and solvent interaction. When an analyte is extracted from a matrix with supercritical CO_2, the analyte accompanies the gaseous CO_2 that exits the restrictor and then mixes with the extraction fluid. The bubbles that form at the restrictor tip are governed by the restrictor internal diameter, the SF flow rate through the restrictor, and the physical properties of the trapping liquid.[37]

The main objective of this work is to demonstrate increased trapping efficiencies by the use of variation of temperature and an ultrasonic bath. By decreasing the temperature of the trapping liquid, the partitioning of the analyte into the solvent phase can be increased, thereby, increasing the trapping efficiency. The ultrasonic bath promotes the physical disruption of the bubble formation and results in the creation of smaller bubbles. The increased surface area of the

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[62] Wenclawiak, W.; Maio, G.; von Holst, C.; Darskus, R. *Analytical Chemistry.* **1994 ,** *66*, 3581.
[63] Langenfeld, J.; Burford, M.; Hawthorne, S.; Miller, D. *Journal of Chromatography.* **1992,** *594*, 297.

bubbles promotes the interaction of the analyte with the solvent phase and should enhance the trapping efficiency.

The compounds ferrocene and iron acetylacetonate were utilized to determine the potential extractability of a nonpolar, iron containing compounds. Because of the nonpolar nature of the molecule, ferrocene should be readily soluble in SC-CO_2 and the analyte matrix interaction should be minimized. Additionally, a study concerning spike placement of ferrocene was designed to determine if recoveries of analytes were affected.

Trapping studies involving three modifications to a liquid trap were explored. The temperature of a liquid trap containing only methanol was varied to optimize the collection efficiency of the system. By decreasing the temperature of the methanol and therefore increasing the viscosity of the solvent, it was thought that the residence time of the decompressing CO_2 and analyte would increase. An ultrasonic bath was then used to disrupt the decompressing bubble formation to increase the trap, solvent and analyte interaction.

A.2 EXPERIMENTAL

All extractions were performed on a Spe-ed SFE from Applied Separations with a 30-mm ID x 100-mm manual restrictor. The fluid was SFC/SFE grade, helium headspaced Carbon Dioxide, obtained from Air Products (Allentown, PA). A 1.67 mL capacity, Keystone (Bellefonte, PA) Extraction vessel was fitted with 2.0 mm frits to contain the extraction media. All extracts were analyzed on a Buck

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[64] Maio, C.; von Holst, C.; Wenclawiak, W .; Darskus, R. *Analytical Chemistry* **1997,** *69,* 601.

Scientific Graphite Furnace Atomic Absorption Spectrometer (GFAAS) with pyrolized coated graphite tubes, using standard conditions. A summary of these conditions is found in Table A.1.

Reagents used for this work are Sea Sand (Fisher Scientific, Fairlawn, NJ), Methanol (EM Science, Gibbstown, NJ), 18 MΩ Water (Nanopure Water Systems), Ferrocene (Sigma Chemical Company, St.Louis, MO), Carbon Dioxide (Air Products, Allentown, PA.), Nitric Acid (redistilled, Fisher Scientific, Fairlawn, NJ), 542 Whatman Hardened Ashless Filter (Kent, England), Iron Acetylacetonate (Iron AcAc) (Sigma Chemical Company, St. Louis, MO)

A.2.1 ANALYTE ANALYSIS

The contents of each extraction cell were carefully collected and volumetrically diluted to 10.0 mL in methanol. Samples were stored at room temperature in closed vials and analyzed within 4 hours.

The ferrocene levels were quantitated by determining elemental iron concentration of each extraction run. The determination was performed by measuring the iron absorbance by GFAAS. (The specific conditions used in the elemental analysis are described in Table A.1.)
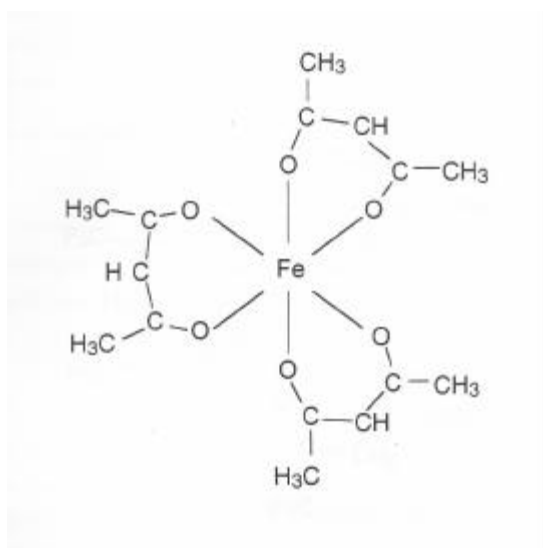
FIGURE A.1: STRUCTURE OF IRON ACETYLACETENOATE

Also called 2,4 pentanedione, iron (III) derivative.

