

**METAL EXTRACTION FROM SOIL SAMPLES BY
CHELATION IN A MICROWAVE SYSTEM**

Urai Chatreewongsin

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Gary L. Long, Co-chairman
Harold M. McNair, Co-chairman
Mark R. Anderson
Paul A. Deck
Andrea M. Dietrich

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By

Urai Chatreewongsin

Gary L. Long, Ph.D., Co-chairman

Harold M. McNair, Ph.D., Co-chairman

(ABSTRACT)

This work involves the combination of chelation and microwave extraction as a technique for extracting adsorbed metals in soil. It has been termed in this work, Chelate Assisted Microwave Extraction (CAME). Unlike other extraction methods, CAME is able to differentiate between anthropological and geological trace metals.

The method has been applied to major sample matrices included three types of soils (Bertie Sandy Loam, Davidson Silty Clay, and Davidson Clayey Loam) as well as sea sand, alumina, and iron oxide. In order to investigate the effects of sorptive coatings on metal concentrations, a sample set was first treated with humic acids, which represented organic coated materials. Afterwards, copper (II) solutions were spiked to both untreated and treated matrices and the efficiencies of recoveries were studied. Metal determinations were performed by Inductively Coupled Plasma-Atomic Emission Spectrophotometry (ICP-AES).

Initial studies of control (untreated soil) and long-term Cu amended (spiked) Bertie Sandy Loam (BSL) indicated that extraction efficiencies were method dependent. Ethylenediaminetetraacetic acid (EDTA) was a primary chelating agent used for method comparisons. Extracting solutions having a pH range from 7.8 to 10.1 did not affect the EDTA-microwave method, but the increase in pH enhanced Cu removal by the EDTA-shaking extraction method which was a conventional chelation using a mechanical

shaker. With the EDTA-microwave method, complete extraction was achieved in a short period of time (20 minutes). Compared to the EDTA-shaking method using two sandy soils, the EDTA-microwave method proved to be more efficient in extracting sorbed metals in soils (5.9 vs. 4.6 $\mu\text{g/g}$ for the control BSL and 37.5 vs. 27.8 $\mu\text{g/g}$ for the amended BSL).

FT-IR and C^{13} NMR were used to characterize extracted and commercially available humic acids. The latter was employed to prepare the humate coated materials. Iron oxide containing materials such as Davidson Silty Clay (DSC), Davidson Clayey Loam (DCL), and iron oxide (Fe_2O_3), showed extremely high adsorption capabilities for humic acids, while sea sand, Bertie Sandy Loam, and alumina (Al_2O_3) provided lower adsorption capabilities for humic acids. Two sets of uncoated and humate coated materials were used for Cu spiking procedures using 0.001 M Cu (II) at pH 5.3 for 24 hour-equilibration. Three original soils and alumina showed high adsorption capabilities for Cu (II). The presence of coated humate helped increase Cu adsorption capabilities of these soils, but diminished the ability of alumina to react with Cu (II) ions. For HA-uncoated matrices, iron oxide had a higher Cu adsorption capability than sea sand but less than real soils. Humate coating improved Cu adsorption capabilities of not only real soils but iron oxide and sea sand as well.

Lastly, the developed method named Chelate Assisted Microwave Extraction (CAME) was further examined and optimized. Several samples were investigated for extraction efficiencies. A two-step extraction, applying 120 W for 5 min followed by 60 W for 10 min, was efficient in removing sorbed metals from different matrices regardless of sorptive oxide and organic components. Concentrations of chelating agents slightly affected percent metal removal; however, there was no significant difference in results obtained using EDTA or diethylenetriaminepentaacetic acid (DTPA). Recoveries of spiked metals (Cu and Cd) ranged from 96 to 107%. Using the untreated (control) Davidson Silty Clay and Bertie Sandy Loam, the comparison of other chelating solutions were made. Clearly, CAME was promising and proved to be a good alternative to classical shaking method for extracting adsorbed metals from soils.

DEDICATION

To my family members, I would never have gone this far without your concerns and supports.

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CHAPTER 1

INTRODUCTION

The determination of metals in soils has been a subject of study for many disciplines in the scientific community. From the interests of the agronomists who consider metal concentrations as nutrients, to the environmental chemists who consider metal concentrations as potentially toxic substances, soils have been widely studied. With the exception of only a few methods, soil determinations require the sample to be converted from a solid to an aqueous form. A liquid extraction technique is one method used to accomplish this conversion and should ideally be a suitably efficient procedure. However, this is often not the case.

Working with soils is usually time consuming and tedious. Accordingly, many methods have been developed to obtain shorter extraction times and to increase the extraction efficiency. For the determination of trace metals in environmental samples, high purity reagents are required to avoid high background levels that could obscure trace analytes. Redistillation has been utilized to concentrated inorganic acids prior to the extraction processes. However, using the redistilled acids still introduces the risk of metal contamination and involves potential human exposure to concentrated acids.

This research has focused on the development of a new extraction technique by combining microwave energy and chelation chemistry using powerful chelating agents. This method was named Chelate Assisted Microwave Extraction (CAME) designed for metal determinations in soils. The goal of this research was to understand the chemistry of metals leached from soil surfaces under microwave application and then to further optimize the conditions for soil analysis. Different types of soils, naturally occurring non-contaminated and metal spiked samples have been employed for evaluation of extraction efficiencies. Also, statistical analysis was applied to comparisons of the different extraction methods and parameters.

1.1 History

In earlier studies, scientists have investigated trace metals in soils as nutrients essential for plants.¹ The mobility and toxicity of heavy metals are controlled by the interactions between metal ions and soil surfaces. Low concentrations of some metals such as Cu, Ni, and Zn, which strongly interact with soil components, may result in nutrient deficiency for living systems. Due to a large human population growth, soils have been subject to increasing sources of metal contamination. Atmospheric deposition, waste disposal, fertilizer and pesticide applications, industrial waste, and nuclear waste are some of the sources of heavy metals. Obviously, these sources can cause metal accumulation in soils. The excessive amounts of heavy metals introduced into the soils can affect soil matrices particularly involving metal-soil interactions, which further affect metal transport. At the interfaces of soils and soil solutions, various competitive reactions occur.^{2,3} Subsequently, it can be said that the quality of groundwater is controlled by soil reactions. Many studies have also shown that as metal concentrations in soils increase, the soils will become toxic to plants and animals. However, the metal toxicity level depends upon the chemical form of the metal that controls both its mobility and reactivity in the environment. Accordingly, speciation methods have been developed.⁴⁻⁶

For environmental analytical chemists, several methods have been developed for treatment of a large number of environmental samples. High efficiency and large sample throughputs are required. In soil studies, there have been two different approaches applied to metal determinations: single and sequential extraction schemes. Single extraction involves only one extracting solution and one step, whereas sequential extraction involves multiple extracting solutions as well as multiple steps. Generally, the techniques applied to soil analysis utilize a single extractant and the metal concentration extracted hopefully directly correlates with environmental available content and is hypothetically used to predict metal uptakes by plants. The single step extractions are performed by using selected extractants having different leaching strengths to determine metals bound to soils in different forms: such as water soluble, ion exchangeable,

carbonate, oxide, organically bound, and mineral lattice. Even with time consuming and tedious work, sequential extractions, e.g. Tessier's and modified methods^{7,8} have been occasionally used to monitor metal distribution in soils and sediments.

Several well-established extraction procedures have been developed, but may be restricted due to particular soil components. The most acceptable methods for metal analysis in soils generally utilize chelation extraction methods. Among them, soil scientists prefer 0.05 M ethylenediaminetetraacetic acid (EDTA) and 0.005 M diethylenetriaminepentaacetic acid (DTPA) to test metal availability for plants.⁹ These reagents are powerful for leaching some anthropological and sorbed metals from soil surfaces. However, both of these extraction reactions are pH dependent and therefore often not possible to provide equilibrium (possible maximum extractable level) within typical experimental time scales, 1-2 hours. To obtain valid data from these methods, it is important to control solution pH, shaking rate, shaking time, and soil/solution ratio for comparison of inter-laboratory studies.

The applications of mineral acids for soil samples have also been widely used. Strong inorganic acids not only extract anthropological or sorbed metals but geological metals as well. The Environmental Protection Agency (EPA) has developed some standard extraction methods using concentrated inorganic acids such as nitric acid and hydrochloric acid with subsequent analysis by atomic spectrometry.¹⁰ Acid digestion methods normally extract total metals from soils and therefore are not able to distinguish between the sorbed and geological metal fractions. Typically, a soil particle has an innermost part of primary minerals, which is usually not involved directly in metal interactions in soil environments. This mineral core, consisting of crystalline layers of aluminosilicate materials, may be subsequently coated with metal oxides and organic matter. Oxide and organic components provide highly active charges and hence enhance metal adsorption capabilities of soil surfaces. High levels of heavy metals introduced into soils can interact with these charged surfaces and then become surface bound fractions, and are considered contaminants.

The first application of microwave techniques for sample digestion occurred in 1975.¹¹ Ten years later microwave applications for analytical extraction work have increased rapidly.¹² Microwave chemistry for sample preparation was reviewed by Kingston and Jassie.^{13, 14} Most of this early work involved the applications of the microwave for mineralization. Total metal contents in a variety of matrices have been explored. In the past few years, another microwave technique has been modified for organic extraction for solid samples. It is called Microwave Assisted Extraction (MAE). Some studies of standard soils showed that MAE had provided good precision and high recoveries of organic compounds compared to other techniques including sonication extraction, Soxhlet extraction, and supercritical fluid extraction (SFE).^{15, 16}

There are several reasons here to choose coupling the technique of chelation and microwave energy for metal determinations in soils. With microwave interaction, removal of metals from specific binding sites should take less time due to faster solubilization and diffusion. Chelation under microwave irradiation may help to stabilize extracted metals in the solution. Complete extraction of adsorbed metals is expected to occur in a shorter period of time under the optimum conditions. This work will investigate the feasibility of the coupling technique and will also evaluate extraction capabilities of adsorbed metals from different soil matrices.

In the experimental section of Chapter 2, there will be a discussion of soil chemistry, the preparation of solutions, conventional extraction methods, a general procedure for microwave extraction, and instrumental operation of spectrometers. Chapter 3 will be concerned with comparative measurements of the EDTA-microwave method and the conventional extraction methods (such as hot acid extraction and EDTA-shaking). Both control and amended (treated) Bertie Sandy Loam will be firstly used for method comparison. Chapter 4 will discuss spectroscopic characterizations of the commercial and extracted humic acids. A commercial humic acid was used in order to have organically based matrices for evaluation of extraction efficiencies. Thereafter, adsorption procedures will be employed to coat the humic acid onto different solid materials. Methods for spiking metals will be done by adsorption procedures. More

extraction condition studies and matrix effect will be explored in Chapter 5. Chapter 6 will involve the studies of the influences of equilibration/aging times (used in Cu spiking procedures) and the extraction temperature (by hotplate heating method) on extraction efficiencies. Lastly, Chapter 7 will contain the overall conclusions of this work.

Before discussing these findings, it is important to describe some details of the following topics: metals in soils, chelation theory, microwave chemistry and some standard extraction methods for metal determination in soils.

1.2 Metals in soils

1.2.1 Soil surface properties

Soil is a complex system consisting of both biological and geological materials. It has been identified as a secondary mineral resulting from the weathering processes of primary minerals and then undergoing chemical transformations. Soil texture normally consists of solution, gaseous, and solid components. Solid components of soil, up to 75% by weight, can be classified into inorganic and organic matter. Typically, soil possesses a core of silicate minerals probably coated further with metal oxides after reprecipitation or neoformation. Likewise, the silicate minerals can interact with organic materials added into soils. Organic tissues from dead plants and animals accumulated in soils may decompose as the time goes by and then undergo polymerization to form humus. Humus or humic substances (HS) are the most interesting parts of organic matter in soils. Previous studies indicate that soil humic substances are amorphous forms of large molecules having molecular weights in the range of 1,000 to >100,000 Daltons.^{17, 18} They contain both hydrophilic and hydrophobic sites. Based on their solubility in aqueous solutions, humic substances can be classified into fulvic acids (FAs), humic acids (HAs), and humins. Generally, fulvic acids with molecular weights up to 5000 Daltons can be dissolved in acidic solutions while humic acids, having higher molecular weights up to 25000 Daltons, are insoluble in acidic conditions. Humins are insoluble in aqueous solutions having a wide pH range due to their huge molecular sizes and low

polarity. Compared to humic acids, fulvic acids contain smaller number of aromatic carbons (about 25% C), straight rather than coil structures, higher charges and therefore higher polarity. Consequently, fulvic acids are more easily leached from soil surfaces by environmental changes. Solid humic acids are able to remain in soils and play an important role in adsorption reactions at soil surfaces. It has also been found that the quantities of metals adsorbed directly correlate to the contents of humic substances present in the soil. For typical soils, organic matter is about 10% by weight and responsible for soil acidity and metal availability to soil environments. There have been many publications and review articles involving the studies of soil humic substances.¹⁹⁻²³

There are two kinds of surface charges in soils: permanent and variable charges. Usually the permanent charges result from isomorphous substitution in aluminosilicate minerals. These reactions involve the replacement of main cations having higher charge by lower charged cations without disturbing the crystal structures. These will produce negatively charged surfaces and normally occur in layer minerals such as kaolinite, smectite, vermiculite, chlorite, and interstratified minerals. Unlike the permanent charges, the variable charges are pH dependent. Generally, the pH-dependent reactions involve hydroxyl (OH) groups on crystal edges or on exposed planes. Some other inorganic groups: such as ammonium (NH_4^+) and thiol (SH), can also be involved. It is believed that the variable charge is dominant in oxide coated soils and organic soils. Dissociation and association of protons will control a net surface charge.²⁴ The pH at which the net surface charge becomes zero, resulting from the negatively charged density equaling the positively charged density, is called the point of zero charge (pzc) of the mineral. Normally, natural soils containing aluminosilicate compounds or oxide coated minerals possess points of zero charge in a range of pH 4 to 8. Compared to pure oxide/hydroxide compounds, natural soils have slightly lower pzc.²

1.2.2 Sources of metals in soils

Anthropological metals added into soils can cause serious problems if the metals weakly interact with soil surfaces or if the metal concentrations are larger than the

adsorption capacity of soil surfaces. Besides microorganisms, soil consists of both organic and inorganic matter. Soil is a natural heterogeneous mixture whose fractions are not well defined. The development of each soil depends upon its environment and climate. Figure 1.1 shows a typical model of metal speciation in soil based on extraction techniques. Generally, there are several kinds of binding sites between metal species and soil components. Some metals may be loosely bound as presented in a water-soluble form. Alkaline and alkaline earth metal ions prefer to form electrostatic interactions with negative surface charges. These metals are classified as exchangeable fractions. Under certain conditions, some may precipitate as carbonate compounds. Metal oxides and organic matter are two major components in soils that can play an important role in metal adsorption. Strong interactions normally occur in these fractions due to inner-sphere complex formations.