The calibration of the GFAAS was accomplished by preparing known standards of ferrocene or $Fe(AcAc)_3$ in methanol. The calibration curve was linear for the range of samples that were quantified.

The determined concentrations were converted to mass amount of analyte by volumetric volumes. From the calculated mass of analyte and the known mass of analyte (from the spike) the efficiencies were calculated.

A.2.2 STATISTICAL ANALYSIS

The extraction data were analyzed for significance using the t-test. The conditions of the test involved the use of a 2 tailed *t*-distribution, at a 95% (α=0.05) confidence, and 8 data points per extraction (degrees of freedom, ν = 7). The calculations were performed using Microsoft Excel '98.

A.2.3 FERROCENE STUDY

Spike placement was studied to investigate the possibility of results being affected by the position of an analyte spike in a packed vessel. Three spike locations were chosen relative to the Keystone (Bellefonte, PA) extraction vessel, Figure A.1. Sea Sand (Fisher Scientific, Fairlawn, NJ) was used as the matrix for the extractions and the sand was used as received.

Approximately 2.5 g of Sea Sand was weighed and placed in a clean extraction vessel. A 0.5 mg spike of ferrocene, dissolved in 100 μL Methanol (EM Science, Gibbstown, NJ) was placed at the top of the extraction vessel, also referred to as the *inlet placement*. A modifier that was determined optimum for

λ=248.3 nm,	Fe atomic line	
Step	**Time**	**Temperature**
Dry	10 seconds	$100^{o}C$
Ash	10 seconds	$150^{o}C$
Atomize	6 seconds	$2200^{o}C$

TABLE A.1: CONDITIONS FOR GFAAS ANALYSIS
This table describes the temperature and time involved in each step of the Graphite Furnace Analysis.
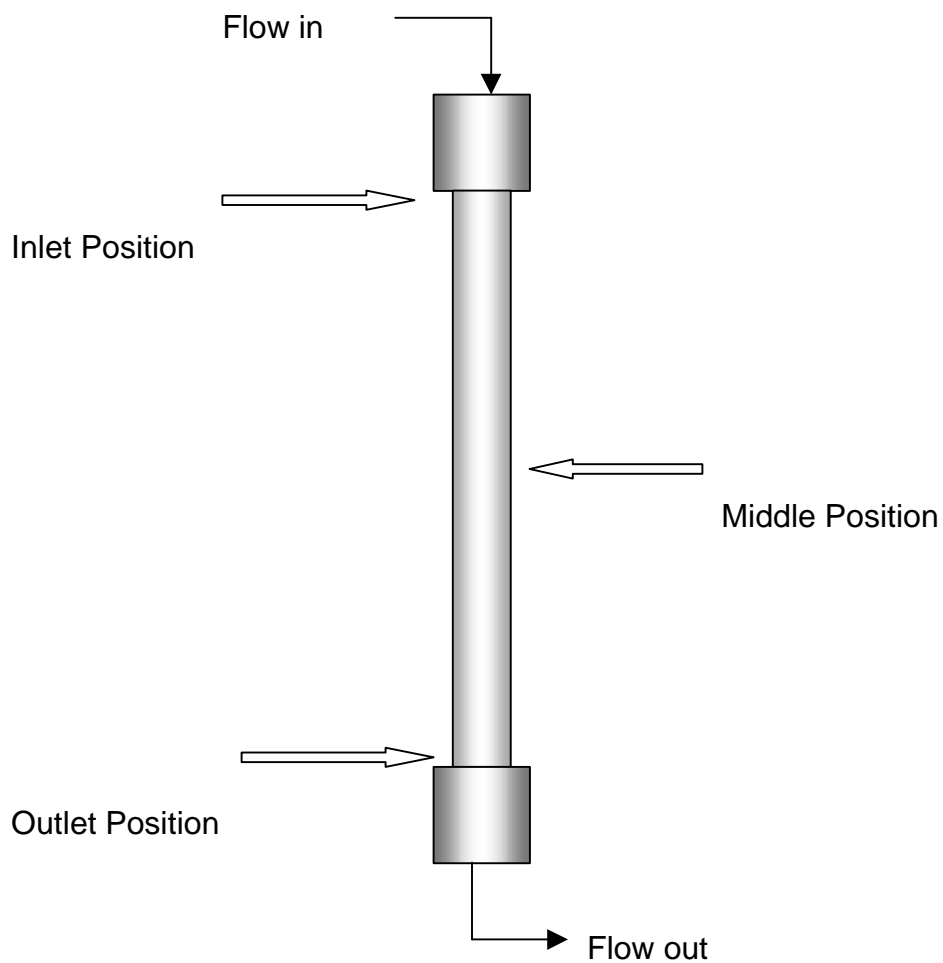
FIGURE A.2: KEYSTONE EXTRACTION VESSEL DISPLAYING THE SPIKING POSITIONS.
This figure demonstrates the spike placement relative to the inlet and outlet of the
extraction vessel.