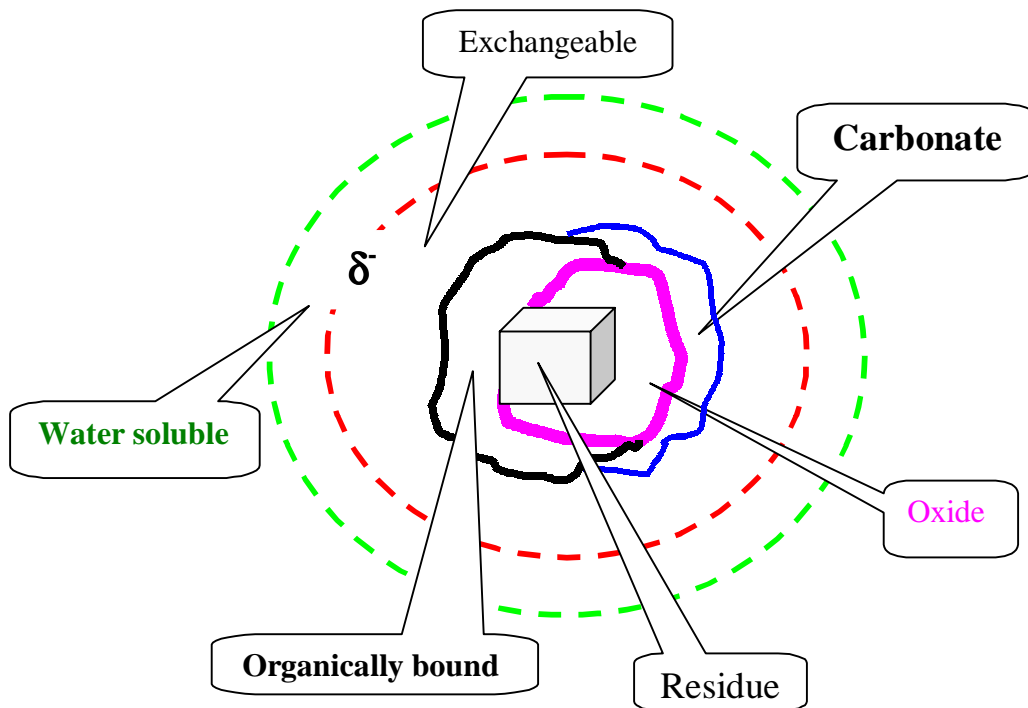
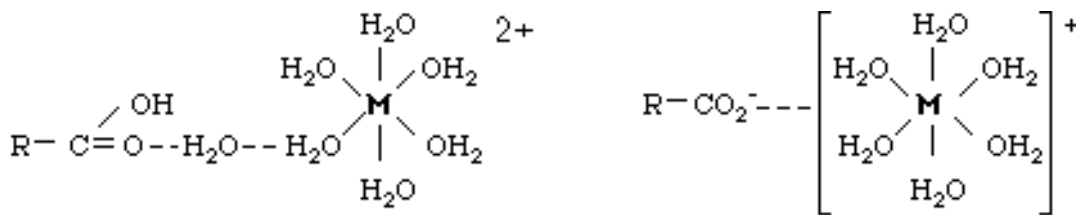
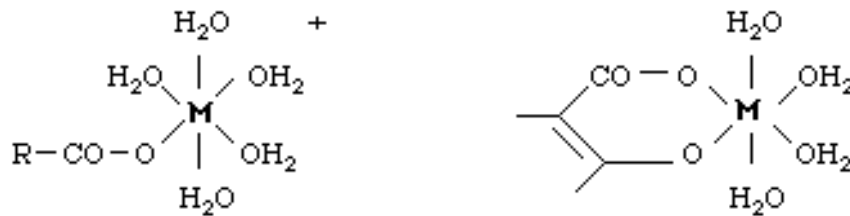


Figure 1.1: A simple model of metal fractions in soils.

As the dominant parts of organic matter, humic substances can provide several kinds of interactions with heavy metals. Figure 1.2 shows possible interactions between divalent metal ions and humic substances. Besides being responsible for electrostatic interactions with alkaline metals, humic substances tend to form covalent bonds directly to some heavy metals like Cu, Ni, and Pb. Strong adsorption sites between humic acids and metals are predominately controlled by carboxylic, phenolic, and amino groups all of which are pH dependent.^{2,17} Metal affinities depend upon electronegativity and charge/radius ratios of metals in both phases: solution and solid. For soils containing low levels of organic matter, layer silicates and oxide compounds will control metal reactions.



Weak interactions



Strong interactions

Figure 1.2: Metals interactions with humic substances.¹⁷

1.3 Chelation theory

1.3.1 Thermodynamic and kinetic properties

For a thermodynamically feasible reaction, the Gibbs free energy change, ΔG , must be negative. It is the free energy difference between the reactants and products of a reversible reaction.

At the standard condition:
$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \quad (1.1)$$

$$\Delta G^\circ = -RT \ln K \quad (1.2)$$

Where ΔH° is the standard enthalpy change and ΔS° is the standard entropy change of the reaction. At the equilibrium point, ΔG° directly correlates to the equilibrium constant (K). The more negative ΔG° , the more the forward direction of the reaction is preferred. A reaction having a large equilibrium constant can cause ΔG° to become more negative.

In order to have a negative ΔG° there are several possible conditions.^{25, 26} Firstly, ΔH° is negative but ΔS° is positive; for example the decomposition of calcium carbonate which provide an exothermic reaction. Secondly, both ΔH° and ΔS° are positive but $|T\Delta S^\circ|$ is larger than ΔH° . The dissolution of solid potassium chloride is an example of this case. The endothermic reaction is driven by the increasing entropy and the temperature at which the reaction is performed. Lastly, both ΔH° and ΔS° are negative but $|\Delta H^\circ|$ is bigger than $|T\Delta S^\circ|$. The example of this case is the Harper process used for the production of ammonia. This reaction is controlled by a larger negative ΔH° (-46 kJ mol^{-1}).²⁵

The possibility of a reaction to occur not only depends upon its thermodynamic properties but also its kinetic properties. With a small negative ΔG° but a large activation energy (E_a) required, the reaction probably will not occur. The Arrhenius equation expresses the rate constant (k) as a function of activation energy and temperature of the

system.

$$k = Ae^{-E_a^\circ/RT} \quad (1.3)$$

Where A is the specific Arrhenius constant of a reaction. Generally, the temperature change will affect the Boltzmann distribution which relates to the ratio of molecules at the ground state and the excited states. At elevated temperatures, the number of molecules possessing energy higher than E_a° are increased.

1.3.2 Adsorption/Desorption

Specific adsorption of metal ions on soils is normally considered consisting of strong interactions since it has been found that added metals are usually tightly bound with soil components. The desorption process is expected to have a much slower rate compared to that of the adsorption reaction. Most adsorption reactions at soil surfaces are exothermic, so the reverse reactions being endothermic will have higher activation energy than the forward reactions (Figure 1.3). Consequently, desorption mechanisms may require some external energy or catalysts to drive the reaction.

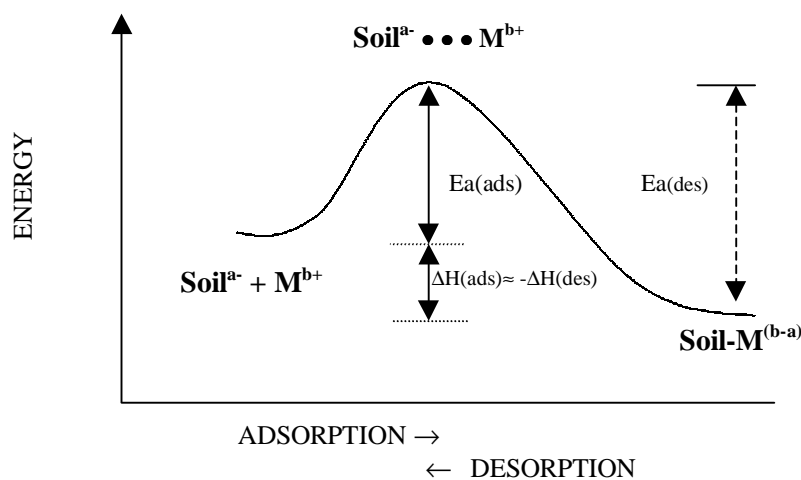


Figure 1.3: Reaction pathways of metal adsorption and desorption at soil surfaces.²

1.3.3 The use of chelating agents

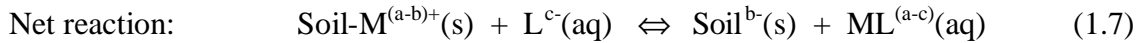
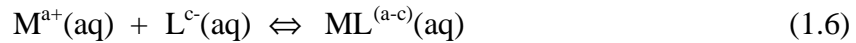
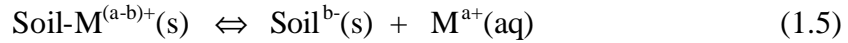
Adding a chelating agent into a soil solution affects the thermodynamic properties of the soil solution system. This is called the chelate effect. Multidentate ligands tend to form more stable complexes than monodentate ligands.

For chelation in a solution, $\Delta G^\circ = -RT \ln \beta$ (1.4)

As the overall formation constant (β) increases, the reaction is more favorable due to the more negative ΔG° . Generally, the result of the forward reaction is the production of more free species in the solution; consequently, there is an increase in the entropy of the system that further drives the forward reaction. Another factor is the local concentrations or unattached ends of multidentate ligands. Once one end is chelated, the other end of the same molecule is more favorably positioned than other molecules. This phenomenon helps to preserve entropy lost in the solution.

Generally, there are two factors that influence the stabilities of metal chelates.²⁶ First is the entropy effect resulting from molecular structures. Unattached longer chains provide more entropy lost once they are chelated with the metal already attached to another end. The other factor is ring strain. Normally, five member rings are more stable than six member rings since a five member ring has bond angles close to sp^3 configuration of a tetrahedral, 109° , that produces low strain to the chelating molecule. For complexes, the number of chelating rings also affect chelate.

For soil studies, chelating agents have been widely used for the determination of surface bound metals. Several reactions can occur in a heterogeneous system. A simple shaking method is simply employed to accelerate interaction between the extractant and the metal. A powerful chelating agent in an aqueous solution may help enhance metal removal from soils. The chelating agent can stabilize extracted metal ions by forming metal chelates in the aqueous solution. Possible reactions are shown below:



where M = Metal ion, L = Ligand, and ML = Metal chelate

Ethylenediaminetetraacetic acid (EDTA) and diethylenetriaminepentaacetic acid (DTPA) are typical reagents used for metal extraction from soils. Both EDTA and DTPA are aminocarboxylic acids containing oxygen and nitrogen binding sites. EDTA is a hexadentate ligand while DTPA is an octadentate ligand. Most divalent metal ions have a coordination number of four, so these two reagents can be applied to a variety of metals. Figure 1.4 shows possible structures of Cu-EDTA and Cu-DTPA. For some applications, DTPA is able to form more stable chelates with metal ions of a coordination number of eight. The higher steric effect of DTPA must be taken into consideration for some complex formations.

The use of chelating agents in soil science has been recognized from Norvell and Lindsay, who published two articles in 1969 about the stability of metal-EDTA and metal-DTPA complexes and the results of EDTA reactions with some transition metals in soils.^{27, 28} From these studies, it has been known that EDTA and its derivatives; such as DTPA and nitrilotriacetic acid (NTA), can produce large stability constants with several heavy metals. Characteristics of some EDTA derivatives (complexanes) are shown in Table 1.1.^{29, 30} In 1978, Lindsay and Norvell further developed a method using DTPA for extraction of some heavy metals such as Zn, Fe, Mn, and Cu in soils.³¹ This method has been generally accepted as a reasonable method for evaluation of trace metals available for plants.

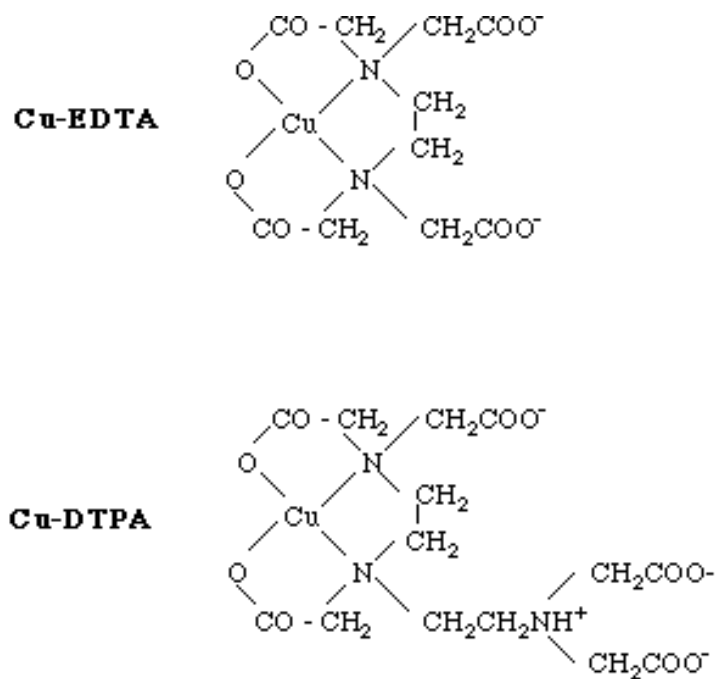


Figure 1.4: Structures of Cu-EDTA and Cu-DTPA chelates.

Table 1.1 (a): Dissociation constants of EDTA derivatives.^{29, 30}

-log K_a	NTA	EDTA	CyDTA	EGTA	DTPA
pK ₁	1.89	1.99	2.43	2.00	2.08
pK ₂	2.49	2.67	3.52	2.65	2.41
pK ₃	9.73	6.16	6.12	8.85	4.26
pK ₄	-	10.26	11.70	9.46	8.60
pK ₅	-	-		-	10.55

Table 1.1 (b); Stability constants of metal chelates.^{29, 30}

Metal	log K_{ML}				
	NTA	EDTA	CyDTA	EGTA	DTPA
Al (III)	9.5	16.13	18.63	13.9	18.4
Cd (II)	9.54	16.46	19.23	16.70	19.31
Cr (III)	> 10	23.40	-	2.54	-
Cu (II)	12.96	18.80	21.30	17.8	21.53
Fe (II)	8.84	14.33	16.27	11.92	16.50
Fe (III)	15.87	25.1	28.05	20.5	28.6
Mn (II)	7.44	14.04	16.78	12.3	15.50
Ni (II)	11.54	18.62	19.4	13.55	20.30
Pb (II)	11.39	18.04	19.68	14.70	18.80
Zn (II)	10.67	16.50	18.67	14.5	18.75

1.4 Microwave chemistry

Due to the commercial development of microwave technologies, microwave energy has been applied to chemistry studies as an alternative to conventional heating. Microwave systems utilize dielectric properties of materials to transform non-ionizing electromagnetic radiation into heat required for driving some reactions. It has been reported that the first simple microwave system appeared in the United States in 1950.^{32,}
¹² The microwave applications for sample preparation have been continuously studied since 1975. As the interactions between microwave energy and materials depend on the physical properties of the molecules, this may provide selectivity for the extraction of metals from soils.

1.4.1 Electromagnetic interactions

Of the electromagnetic spectrum as shown in Figure 1.5,^{12, 13} the microwave region has frequencies from 3×10^2 to 3×10^5 MHz and the wavelengths range from 1 m to 1 mm. The microwave region is between the infrared and radiowave regions. Due to communication regulations, only four frequencies have been approved for industrial, medical, and laboratory uses: 915 ± 25 , 2450 ± 13 , 5800 ± 75 , and 22125 ± 125 MHz.

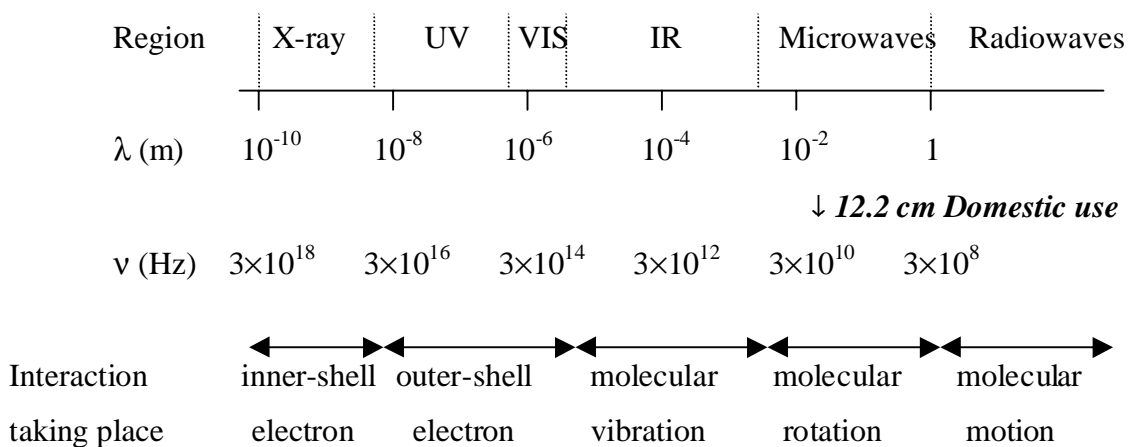


Figure 1.5: Electromagnetic spectrum and molecular interactions.^{12, 13}

Typically, domestic and analytical microwave systems have been constructed at a fixed wavelength of 2450 MHz. Accordingly, uses of microwave for sample preparation have been limited due to the allowed microwave frequencies. Working with these microwave instruments, microwave intensity and irradiation time can be varied.

1.4.2 Dielectric polarization

As mentioned above, the major factors of microwave heating involve dielectric properties of materials. This depends upon the ability of an electric field to polarize charges of a target material. Dielectric materials are able to store electric energy by displacement of the positive and negative charges under an applied electric field and against the atomic and molecular attractions. Totally, there are four kinds of dielectric polarization of a material.¹²⁻¹⁴

$$\alpha_T = \alpha_E + \alpha_A + \alpha_O + \alpha_{SC} \quad (1.8)$$

The total dielectric polarization (α_T) is a summation of all polarization possible occurring during interactions with electromagnetic radiation. The electronic polarization (α_E) arises from the realignment of electrons around nuclei; and the atomic polarization (α_A) results from the relative replacement of nuclei due to the unequal charges of the molecule. Orientation or dipolar polarization (α_O) is from the orientation of permanent dipoles by the applied electric field. Space charge polarization (α_{SC}) occurs due to the restriction of charge displacement and hence there are charge interfaces built up in the materials containing ionic charges. The last phenomenon is known as the Maxwell-Wagner effect.

With electromagnetic radiation, the response of a material depends upon the time scale used for each polarization mechanism relative to the applied frequency. In the microwave region, electron and atomic polarization are much faster than the applied field, so these effects do not contribute to the dielectric heating of the material. Only dipolar and space charge polarization mechanisms have the same time scale that can synchronize with the applied field. Consequently, these two mechanisms are responsible

for interactions between the polarized material and the applied field. Due to some phase lag resulting from the molecular-electromagnetic interactions, adsorption of microwave energy normally produces Joule heating. Polarizability is represented by real permittivity (ϵ') also called dielectric constant. The larger the dielectric constant the more electromagnetic radiation can be stored in the material.

1.4.3 Dielectric loss

Microwave heating of a material depends upon the dissipation factor of the target material, $\tan \delta$. The dissipation factor or loss tangent represents the ability of a material to convert electromagnetic energy into other forms of energy. In this case, $\tan \delta$ can be derived as a ratio of the material's dielectric loss or loss factor (ϵ'') to its dielectric constant.

$$\tan \delta = \frac{\epsilon''}{\epsilon'} \quad (1.9)$$

Loss tangent can occur in a dielectric material which conducts an electric current and also has a loss current (I_l) in phase with the applied field. The total current flowing through a capacitor will be out of phase by the angle $\theta < 90^\circ$ or by the loss angle δ against the charging current (I_c).

$$\tan \delta = \frac{I_c}{I_l} = \frac{1}{\omega RC} \quad (1.10)$$

The ω is a radial frequency of the applied field which equals to $2\pi f$. R and C are resistance and capacitance, respectively. The relationship between the dielectric constant and the dielectric loss can be expressed by complex permittivity (ϵ^*).

$$\epsilon^* = \epsilon' - j\epsilon'' \quad (1.11)$$

where j equals $\sqrt{-1}$ and ϵ'' (loss factor) often called imaginary permittivity. The ϵ' is a measure of a material's ability to store the applied microwave while ϵ'' is a measure of the material's ability to convert the adsorbed energy to heat. In a real system, the relationship between the dissipation factor and the radial frequency is different from theoretical behavior as show in Figure 1.6. Maximum dissipation factor can be observed at only a specific frequency.

1.4.4 Penetration depth

Once exposed to microwave irradiation, the dielectric sample will adsorb the applied energy and thereby, heat will be produced. The adsorption rate depends upon the sample's dissipation factor which is inversely proportional to the microwave penetration into the sample. For example, metals which are reflective materials provide extremely low microwave penetration and some transparent materials can have infinitive penetration.

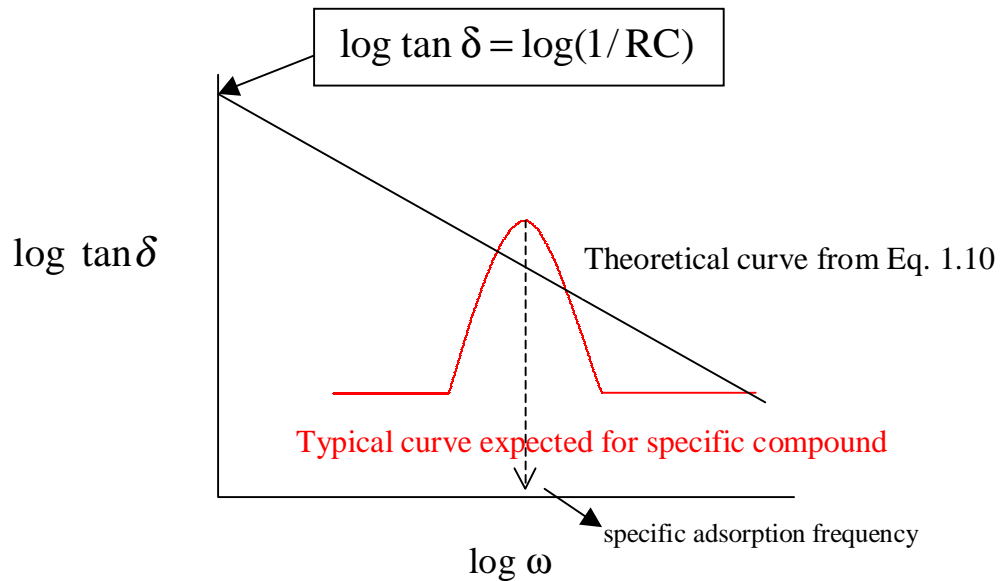


Figure 1.6: Graphic relationship of the dissipation factor and the radial frequency¹².

Since the dielectric constant and the dielectric loss are frequency dependent, each material has a specific frequency at which it can have maximum adsorption as shown in Figure 1.7. For instance, water has maximum adsorption at about 20 GHz. The maximum adsorption at this frequency will provide heating at outer layers of the processed material with low penetration depth, D_p . The approximate relationship of penetration depth, microwave wavelength, and a given material's dielectric properties are shown below.

$$D_p \approx \frac{\lambda_o \epsilon'}{2\pi \epsilon''} \approx \frac{\lambda_o}{2\pi \tan \delta} \quad (1.12)$$

D_p is the depth into the processed material where the power is 1/e of the value at the surface and λ_o is the wavelength of the microwave energy. According to the above equation, the larger the wavelength the more penetration will occur. With most domestic microwave ovens, applied electromagnetic frequency is restricted to 2.45 GHz which is much lower than the maximum adsorption frequency of water at 20 GHz. This design allows deeper penetration of the applied energy into the water containing materials at a longer wavelength.

1.4.5 Relaxation time

Dielectric relaxation time (τ) is defined as the time required for the dipole molecules to rearrange to 63% or 1/e of their original disorder.¹² When the radial frequency equals to $1/\tau$ (radians/second), maximum energy conversion will be produced at $\omega = 2\pi f = 1/\tau$. In practice, it has been found that the relaxation spectra of liquids and solids are flatter than the predicted values due to molecular interactions with surroundings.¹² These will affect the specific ω and the dissipation factor of a material of interest.

1.4.6 Temperature effect

Since ϵ'' changes slightly with frequencies, microwave irradiation at frequencies away from $\omega = 2\pi f = 1/\tau$ will improve penetration of the applied energy into the bulk material. Consequently, the entire region can be heated simultaneously rather than only outer layers due to the maximum adsorption at the surface. The rate of temperature change can be expressed as:

$$\frac{\delta T}{\delta t} \approx \frac{A\epsilon''fE^2}{\rho C_p} \quad (1.13)$$

where,

A	=	a constant
ρ	=	the density of a material
f	=	the electromagnetic frequency
E	=	the electromagnetic field intensity
C_p	=	the specific heat capacity of the material

For instrumental designs for analytical work, microwave intensity has been made variably. Considering the above relationship, the temperature effect is material dependent. As the temperature changes, the dielectric relaxation time, the dissipation factor and the penetration depth will also change. For water, its dissipation factor will decrease as temperature is increased. On the other hand, most organic molecules and solids have higher dissipation factors with increases in temperature since the temperature change will affect sample viscosities.