ferrocene extraction[59] was then added. The modifiers were 20 μL Methanol and 20 μL 18 MΩ Water (Nanopure Water Systems.) The vessel was lightly tapped on the laboratory bench to ensure packing of the sand.   The vessel was then placed into the SFE system and brought to equilibrium.

The vessel temperature for the extractions was $50^{o}C$ and the outlet valve temperature was set at $100^{o}C$. The pressure was maintained at 100 ATM for 5 minutes (static time), then the outlet valve was opened and a 5 minute dynamic time followed[59]. The collection solvent was 9 mL of 100% Methanol and the trap was cooled in an ice water bath. All traps were then brought to a volume of 10.0 mL with class A volumetric glassware.

These extractions were repeated for a total of *n=8* at each spike location. For the *middle placement,* 1.25 g Sea Sand was weighed and placed in a clean extraction vessel. The spikes were placed as described above and the remaining 1.25 g Sea Sand was then placed in the vessel. The *outlet placement* required the spikes to be placed in the clean extraction vessel and 2.5 g Sea Sand placed on top of the spikes. The percent recovery of all extractions was determined by a comparison of the absorbance of the extracts with a standard solution of 0.5 mg Ferrocene dissolved in 10 mL Methanol. For the standard, an empty extraction vessel was brought to the same parameters as the extraction and the CO_2 was bubbled through the solution for 5 minutes. The standard solution was then brought back to volume with an addition of methanol.

			t_{calc}	t_{crit}	Different or Same
Control	vs.	Inlet	10.08	2.01	Different
Control	vs.	Middle	10.38	2.01	Different
Control	vs.	Outlet	1.65	2.01	Same
Inlet	vs.	Middle	0.06	2.01	Same
Inlet	vs.	Outlet	6.76	2.01	Different
Middle	vs.	Outlet	6.86	2.01	Different

n=8 for all extractions

TABLE A.2: STATISTICAL ANALYSIS OF SPIKING STUDY OF FERROCENE EXTRACTIONS
A Two tailed t-test for means was performed on the recoveries obtained through the various spike placements. At a 95% confidence the null hypothesis, the means were similar, was only proven between the mean recoveries of the control / outlet and the inlet / middle.

A.2.4 TRAPPING METHOD STUDY

The *middle placement* of the spike was deemed the most representative of an environmental sample that contain contaminants and in the need of better recoveries, Table A.2. The same method described above was used for vessel packing and extraction. The trapping method was varied. Three different trapping methods were compared. A *Regular* trapping method is described as immersing the methanol trap in an ice water bath. A *Cold* trapping method is the trap immersed in a dry ice/acetone bath. The *Ultrasonic* trapping method involves the trap immersed in an ice water bath contained in an ultrasonic bath (Fisher Scientific, Fairlawn, NJ).

A.2.5 THE EXTRACTION OF IRON ACETYLACETONATE

A spike of 0.5 mg of Iron Acetylacetonate, Figure A.2, was placed in the *middle* position, as described above and trapped using the *ultrasonic* trapping method. The same modifier was used for these extractions, 20 µL methanol and 20 µL distilled-deionized water. The vessel temperature was maintained at 80$^{\text{o}}$C and the restrictor temperature was maintained at 100$^{\text{o}}$C. The pressure was held at 150 ATM.[69] The sand was preserved for further analysis.

The retained sand was washed with 1% Nitric Acid and filtered in a 542 Whatman Hardened Ashless Filter (Kent, England). The eluents were diluted to 10.0 mL and analyzed with GFAAS. The resulting absorbances were compared

with a concentration curve and back calculated to determine the amount of iron

containing compounds extracted from the sand using ambient conditions.

A.3 RESULTS

A.3.1 SPIKING STUDY

The spiking study, using the "regular" trapping technique, determined that, as

expected, the *inlet* provided the poorest results with a recovery of $35.3 \pm 0.7\%$. The

middle placement provided a recovery of $60.1 \pm 0.5\%$. The *outlet* placement

showed a recovery of $87.4 \pm 1.3\%$. All of these values are statistically different, see

Table A.2. The outlet value is statistically the same as the standard solution. It is

shown that spike placement does affect recoveries of analytes.