The energy adsorbed gives rise to an increase in temperature of a substance. In order to estimate the conditions in the microwave system, the useful relationship is expressed below:

$$P_{\text{ads}} = \frac{KmC\Delta T}{t} \quad (1.14)$$

where,

P_{ads}	=	the adsorbed power in W
m	=	the material mass in gram
t	=	the irradiation time in second
C	=	the specific heat capacity in cal/g °C
K	=	the conversion factor equal to 4.184 J/cal
ΔT	=	the temperature change in °C

1.4.7 Conduction loss

Electromagnetic irradiation of a solution containing ionic species can induce ionic migration which leads into conduction loss, I^2R , in the processed solution. All ions contribute to conduction in the system. However, there are several parameters including ionic size, charge, and conductivity which can affect interactions between ions and solvents. In addition, ionic conduction is determined by ion concentration, ion mobility, and temperature. The higher the ionic concentration, the larger the dissipation factor of the ionic solution. Table 1.2 shows the effect of NaCl concentrations on the dissipation factors of aqueous solutions at different electromagnetic frequencies.

Table 1.2: The effect of ionic concentration on dissipation factor.^{13,14}

Molal NaCl	Dissipation factor ($\tan \delta$)		
	300 MHz	3,000 MHz	30,000 MHz
0.0	0.0155	0.1564	0.54
0.3	-	0.4350	-
0.5	-	0.6250	-

1.4.8 Contribution of dipole and ionic conductions

For small molecules, as temperature increases the contribution of dipole rotation decreases as the ionic conduction increases. Accordingly, the dielectric loss due to dipole rotation will initially control the heating mechanism and at higher temperature the dielectric loss due to ionic conduction will play a significant role. As mentioned earlier, concentrations and mobilities of ionic species in the sample are also major factors. With low ion mobility and low concentration, the heating mechanism will be controlled by dipole rotation and the heating time will depend upon $1/\tau$. The heating time will be minimized at $\omega = 1/\tau$. Usually, the microwave frequency can not be freely varied, so working at ω away from $1/\tau$ will result in an increase of the heating time. At high ionic concentration with high mobility, the dielectric loss due to conduction will predominate and thereby the heating mechanism will be independent of the dielectric relaxation time. As can be seen in Table 1.2, the conduction loss resulting from the addition of NaCl will dominate the dielectric loss due to dipole rotation of water molecules. In this case the dissipation factor will increase and the heating time will decrease.

1.4.9 Physical properties of materials

Several parameters such as shape, size, and viscosity can also affect microwave adsorption. Microwave energy is able to penetrate and then heat the target material inside. In this case, the temperature inside the heated content is usually higher than outside. This can be seen in some solids having low thermal conductivity. In addition, it can happen with liquids due to slow rates of heat transfer. The hot spots inside have a lower density than the outside region; consequently, superheating is normally observed in microwave systems.

Like other electromagnetic interactions, boundary conditions are important due to the differences of dielectric properties. The applied electromagnetic field will be changed in direction at the boundary of the material and its surroundings. If the target has a dimension in the range of microwave wavelength applied, the bending of the

electric field can provide higher intensity of the electric field inside the target. In reality, this might have more effect at the corners of the material. For spherical materials, their centers will have high intensity of the microwave field due to low reflection. The distribution of microwave energy inside the target materials is very complicated and is quite difficult to predict. Boundary conditions often change during the microwave processes. The dielectric properties alter with temperature and also microwave heating can give rise to structure changes.

1.5 Extraction methods for metal determination in soils

As mentioned earlier, sample preparation is the first important step for an analytical process. There have been several extraction methods developed for metal determination in solid matrices. In soil science, there are two classes of accepted sample preparation techniques: digestion methods and extractive methods. The choices of extractants and physical methods used are dependent of the points of view of the interested scientists. In order to investigate metal bioavailability, soil scientists have preferred chelation extraction methods to digestion methods because they believe that suitable chelating agents can provide direct correlation of the extracted metal concentrations to those levels naturally transferred into other systems. These extractive methods can also provide higher selectivity than the digestion methods using strong acids since mild extraction methods can liberate specifically bound metals. Besides being used in a single extraction scheme, EDTA has also been applied to some sequential extraction procedures and even used as a modifier for some extraction methods.³³⁻³⁹

1.5.1 Conventional Acid Dissolution (CAD)

Unlike chelation extraction methods, high concentrations of strong inorganic acids used for mineralization usually provide total dissolution of matrices. This causes lower selectivity, but is utilized for multi-element analysis. A hot plate has been used as a heat source, and this technique is the so-called “hot acid digestion technique.” In general, inorganic/mineral acids such as nitric acid (HNO₃) and hydrochloric acid (HCl)

are mainly used to dissolve trace metals from soil matrices. To obtain complete digestion of silicate compounds, hydrofluoric acid (HF) may be required. The digestion procedure is performed at elevated temperatures close to the boiling point of the acid solvent for a certain period of time. After being allowed to settle, the extracted mixture is centrifuged or filtered to remove non-dissolved residues. Recently, there have been several EPA methods such as EPA 200.7, 200.8, and 200.9 developed for determinations of trace metals using different detection methods.^{40, 41} These methods have been modified from EPA 200A and 200.2 using atomic absorption spectrometry (AAS) and inductively coupled plasma-atomic emission spectrometry (ICP-AES) as detection methods. However, the old version EPA 200A is still acceptable for analysis of some trace metals in normal soils. EPA has again revised EPA 3050 method to a new EPA 3050B method in 1994. Some characteristics of EPA 200A and EPA 3050B are described below. These conventional digestion methods normally require several steps and therefore consume total time up to 2 hours.

EPA 200A method, a conventional hot acid extraction, has been employed for the determination of trace metals from soils using a mixture of nitric acid and hydrochloric acid heated by a hot plate. The detail of this method can be seen in Chapter 2. It appears that this method is suitable for general soils. The extracts are subsequently analyzed by atomic spectrometry.

EPA 3050B method is a revised hot plate procedure using nitric acid and hydrogen peroxide. It has been applied to waste solid samples: such as sludges and soils. The use of hydrogen peroxide is effective for highly organic based samples. Metal determinations in the final extracts can be done by flame atomic absorption spectrometry (FAAS), ICP-AES, or inductively coupled plasma-mass spectrometry (ICP-MS).

1.5.2 Microwave Acid Digestion (MAD)

Since 1994,¹² the applications of microwave energy for sample preparation reported have been increased rapidly because of its advantage of time reduction. These

microwave methods have been accepted worldwide by analytical laboratories. Up to now, EPA has released some microwave acid digestion methods for solid samples using the close-vessel systems specially designed for acid extractions as described below.^{41, 42}

EPA-3051 method has used only concentrated HNO₃ in a microwave system to digest sludges, soils, and sediments for metal determination by FAAS, graphite furnace atomic absorption spectrometry (GFAAS), ICP-AES, or ICP-MS. The microwave closed-vessel system requires fluorocarbon digestion vessels capable of withstanding pressure up to 7.5 atm (110 psi) and a temperature-monitoring device. Soil sample size is about 0.5 g. With microwave power of 600 to 1200 W, the sample mixture is heated within 5.5 min to reach 175 °C and then maintained at 170-180 °C for 10 min. Total time used for this method is less than one hour. This method can be used as an alternative to EPA 3050B.

EPA-3051A is a revised method of EPA-3051.⁴² A mixture of concentrated HNO₃-HCl (3:1 v/v) can be used instead. The addition of concentrated hydrochloric acid helps stabilize and improve the recoveries of some analytes such as Fe, Al, Ba, Sb, and Ag.

EPA-3052 method has been designed for total dissolution of samples using HNO₃ and HF combined with microwave energy to dissolve silicate containing materials and organically based matrices: such as oil contaminated soils, sludges, sediments. Metal determinations can be performed by FAAS, cold vapor atomic absorption spectrometry (CVAA), GFAAS, ICP-AES, or ICP-MS. Besides a higher pressure of 30 atm (435 psi), temperature and time required are in the same scales as those used for EPA 3051 method.

1.5.3 Leaching methods

From the point of view of soil scientists, single extraction procedures are the major method for diagnostic purposes including the prediction of deficiency/toxicity of trace metals for living systems. These methods are not designed to provide a total metal

analysis, but rather to provide a speciation analysis. The extractant methodology is reliable for some types of metals and sample matrices. Normally, alkaline salt solutions such as 0.1 M NaNO₃, 0.05 M MgCl₂, and 0.05 M CaCl₂ are employed to extract heavy metals weakly bound to soil surfaces as physisorption: such as Van der Waal, H-bonding and electrostatic interactions. With these salt extractants, low levels of some metals: such as Cu, Ni, and Zn, can be detected. Moderate concentrations of more powerful extractants such as 0.005 M DTPA and 0.05 M EDTA are designed for the speciation of organically bound metals. It has been reported that EDTA worked better than DTPA for calcareous soils.⁴³ These acceptable chelation methods may be able to provide the difference between two major metal fractions: all adsorbed metals and geological metals in mineral lattices, but these methods may need very long extraction times to achieve completion for adsorbed fractions. Some examples of extractants having selective extracting strengths are shown in Table 1.3.

Table 1.3: Extractants used to define metal fractions in soils. ^{7, 9, 33, 36, 43}

Metal fraction	Extractant
Water soluble	Water
Exchangeable	0.05 M CaCl ₂ , 1 M MgCl ₂ , 1 M KNO ₃ , 1 M NH ₄ Oac, 1 M NaOAc (pH 8)
Carbonate	1 M NaOAc/HOAc (pH 5)
Organically bound	0.005 M DTPA, 0.05 M EDTA, 0.1 M Na ₂ P ₂ O ₇ , 1 M HNO ₃ /H ₂ O ₂
Bound, occluded in oxides	NH ₂ OH•HCl/HOAc, Na ₂ S ₂ O ₄ /Na-citrate/H-citrate, Acid (NH ₄)C ₂ O ₄ , Na-dithionite
Residual/primary minerals	HNO ₃ /HF, HClO ₄ /HF

In soil laboratories, conventional chelation methods are performed at room temperature and consume large sample sizes: 10 g for DTPA and 2-5 g for EDTA. Normally, non-steady state extractions (incomplete reactions) occur within the experimental time scales of 1 to 2 hours.

1.6. Reviews of current microwave applications for metal determination in environmental samples

Since 1975¹¹ after Samra published an article about acid digestion of biological samples using a domestic microwave, it has taken time about a decade to develop microwave instruments for analytical purposes. In 1985, the first laboratory multimode cavity microwave system IR100 was introduced by CEM Company. A year later, the focussed microwave system was announced. Microwave-based sample preparation has been increasingly used since 1986 after the first conference of microwave dissolution for sample preparation took place.¹³ As is known most of early work have involved investigations of extraction recoveries obtained by microwave dissolution compared with those obtained by classical methods using conductive heating. Several microwave systems designed and a variety of acids have been studied. Microwave systems have been invented and applied to different purposes. Available microwave systems are close-vessel systems, open-vessel systems, and bomb systems. As mentioned earlier, Kingston (Skip) and Jessie have studies sample preparation using microwave instruments for many years.^{13, 14} Due to the need of fast and efficient methods to work with lots of current environmental samples, the applications of microwave energy have been studies continually. Currently, hundreds of citations can be found for the applications of microwave digestion and microwave extraction to a variety of sample matrices. For metal analysis in environmental samples, all reports found have involved microwave-assisted dissolution of different matrices prior to analysis using atomic spectrometry or mass spectrometry. Major inorganic acids such as HNO₃, HCl, and HF have been employed. Other acids such as HClO₄, H₂SO₄, H₃BO₃ have also been used to adjust the extracting conditions. The effect of acid mixtures on metal determination has been investigated.⁴⁴⁻⁴⁷ Besides metals, microwave assisted digestion has also been used for

determination of soil organic carbon using colorimetric measurement.⁴⁸ Even though some standards EPA methods have been addressed, comparisons between developed microwave digestion and conventional methods have still been studied.⁴⁹⁻⁵¹ In addition to soils, other environmental samples such as sediments, sludges, soil amendments, fertilizers, marine samples, plants, and natural waters have been subjected to microwave digestion techniques.⁵²⁻⁵⁸ Lately, microwave-assisted distillation methods have been developed for determination of arsenic in soils and food products.^{59, 60}

For analytical soil chemists, the major purposes for developing extraction techniques are to obtain safe, cheap, fast and selective methods. Because of this, a microwave instrument applied as an energy source is interesting. This dissertation will report studies combining chelation extraction and microwave heating for the determination of metals (Cu, Cd, Fe, Ni, Cr, Zn, and Pb) from soils. The selected extractants were anticipated to provide high selectivity of metal fractions in soils while the microwave radiation was expected to accelerate the extraction mechanisms and thereby reaching the steady state level in a reduced time scale. Compared to concentrated inorganic acids, EDTA derivatives were expected to provide safer conditions in the open microwave system. At this time, no details of standard chelation-microwave extraction methods for routine analysis have been addressed in the literature.

CHAPTER 2

EXPERIMENTAL

In this research, several tasks were performed to investigate the efficiencies of the coupling of chelation and microwave energy in the so-called Chelate Assisted Microwave Extraction (CAME) technique. First, sandy soils were used to test the feasibility of the CAME technique compared to hot acid extraction and conventional chelation. Two other control soils, Davidson Silty Clay and Davidson Clayey loam, used here contains large quantities of Fe/Mn oxides/hydroxides. Unfortunately, organic soils were not available, so the preparation of simulated organic materials was done by adsorption procedures. Then, metal spiked samples were prepared using adsorption processes as well. Different soil matrices were then employed to determine extraction capabilities for adsorbed metals. Copper was used in testing CAME since among trace metals it normally forms strong interaction with soils. So if copper, a strongly bound metal, can be extracted, then CAME should be able to work well with other trace metals as well. Also, Cu and Cd contaminated soils were prepared to explore the method efficiencies for co-extracted metals. The choice of chelating agents used with CAME was also investigated to verify suitability for each metal. Moreover, the influence of the equilibration time used for Cu spiking procedures prior to extraction was studied. Extraction temperature effect was also investigated to compare the benefit of microwave heating with hot plate heating.

2.1 Chemicals

Most reagents used for this work were of analytical grade. ACS Plus grade nitric acid was purchased from Fisher Scientific Company (Raleigh, NC). Reagent grade hydrochloric acid was obtained from E.M. Science (Gibbstown, NJ). High purity ethylenediaminetetraacetic acid (EDTA 99.99%), nitrilotriacetic acid (NTA 99%) diethylenetriaminepentaacetic acid (DTPA 97 %), citric acid (98%), oxalic acid (98%), ethylenebis(oxyethylenenitrilo)tetraacetic acid (EGTA 97%), triethanolamine (TEA

97%), diethylenetriamine (DETA 98%), and triethylenetetraamine (TETA 98%) were purchased from Aldrich Chemical Company (Milwaukee, WI). Fisher Scientific (Fair Lawn, NJ) supplied 1,2-Cyclohexylenedinitrilitetraacetic acid (CyDTA 98.0-100.0% A.C.S. grade) as well as solutions of metal standards for spectrometry and analytical grade copper chloride. Analytical grade cadmium chloride was obtained from Baker Chemical Company (Phillipsburg, NJ). Argon gas used for inductively coupled plasma atomic emission spectrophotometer (ICP-AES) and for graphite furnace atomic absorption spectrometer (GFAAS) was purchased from Air Products and Chemical Company (Allentown, PA). A commercial humic acid as sodium salt produced by Acros Organic Inc. was obtained from Aldrich Chemical Company. Washed and ignited sea sand was obtained from Fisher Scientific. Fine powder alumina (Particle size < 10 μm) and iron oxide (Particle size < 5 μm) were purchased from Aldrich Chemical Company.

2.2 Soil samples

Soil samples were obtained from the Crops Soil and Environmental Science Department at Virginia Polytechnic Institute and State University. There were three different control soil samples: Bertie Sandy Loam (BSL), Davidson Silty Clay (DSC), and Davidson Clayey Loam (DCL). The Bertie soil was collected from the Coastal Plain of Virginia while the Davidson clay and Davidson loam came from the Ridge Region of Virginia. The other sample studied here was the amended Bertie Sandy Loam, which was treated with CuSO_4 for a period of time during 1980-1983. These samples have already been used to study metal availability for plants. The details of some characteristics of these soils can be seen in Table 2.1.⁶¹⁻⁶³

TABLE 2.1: Characteristics of the selected control soils.⁶¹⁻⁶³

Property	BSL	DSC	DCL
pH	6.3	6.7	6.3
Organic matter (wt %)	1.7	1.3	1.8
CEC (meq/100g)	5.6	11.2	12.5
Fe content (wt %)	0.3	5.5	-
Particle size distribution (%)			
Sand	68	11	15
Silt	26	40	47
Clay	6	49	38
Mineral suite of < 2 μm clay fraction	I ₁ , K ₂ , Q ₃ , M ₄	K ₁ , I ₂ , M ₃ , Q ₃ , G ₄ , F ₅	K ₁ , I ₂ , MO ₃ , Q ₄ , F ₅

I = intergrade minerals, K = kaolinite, Q = quartz, M = mica, G = gibbsite, MO = montmollinite, F = feldspar, CEC = cation exchange capacity

2.3 Acid distillation

High purity acids are required for trace metal analysis in order to avoid high background possibly obscuring the trace analytes. Some standard EPA methods, such as EPA 200A and EPA 3050B, recommend double distilled acids for this purpose. Metal levels in acid solvents used have to be lower than the method detection level. In this work, a laboratory constructed sub-boiling system was employed for distillation of ACS grade acids. The still was made from Teflon compartments. Concentrations of Cr, Cd, and Fe in the redistilled acids were determined to evaluate the efficiency of the still system.⁶⁴ The results are shown in Appendix A.

2.4 Apparatus

Apparatus used in this study included a Fisher Thermanix® hot plate model 600T, a Clay Adama Dynac centrifuge, a Lab Line junior orbit shaker, a Mettler AB204 digital balance, a Fisher accumet® model 10 pH meter, and a Prolabo Soxwave 100 microwave extractor (Paris, France).⁶⁵ All Nalgene™ polyethylene storage bottles and glassware were cleaned thoroughly with Sparkleen™ and then soaked with 20% nitric acid for 4 hours. Afterwards, they were rinsed once with distilled water and three times with distilled-deionized water. All cleaned containers were allowed to dry.

2.5 Microwave extractor

The microwave system used for this work was an open focused Prolabo Soxwave 100 system. Calibrated maximum microwave energy output was 300 watts at 2450 MHz. Nine steps of increasing applied energy could be selected. For each step, the power could be varied from 10 to 100% with 5% interval and the irradiation time could be adjusted from 0 to 60 min with 1-min increments. All microwave parameters were controlled from the operation unit. A water condenser system was designed to prevent the vaporization and loss of solvents and analytes. Microwave vessels of this model were made from borosilicate and had a capacity of 250 mL. The design allowed microwave

energy to be focused on the sample sitting at the bottom of the vessel. Compared to closed vessel systems, less energy is required and sample heating is more uniform and reproducible. The specifications of the Soxwave 100 are described in Table 2.2. The simple schematic diagram of the Soxwave 100 is also shown in Figure 2.1.⁶⁵ For optimum conditions, it has been found that microwave power levels should be adjusted to provide gentle refluxing without sputtering and possible loss of the sample.

2.6 Conventional acid dissolution/Hot acid extraction

All hydrochloric and nitric acid was distilled by the system mentioned above. The acid extraction known as EPA 200A method was employed for the determination of total trace metals in soils. Two acid solutions were prepared for this experiment. The straight HCl was diluted to 20% by adding 1 part of acid to 4 parts of distilled-deionized water while the straight HNO₃ was diluted to 50% by adding 1 part of straight HNO₃ to 1 part of distilled-deionized water. The two acid solutions (20% HCl and 50% HNO₃) were kept in glass storage bottles for further use.

At the beginning of the procedure, 0.5 g of a soil sample was accurately weighed into a 250 mL beaker. Then 4.0 mL of 50% nitric acid and 10.0 mL of 20% hydrochloric acid were pipetted into the beaker. The sample beaker was covered with a watch glass and placed on a hot plate. In order to monitor and circulate the temperature generated by the hot plate, another beaker containing 50 mL of water was placed at the center of the hot plate. The temperature was slowly increased to 85 °C and then maintained at this level for 30 minutes. The sample beaker was removed from the hot plate. The digested mixture was transferred into a 100 mL volumetric flask and then adjusted to volume with distilled-deionized water. The diluted sample was then centrifuged at 2500 rpm for 10 min. Finally, the clear extract was separated and kept in a cleaned storage polyethylene bottle.

TABLE 2.2: Specifications of Prolabo Soxwave 100 microwave extractor.

Microwave frequency	2450 MHz
Pressure control	Atmospheric
Maximum power	300 W
Number of operational steps	9
Maximum time of each step	60 min
Time increment	1 min
Minimum power setting	10%
Power increment	5%
Microwave vessel material	Borosilicate glass
Microwave vessel volume	250 mL
Sample size	1-10 g

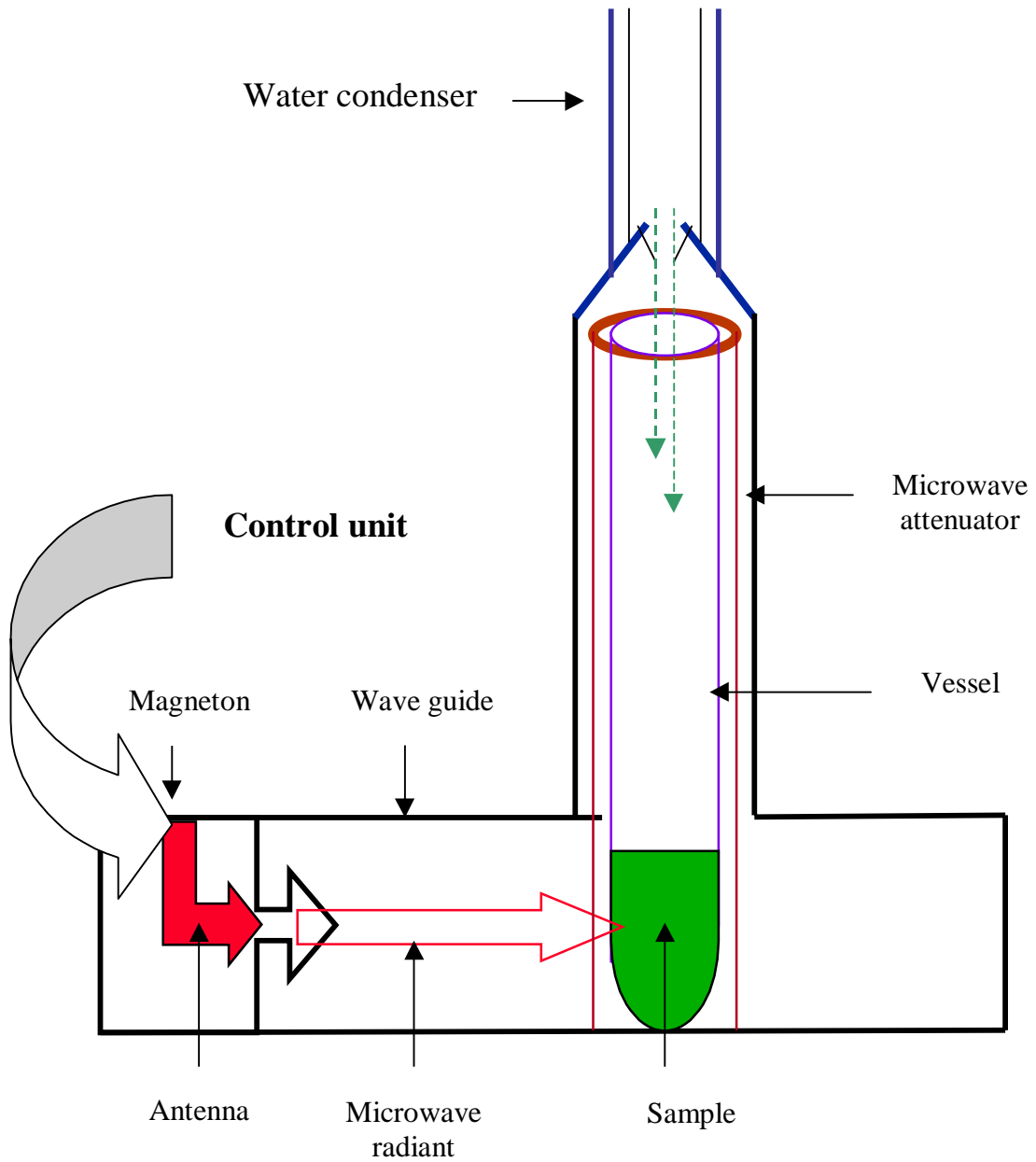


Figure 2.1: Schematic diagram of Prolabo Soxwave 100 open focused microwave extractor.⁶⁵

2.7 Chelation extraction

The EDTA-shaking method was used for comparative measurements. Generally, a soil sample of 0.5 to 2.0 g was accurately weighed into a 125 mL conical flask. Next, 10.0 mL of an EDTA solution of pH 7 were pipetted into the flask. The sample flask was covered with Parafilm and then shaken at room temperature for 1 hour at 150 rpm by using the Lab Line junior shaker having a stroke of 3 cm. The sample mixture was then filtered through Whatman No. 42 filter paper. The filtrate was stored in a clean plastic bottle for later metal analysis.

2.8 Chelate Assisted Microwave Extraction (CAME)

An accurately weighed soil sample of 0.5 to 1.0 g was mixed with 10.0 mL of the extracting solution in a cleaned microwave vessel. Microwave conditions were then adjusted via the control unit. After microwave irradiation time has elapsed, the sample mixture was filtered through Whatman No. 42 filter paper into a volumetric flask to obtain a clear filtrate. To minimize sample loss, distilled-deionized water was used to rinse the sample from the extraction apparatus and was collected in the sample flask. The exact volume of the extract, 50 mL or 100 mL, was adjusted using distilled-deionized water. Afterwards, the final extract was transferred into a plastic bottle for further analysis.

2.9 Conventional heating-chelation extraction

An accurately weighed soil sample of 0.5 g was added into a 50 mL erlenmeyer flask. Next, ten milliliters of an extracting solution were pipetted into the sample flask, which was then covered with a watch glass. The sample flask was placed on a hot plate used as a heat source. Temperature was measured for 60 min. After the solution began to boil, the heating was adjusted in order to avoid sputtering, but to maintain constant temperature. After each extraction, the sample mixture was filtered through Whatman No. 42 filter paper into a volumetric flask. Distilled-deionized water was used to rinse

the extraction flask and to adjust the final volume of the extract. The clear filtrate was then transferred into a plastic storage bottle.