A.3.2 TRAPPING STUDY

The recoveries for the various trapping methods were found to be; *Regular*,

$60.1 \pm 0.5\%$; *Cold*, $38.3 \pm 0.5\%$; *Ultrasonic*, $93.2 \pm 0.6\%$, Figure A.3. All of these

recoveries were found to be statistically different. The *Ultrasonic* trapping method

was found to be statistically similar to a standard prepared in the same manner as

the previous standards, statistics contained in Table A.3. For this system, it is found

that by sonicating a methanol trap the recovery of analyte increases. It was

speculated that the sonication physically broke up the decompressed CO_2 bubbles.

This speculation was confirmed by decompressing CO_2 into a 100-mL beaker with

approximately 60mL of methanol and taking photographs both with and without

n=8
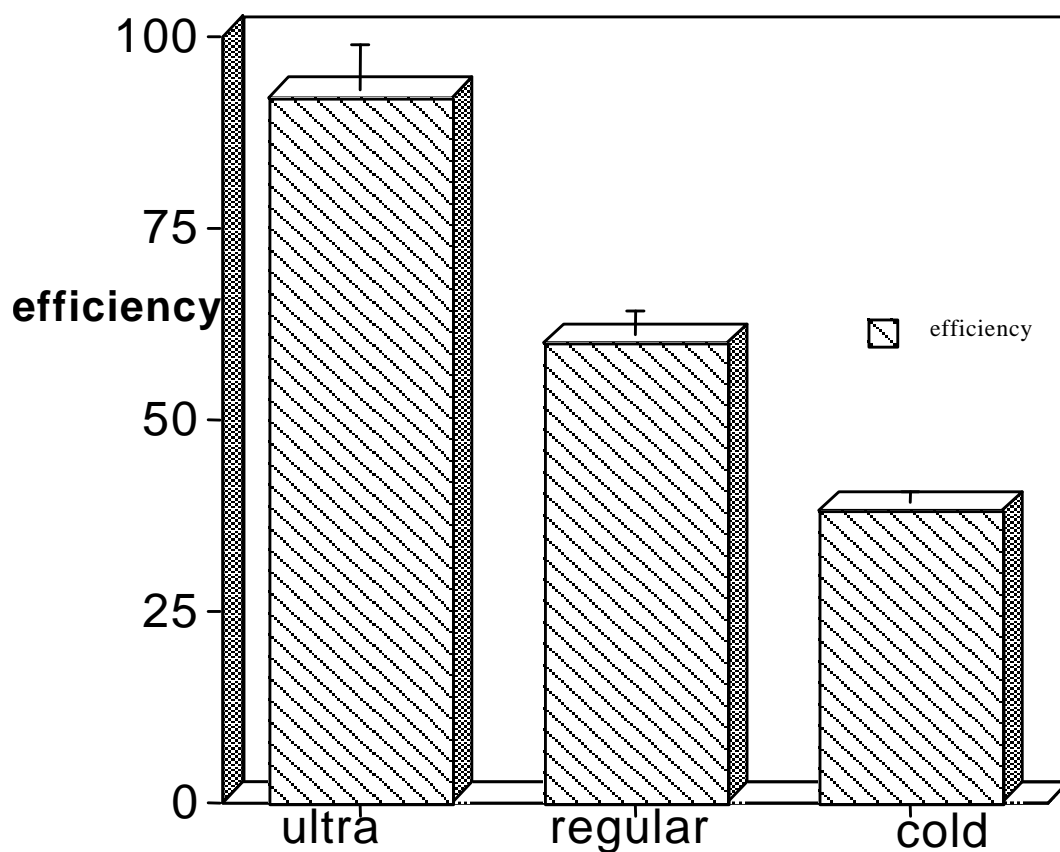
FIGURE A.3: RECOVERIES OF FERROCENE FROM SEA SAND, %EFFICIENCY VS. TRAPPING
   **METHOD**

This graph visually represents the increased recoveries obtained by the addition of
the ultrasonic bath to the trapping procedure.