2.10 Extraction of humic acids from soils

Three soil samples (Bertie Sandy Loam, Davidson Silty Clay, and Davidson Clayey Loam) as described in the soil background section were extracted using an alkaline extraction method described elsewhere.⁶⁶ Twenty grams of each soil were suspended in 200 mL of 0.1 M NaOH solution in a screwed-cap plastic bottle which was shaken using a mechanical shaker for a specific time period. After 24-hour extraction, the sample mixture was filtered through Whatman No. 542 filter paper to remove the solid residue. Then the filtrate was acidified to pH 1 to precipitate out extracted humic acids. Reprecipitation was performed to obtain better purity of the extracted humic acids, which were allowed to dry before being studied further.

2.11 Spectroscopic studies of humic acids

Comparative investigation of the Acros Organic humic acid and the extracted humic acids was performed using infrared spectroscopy (IR) and nuclear magnetic resonance spectroscopy (NMR). For this research, a Perkin Elmer model 1600 IR unit was adapted for a solid state study using a Diffusion Reflectance Infrared Fourier Transform (DRIFT) technique. All humic acids were investigated by DRIFT. A solid state C^{13} NMR as Cross Polarization Magic Angle Spin (CPMAS) technique was performed by a Bruker model MSL 300/200 at 75 MHz and applied to the commercial humic acid and the extracted humic acid from Bertie sandy loam. The ratio of absorbance at two wavelengths (465 nm and 665 nm), E_4/E_6 , was measured by Perkin Elmer Lambda 4 UV-VIS spectrophotometer. This measured ratio (<5) helped confirm that the majority of this commercial humic substance was humic acid.⁶⁶

2.12 Matrix preparation

2.12.1 Preparation of organic coated humate materials

Batch experiments were performed to prepare organic coated matrices. Fifty grams of each original soils as well as sea sand, alumina, and iron oxide were individually equilibrated with 150 mL of 2000 mg-humic acid/L at pH 6 for 48 hours. UV-VIS spectroscopy was then employed to quantify adsorbed humic acids on each original substrate.⁶⁷ The organic coated materials were allowed to dry before being used in the next experiment.

2.12.2 Preparation for Cu adsorption on original materials (Cu-substrates) and on organic coated materials (Cu-HA-substrates)

To obtain Cu-spiked samples for evaluating extraction efficiencies, adsorption procedures were applied at room temperature. A solution of 0.001 M CuCl₂ at pH 5.3 was prepared as an initial equilibrating solution. Then, Cu spiking procedures were performed at a ratio of 20 g-material per 200 mL-Cu (II) solution for 24 hours. The mixture was centrifuged, filtered and analyzed to determine Cu concentration left after the adsorption process. Determination of Cu was performed using ICP-AES. The differences between initial and final filtrate Cu concentrations represented the amounts of Cu adsorbed onto each matrix. The resulting matrices, Cu-materials and Cu-HA-materials, were allowed to dry and then ground before being stored in plastic bags for further extraction studies.

2.13 Metal determinations by atomic spectrometry

2.13.1 Graphite Furnace Atomic Absorption Spectrometry (GFAAS)

For the determination of low metal levels such as impurities in acids and reagents, a GFAAS Buck Scientific model 200A connected to a Linseis model L6522B recorder

was employed. Manual injections were performed using 10 μ L of a sample with and without modifiers.⁶⁴

2.13.2 Inductively Coupled Plasma-Atomic Emission Spectrophotometry (ICP-AES)

The ICP-AES used for this research was a Perkin Elmer model 6000 generating a high radio frequency (rf) of 27.12 MHz with a variable radio frequency power up to 2.5 kW. With a demountable quartz torch and an alumina line tube (2.5 mm i.d.), the argon plasma flow rate was set at 12 L/min with the auxiliary argon gas flow rate of 0.4 L/min. For aqueous solutions, the applied rf power generated from a Cu tesla coil was set at about 1.25 kW. Before the plasma could be started, the water cooling system was set at a flow rate of 0.5 L/min to prevent overheating of the rf coil. For the sample uptake, the nebulization system consisted of a concentric Meinhard nebulizer type C (J E Meinhard Associates Co, Santa Ana, CA) and a single pass spray chamber built in our laboratory. The optimum nebulization pressure was obtained at 34 psi with the consumption rate of 2.8 mL/min of Argon gas. The monochromator of the ICP model 5000 was of a Czerny-Turner type with a 0.40 m-focal length. Resolution for the ICP was 0.05 nm/mm and the nominal bandpass was 0.02 nm. Some specifications of the ICP-AES system used are given in Table 2.3.

2.14 Analytical process

In this research, analytical tasks were begun with soil collection and then proceeded to matrices (organic and metallic) applied to the original soils. These prepared soil matrices would be further subjected to extraction procedures to remove metals. Next, the extracts were separated using centrifugation or filtration. Standard solutions were then prepared to match the solvent effect used for each extraction method. Metal determinations were mainly performed using ICP-AES. Data treatment and statistical analysis were then applied to compare the obtained results. A simple scheme of the analytical process is shown in Figure 2.2.

TABLE 2.3: Specifications of ICP-AES spectrometer.

Spectrometer	Perkin Elmer ICP 5000
ICP generator	Perkin Elmer 6000, 27.12 GHz
Plasma power	1.25 kW
Data station	Perkin Elmer 7500
Printer	Perkin Elmer PR-210
Acquisition software	Perkin Elmer series 7000, idris system
Torch	Perkin Elmer demountable quartz, 0.3 mm i.d. tip
Nebulizer	Meinhard, concentric type C
Plasma/auxiliary gas	Argon 12 L/min and 0.4 L/min, respectively
Consumption rate	2.8 mL/min
Viewing height	15 cm
Wavelength (nm)	
Cd	214.44
Cr	267.72
Cu	324.75
Fe	259.94
Ni	231.60
Pb	220.35
Zn	213.86

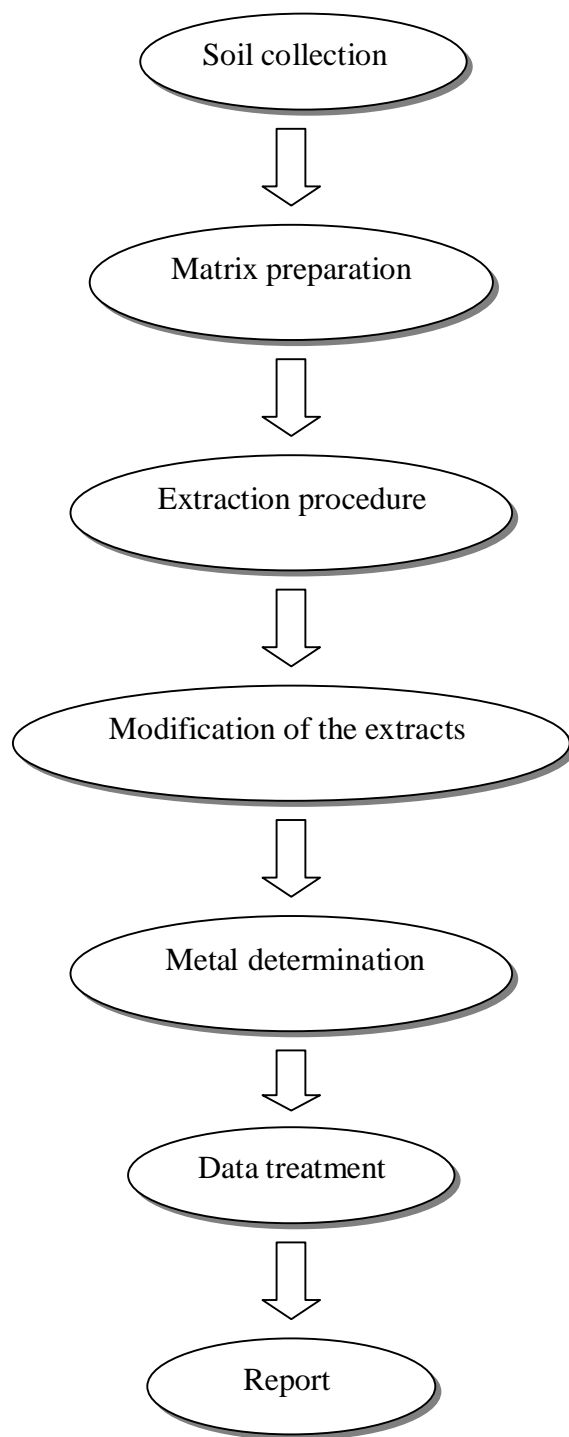


FIGURE 2.2: Schematic diagram of the analytical process.

CHAPTER 3

EDTA-MICROWAVE METHOD & SANDY SOILS

Initial studies explored in this research have involved method development of Chelate Assisted Microwave Extraction (CAME). The combination of the powerful chelating agent EDTA and microwave heating (EDTA-CAME) was first used to liberate adsorbed copper from natural sandy matrices. It has been believed that sandy aluminosilicate soil, compared to organic and oxide coated soil, is the weakest sorbent for metals. In this chapter, two sets of Bertie Sandy Loam (BSL), control and amended, were examined.

A major concern of soil scientists on Cu behaviors in soils results from Cu-surface interactions. Among Cu species, the Cu (II) is the main ion involved in mobility and transport in soil-plant systems. As is well known, Cu (II) is a small ion (radius 0.81 Å) possessing high electronegativity (2.0). Cu (II) ions, therefore, strongly adsorb onto negatively charged surfaces and as well as to oxide and organic components in soils. Because of this, Cu (II) concentrations available to plants could fall to deficient levels, hence soil amendment is used to overcome this problem. The original BSL used here was called a “control” while the other, the “amended BSL”, was prepared by adding CuSO₄ to the original soil. Both BSL samples had already been subjected to a study of Cu (II) availability for plants in previous studies.⁶¹ However, it was believed that the residual copper left in the amended BSL after long-term interactions with sandy particles should be higher than that in the control soil. Strong interactions between Cu (II) ions and soil matrices are expected to be a good measure for evaluating the extraction efficiency of a developed method. Because the amended BSL had higher level of Cu (II), it was used for further extraction studied here. At the last section, both sample sets (control and amended) were employed for comparative measurements using different extraction techniques including the hot acid extraction, the conventional chelation (shaking extraction) and the EDTA-microwave method. Cu (II) determinations were

performed at 324.75 nm using Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES). For each extraction method, the extracting solution was added into the standard solutions to overcome the solvent effect possibly encountered with the detection technique.

3.1 The pH effect

For the first experiment, a series of 0.05 M EDTA solutions having a pH range from 7.75 to 10.08 at an ionic strength of 0.04 Na₂CO₃/NaHCO₃ were prepared in distilled-deionized water. Two grams of an accurately weighed amended sample were added into a clean microwave vessel. Then 10.0 mL of an EDTA solution were pipetted and used to leach the sample. Each extraction was performed using a microwave power of 90 W (30%) for 3 min. After microwave application, the extracted mixture was transferred into a 100 mL volumetric flask. Distilled-deionized water was used to thoroughly rinse all parts of the extraction apparatus, and the rinsing water was collected in the sample flask. A clear extract was obtained by filtration/centrifugation.

For the classical shaking method, chelation extraction was carried out at room temperature. Also, the same soil/solution ratio of 2 g-soil/10 mL-extracting solvent was employed for the shaking method at 3 cm-stroke and 150 rpm for 1 h. Filtration and centrifugation was also required to separate the clear extract from the solid residue. All resulting extracted solutions were analyzed for Cu by ICP-AES. These results are given in Figure 3.1.

As can be seen in Figure 3.1, the amount of extracted Cu was dependent upon the pH for the classical shaking chelation technique, whereas pH did not affect the extraction of the EDTA-microwave method. It is evident that the EDTA-microwave method provided better extraction efficiencies than the classical shaking method. At higher pH, more dissociation of EDTA molecules will occur. The known effective EDTA species, HY³⁻ and Y⁴⁻, can stabilize extracted Cu species as dissolved complexes rather than those bound onto the soil surfaces. Moreover, at higher pH soil surfaces possibly contain a

larger number of negative surface charges, thus Cu-EDTA complexes possessing negatively charges will prefer to stay in the aqueous solutions.

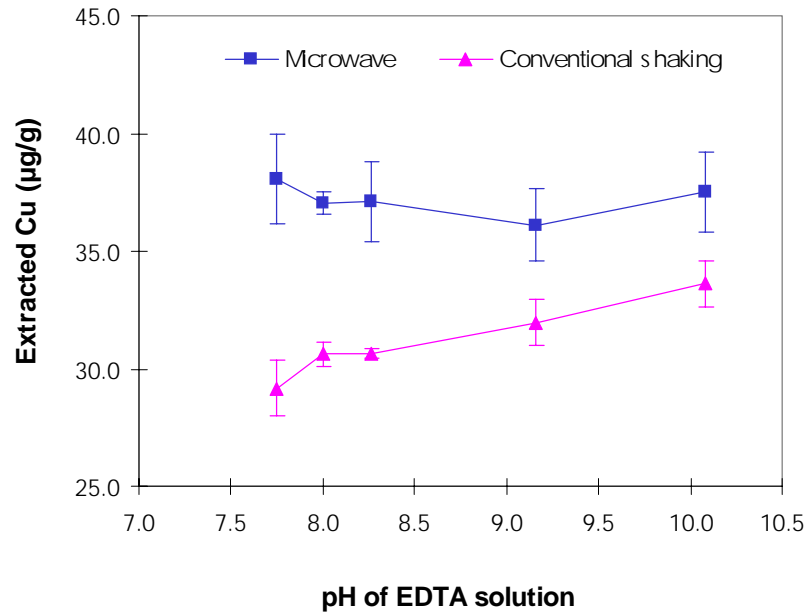


FIGURE 3.1: Cu removal from amended Bertie Sandy Loam as a function of pH and extraction method. Each data point represents the average of 3 extractions. Error bars reflect the confidence intervals of the mean values.

From Figure 3.1, the extraction profile obtained from the EDTA-microwave method illustrates a steady level of extracted Cu concentrations whose average is higher than that obtained from the conventional chelation at any pH. With two-way ANOVA statistical analysis without replications at 95% confidence, it has been found that there is a significant difference between the two extraction methods ($F_{\text{cal}} = 41.58 > F_{\text{crit}} = 7.71$), whereas there is no significant difference between pHs ($F_{\text{cal}} = 0.58 < F_{\text{crit}} = 6.39$). Despite the increase of the extraction efficiencies of the conventional method with increasing pH, this statistical test has been unable to detect whether the different pHs give significantly different results. However, one-way ANOVA applied to a data series

obtained from the shaking method proves that the different pH values have significantly different efficiencies ($F_{\text{cal}} = 70.12 > F_{\text{crit}} = 3.48$).

It is most likely that the applied microwave energy can affect the kinetically controlled reactions, and thereby accelerate the overall mechanism of Cu leaching from the irradiated soil surfaces. Probably, the energy transfer by microwave interactions is the major factor to produce the difference between the EDTA-microwave method and the EDTA-shaking method. In order to reach the same extractable level as obtained from the EDTA-microwave method, the conventional chelation may require much higher EDTA concentration, longer extraction time and/or extremely high pH.

3.2 Solvent effect

The next experiment was designed to elucidate whether the better extraction efficiency of the EDTA-microwave (CAME) resulted from the presence of EDTA or water and $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$ in the extracting solution. Although water is a good microwave absorbing solvent, the carbonate species may also enhance ionic conduction heating during microwave adsorption. This examination was performed using three solutions including distilled-deionized water, $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$ solution, and EDTA in $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$ solution as extractants in the microwave extractor. Here we investigated the extraction efficiencies as a function of a microwave profile of 90 W for 3 min followed by 30 W for 17 min. A ratio of 1 g- amended BSL/1- mL- solution was used. The variation of times was done to maintain the boiling condition of the mixture without strong sputtering and possible loss of the sample. Figure 3.2 implies that each extracting solution provides an extraction level related to a different adsorbed Cu fraction in the amended soil. EDTA showed better extraction efficiency than the carbonate buffer and water, respectively. Generally, water with a shaking method rarely produces detectable levels of trace metals in soils. This study indicates that the applied microwave energy easily helps faster water-Cu exchange reactions. Microwave energy may help accelerate the forward reaction of the water exchange reaction for Cu in diffused and most loosely bound fractions due to the heating contributed from water molecules and

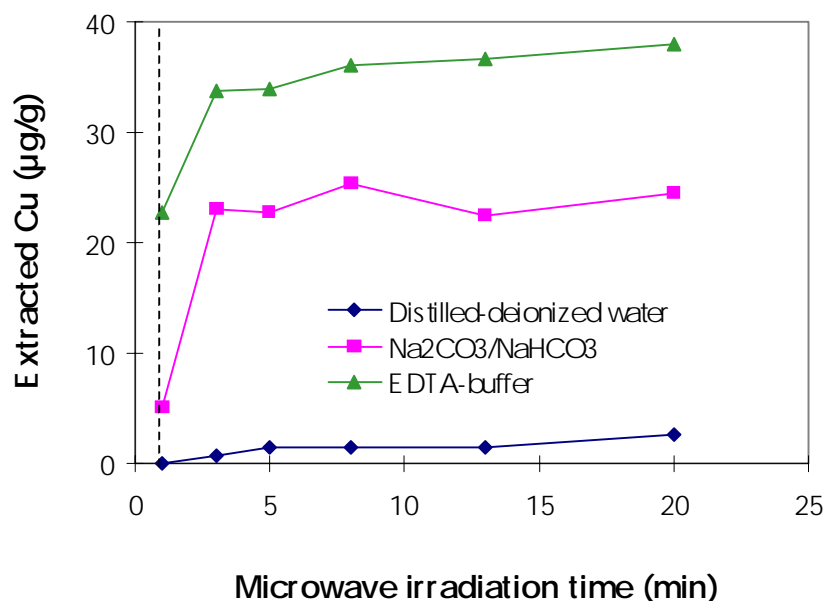


FIGURE 3.2: Extraction efficiencies as a function of microwave profile and solvent (90 W for 3 min followed by 30 W for longer times). The soil mixture boiled within 1 min-exposure.

due to some direct microwave adsorption at the soil surfaces. Compared to water molecules, the carbonate species could help to desorb another Cu fraction, which possesses intermediate binding sites of electrostatic interactions between Cu species and negatively charges of aluminosilicate surfaces or organic components. The presence of Na⁺ ions combined with microwave energy could help desorb the exchangeable Cu fraction in a short extraction time. The EDTA solution should help in removing the Cu fractions bound at the stronger binding sites in soil at which coordinated covalent bonds occur. The data in Figure 3.1 allow the surface bound Cu to be classified into three different fractions including water soluble-, exchangeable-, and chemisorption fraction. The time required for a steady-state extraction appears to be only about 15 to 20 min with microwave irradiation.

3.3 Microwave conditions

3.3.1 Microwave irradiation power

Optimization of microwave conditions was investigated with the respect to the effect of microwave irradiation power. First, the microwave irradiation time was fixed for 3 min-exposure while the microwave power was varied from 30 to 300 W. It was found that the extraction efficiencies increased when the power was up to 100 W, and then varied slightly at higher power till 300 W, as shown in Figure 3.3. This implies that within 3 min of microwave irradiation the power exceeding 100 W could effectively remove all exchangeable Cu concentrations and a part of chemisorbed Cu fraction. To leach out all chemisorbed Cu species, it may require more time for this system.

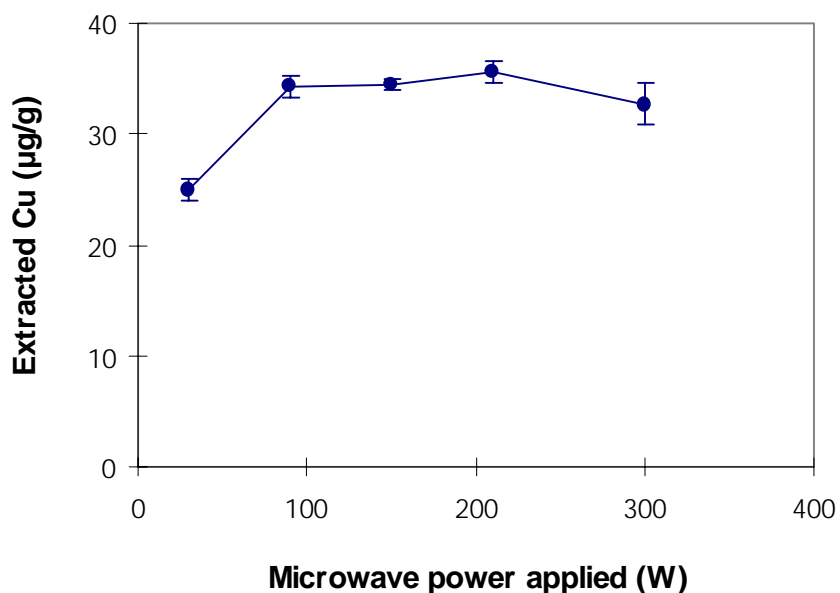


FIGURE 3.3: Extraction efficiencies as a function of microwave power (n=3), time 3 minutes.

3.3.2 Microwave irradiation time

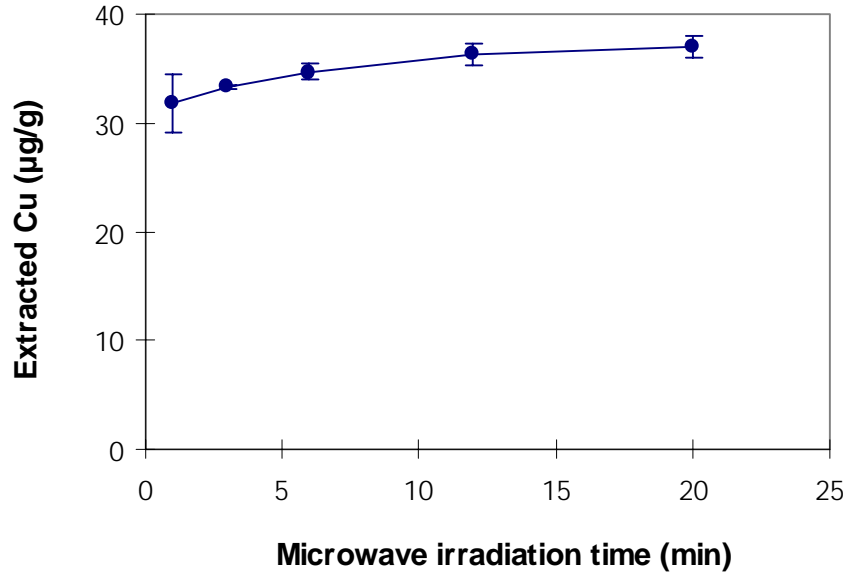


FIGURE 3.4: Extraction efficiencies as a function of microwave irradiation time (n=3), power 300 W.

The next study was performed using a fixed microwave power of 300 W and the varied irradiation times from 1 to 20 min. Figure 3.4 shows that a large extent of extraction could be achieved within first few minutes. It can be explained that the initially extractable Cu concentrations could possibly result from non-bound and loosely bound fractions (exchangeable metals) that were readily leached out within 1-2 min. These outer-sphere Cu complexes were expected to be completely extracted. Afterwards, the stronger interactions between Cu ions and soil components as chemisorbed fractions would therefore be involved. The order of extracted species coming off the sample is possible as follow: (1) unbound surface metals, (2) bound surface metals: physical adsorbed and chemisorbed fractions, (3) geological metals in crystal structures. The fraction 1 and 2 are considered as anthropological metals, which were expected to be

completely extracted by the EDTA-microwave method. With the extraction profile selected, metals in enthalpically controlled region (fraction 1 and 2) were totally leached out within 15 minutes. The following steady-state level may be kinetically controlled, and the extended extraction time did not show any influence on the post extraction period. However, the maximum of the extracted Cu concentration was $37.5 \pm 1.7 \mu\text{g/g-soil}$, which was not significantly different from the total Cu concentration ($40.1 \pm 6.7 \mu\text{g/g}$) in the amended Bertie Sandy Loam using the classical hot acid extraction. It is proposed that after the extraction equilibrium occurs at the steady-state level of the EDTA-microwave method the combination of EDTA strength and microwave energy does not affect Cu stability in the soil residue. The later experiments of comparison measurements performed would help to describe this issue.

3.4 Comparative measurements of Cu determinations in sandy soils

From previous studies, it has been observed that the strong microwave power at 300 W can induce violent sputtering of the sample mixture inside the extraction vessel after less than 1 min. We observed this problem and the resultant possible loss of sample due to violent sputtering. Comparing methods using total 20 min-microwave exposure, the results obtained from 1 step extraction (300 W) and 2 step-extraction (90 W 3 min + 30 W 17 min) were not significantly different. Because of this, a two-step extraction of milder condition using 90 W for 3 min followed by 30 W for 17 min was chosen for comparing the extraction efficiencies of the EDTA-microwave technique and conventional methods. The procedural details of the hot acid extraction and the shaking chelation were described in Chapter 2. Table 3.1 shows confidence limits of Cu concentrations extracted from the control and the amended Bertie Sandy Loam. It was found that the EDTA-microwave method provided better extraction efficiencies than the EDTA-shaking method but worse than the hot acid extraction method. It can be explained that Cu extractabilities of the EDTA-shaking method may be obtained at non-equilibrium (incomplete) conditions, which give rise to Cu concentrations lower than that observed by other methods. There is no surprise that the control BSL contained lower Cu concentration than the amended BSL that could be observed from all extractions shown

in Table 3.1 and Figure 3.5. More importantly, the differences of extracted Cu concentrations obtained from the hot acid extraction method and the EDTA-microwave method were quite close, 3.0 ppm (8.9-5.9) for the control BSL and 2.6 ppm (40.1-37.5) for the amended BSL, respectively. Therefore, a question has been addressed if these differences probably resulted from the presence of Cu species in the mineral residues geologically formed in the control BSL.

TABLE 3.1: Comparison of extraction methods using sandy soils (95% confidence limits, n = 3)

Soil sample \ Method	μg -extracted Cu/g-soil		
	EDTA-shaking	EDTA-microwave	Hot acid
The control BSL	4.6 ± 0.2	5.9 ± 0.5	8.9 ± 5.0
The amended BSL	27.8 ± 1.7	37.5 ± 1.7	40.1 ± 6.7

Up to this point, the initial studies of the sandy soils indicate that the combination of EDTA and microwave energy offers a good opportunity to monitor all naturally adsorbed and anthropological Cu, but not total Cu concentrations. It seems likely that some concentrated inorganic acids are still required for only geological studies of metal resources in minerals but not necessary for the assessment of metal toxicity for environmental studies. Because the geological metals in lattice crystal forms not directly contact daily environmental changes and they may take a very long time to be transformed to other forms that can move into soil solutions.