			Recoveries (% of applied compound)	t_{calc}	Same or Different
Regular	Vs	Cold	60.1 ± 0.5 v. 38.3 ± 0.5	10.1	Different
Regular	Vs	Ultrasonic	60.1 ± 0.5 v. 93.2 ± 0.6	13.5	Different
Regular	Vs	Control	60.1 ± 0.5 v. 100.0 ± 0.3	17.3	Different
Cold	Vs	Ultrasonic	38.3 ± 0.5 v. 93.2 ± 0.6	19.8	Different
Cold	Vs	Control	38.3 ± 0.5 v. 100.0 ± 0.3	47.2	Different
Ultrasonic	Vs	Control	93.2 ± 0.6 v. 100.0 ± 0.3	0.76	Same

n=8 for all extractions

TABLE A.3: STATISTICAL ANALYSIS OF TRAPPING STUDY EXTRACTIONS OF FERROCENE
A statistical analysis of the trapping methods was applied to determine if any of the trapping methods obtained results statistically similar to the control. A Two-tailed t-test for means was employed at the 95% confidence to make this determination. The t_{crit} for this test was 2.01. The null hypothesis, the two methods were the same, was only proven in the case of the ultrasonic trapping method and the control.
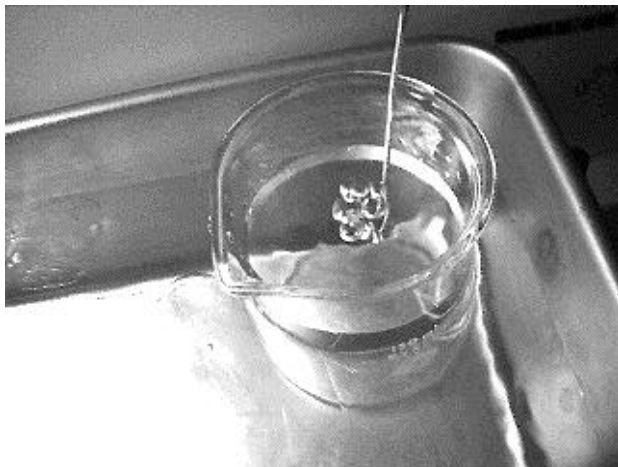
A   B

FIGURE A.4: PHOTOGRAPHS OF (A) SONICATED DECOMPRESSING CO_2 AND (B) NON-SONICATED DECOMPRESSIONG CO_2
These photographs display the difference in bubble size between the sonicated (A) decompressing liquid and the non-sonicated (B) decompressing liquid.

sonication, Figure A.4.   The diameters of the bubbles with and without sonication

were compared using a one tailed *t*-test for means. The sonicated bubbles were

found to be statistically smaller, 50% smaller, than the non-sonicated bubbles

(t_{crit}=1.86, t_{calc}=2.65.) It is determined that trapping can be enhanced by the

sonication of a liquid trap during the collection step of a supercritical fluid extraction.

A.3.3 IRON ACETYLACETONATE

The Iron AcAc extractions provided 12.8 $\pm$ 0.7% of applied analyte, using the

ultrasonic trapping method, *n=6.* Using the *regular* trapping method, the recovery

dropped to 6.4 $\pm$ 1.1%, *n=9.* It was determined that 0.2 mg, or 42% of spiked

solution was removed from the sand using dilute nitric acid.

It has been determined that the remainder of the iron acetylacetonate either

decomposed in the extraction vessel or was strongly bound to the active silanol

sites of the sea sand.

A.4 CONCLUSIONS

Supercritical Carbon Dioxide was investigated to demonstrate the

ineffectiveness of carbon dioxide for removing a preformed, non-fluorinated metal

chelate from sea sand. Using previously determined optimum time, temperature

and pressure conditions, the spike placement of ferrocene was investigated. This

study provided a statistical difference in recovery of analyte by the altering of the

position of the analyte in the vessel. The trapping method was then optimized to

insure complete recovery of extracted analyte. The most desirable trapping method

was 9 mL methanol in a glass trap immersed in an ice water bath contained in an ultrasonic bath. The ultrasonic bath provided a physical disruption of the carbon dioxide/analyte bubble formation from the outlet tube and therefore greater analyte/trap solvent interaction. A 67% increase in analyte recovery was noted with the addition of the ultrasonic bath to the trapping procedure. This addition brought recovery of the analyte, ferrocene, to near unity. The preformed metal chelate, iron acetyl acetonate, was then studied for extractability. Previously determined optimum time, temperature and pressure conditions were utilized with the aforementioned optimized spike placement and trapping methods. With conditions appropriate for the new analyte, recovery of the iron acetyl acetonate did not exceed 13% of the total spike applied. It was determined that a different method of metal contaminant analysis was necessary for non-fluorinated metal chelate removal.

Vita

Karen L. (O'Leary) Marshall was born on November 15, 1974 in Fairfax, Virginia. She obtained her Bachelor of Science degree in Chemistry from Virginia Polytechnic Institute and State University on May 13,1995. Ms. Marshall studied for a year at the Pamplin School of Business in the M.B.A. program before returning to the Chemistry Department to pursue a Ph.D. in Chemistry.