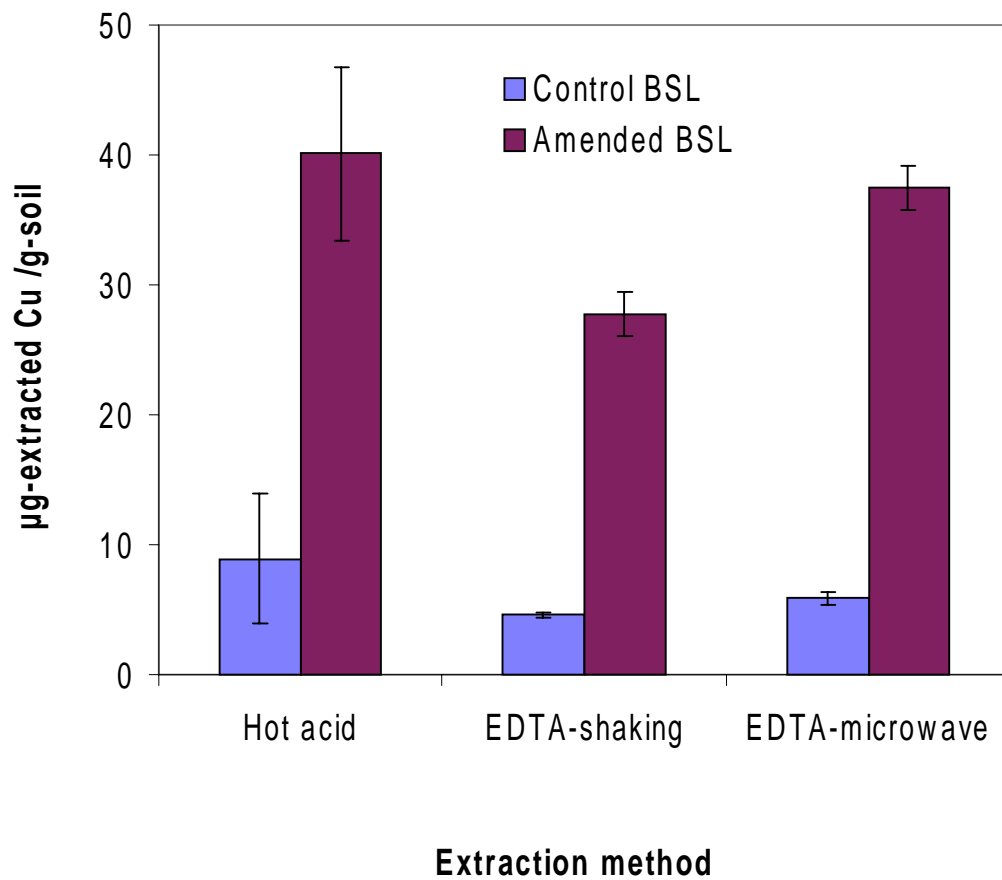


FIGURE 3.5: Extracted Cu concentrations from control and amended Bertie Sandy Loam using different extraction methods (95% CI, n=3).

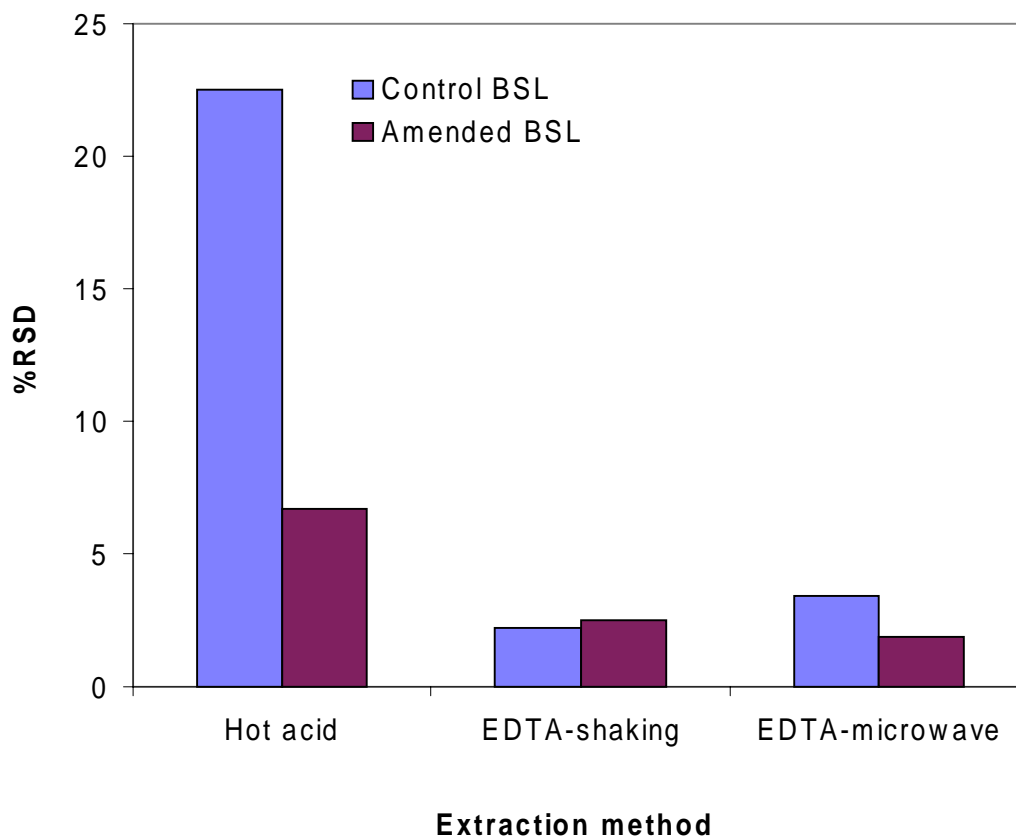


FIGURE 3.6: Relative standard deviation of extracted Cu concentrations from control and amended Bertie Sandy Loam using different extraction methods (95% CI, n=3).

Figure 3.5 helps to clearly illustrate that the amended BSL contained higher Cu level than the control BSL. This results from the long-term application of CuSO_4 during 1980 to 1983. Most of spiked Cu contents probably still remain as anthropological Cu and we can prove that it was successfully extracted by the EDTA-microwave method.

In order to compare the precision of extraction methods, the relative standard deviation of the extracted Cu levels from the control and the amended samples were calculated. The results shown in Figure 3.6 indicate that a solvent containing high concentrations of mineral acids, HNO_3 and HCl , do affect the detection efficiency of the ICP-AES. High concentrations of HNO_3 and HCl will cause high solvent viscosity, hence the smaller detection signals. Even though all standard solutions were prepared by adding the extracting solvent to overcome this solvent effect, some metal contamination from the redistilled acids can cause errors. Extraction using EDTA with either the shaking method or the EDTA-microwave method provides better precision than the hot acid extraction.

From Table 3.1, statistical tests were applied to the results obtained from the amended BSL. The conclusion of t-test for the comparison of means is given in Table 3.2. All pairs except the hot acid method and EDTA-microwave method show significant differences of the means. This is suspected the result from the small amount of Cu (2.6-3.0 $\mu\text{g/g}$) occupying in the mineral lattice relatively compared to the anthropological Cu concentration (37 $\mu\text{g/g}$) in the amended BSL. Considering method precision, the variance of the EDTA-microwave method is not significantly different from those of the EDTA-shaking method and the hot acid extraction method since the calculated F values are lower than the F critical values. However, the variance from the hot acid extraction is much different from those of the other two methods. This implies that these differences may be not influenced from random variation, but systematic variation especially from the solvent effect of the hot acid extraction. Because of using the long-term amended BSL, extraction efficiencies here have been reported as part-per-million (ppm or $\mu\text{g/g}$), not % recovery.

TABEL 3.2 Statistical results of extraction methods applied to the amended Bertie Sandy Loam.

Method	(1) EDTA-shaking	(2) EDTA-microwave	(3) Hot acid
(1) EDTA-shaking			
Mean	-	Different, $t_{cal} = 13.86$	Different, $t_{cal} = 6.24$
Precision	-	(1) \approx (2), $F_{cal} = 1$	(1) \approx (3), $F_{cal} = 14.88$
(2) EDTA-microwave			
Mean	Different, $t_{cal} = 13.86$	-	Not different, $t_{cal} = 1.32$
Precision	(2) \approx (1), $F_{cal} = 1$	-	(2) \approx (3), $F_{cal} = 14.88$
(3) Hot acid			
Mean	Different, $t_{cal} = 6.24$	Not different, $t_{cal} = 1.32$	-
Precision	(3) \approx (1), $F_{cal} = 14.88$	(3) \approx (2), $F_{cal} = 14.88$	-

- Comparison of means uses t-test and comparison of precision uses F-test.
- Two-tailed t-test at 4 degrees of freedom, $t_{crit} = 2.78$.
- Two-tailed $F_{crit} = 39.00$ and one-tailed $F_{crit} = 19.00$.

3.5 Strong inorganic acids in the microwave extractor

One additional experiment has been performed using the hot acid extraction method to an open focused microwave extractor Prolabo Soxwave 100. As a function of irradiation time from 1 to 20 min, the extracted Cu concentrations obtained from the amended Bertie Sandy Loam are given in Figure 3.7. It is important to point out that this experiment was very risky due to vigorous vaporization of some gaseous acidic species during absorption of microwave energy. Unfortunately, we had no a closed-vessel

microwave extractor designed for a strong inorganic acid extraction to compare the results. However, with the opened system it was observed that extremely fast extraction could occur within 1 min at 100 % of power, and there was no significant improvement of extraction efficiency when irradiation time was increased from 1 to 20 min. Each data point was obtained from a single extraction and determined for Cu by ICP-AES for 5 replicates. The application of the t-test to the data indicated that there was a significant difference between means of 1-min and 5-min extraction ($t_{\text{cal}} = 2.82 > t_{\text{crit}} = 2.57$). This could result from systematic error and metal contamination from inorganic acids used. Nevertheless, the average extracted Cu concentration from the amended Bertie Sandy Loam seemed to be approximately 40 $\mu\text{g/g}$, which was consistent with the mean obtained from the hot acid extraction method.

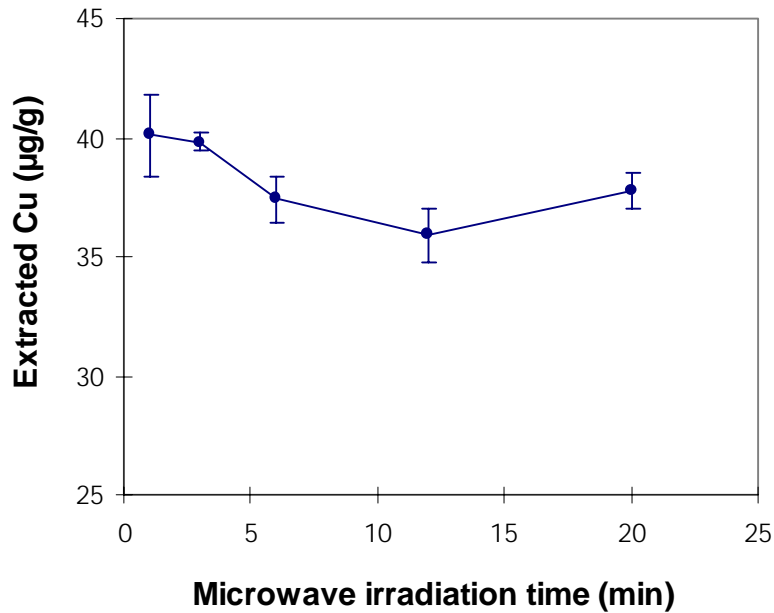


FIGURE 3.7: Strong inorganic acids in Prolabo Soxwave 100 using 100% power on the amended BSL. Each data point represents the average of five readings from ICP-AES. Error bars reflect the 95%CI of the mean values.

3.6 Summary

The preliminary tests have indicated that the extraction efficiency was method dependent. Conventional shaking chelation at room temperature for 1 hour-extraction could not effectively extract total adsorbed Cu fractions from the soils. On the other hand, the maximum steady-state level was obtained in a short period of time with microwave application. From the microwave extraction profile, the maximum extractability occurred within 15 to 20 min using a microwave irradiation of 90 W for 3 min followed by 30 W for longer time. Using 0.025 M EDTA combined with 20-min microwave application on two sets of Bertie Sandy Loam, it can be seen that the EDTA-microwave method was able to liberate total adsorbed Cu fractions from soil components.

This extraction methodology developed here was a static process. The extracting strength of EDTA was proven to be helpful for the differentiation between labile and immobilized metals in soil environments. The EDTA-microwave method appears to be feasible with a short extraction time. Up to this point, one question has been addressed, "Could this coupling technique work well with other kinds of soils?" To answer this question, later experiments would be studied on organic coated and oxide rich materials. The next chapter will involve characterizations of humic acids and matrix preparation.

CHAPTER 4

HUMIC ACIDS & MATRIX PREPARATION

The experiments performed in this chapter involves characterizations of humic acids (HAs) and the preparation of Cu-spiked materials. Initially, the extracted and the commercial HAs were structurally elucidated by spectroscopic techniques such as UV-VIS spectrometry, NMR spectroscopy, and IR spectroscopy. The commercial HA was employed to prepare organic based matrices. These investigations helped foster a better understanding of HA structural properties and how they are dependent upon their natural sources.

In natural systems, there are several types of soil materials depending upon soil components. The soils used in this work consisted of one sandy soil (Bertie Sandy Loam) and two oxide rich soils (Davidson Silty Clay and Davidson Clayey Loam) all which contained low Cu concentrations. Accordingly, simulated organic materials were prepared for supplementing these soils. All procedures used for the preparation of HA coated matrices and the Cu-spiked samples utilized adsorption techniques. The available commercial HA representing a soil HA was used to prepare highly organic containing samples as HA-materials of six solids including sea sand, alumina, iron oxide, Bertie Sandy Loam (BSL), Davidson Silty Clay (DSC), and Davidson Clayey Loam (DCL). Lastly, the adsorption experiments were employed to coat Cu onto two sets of solid matrices, including six original (uncoated HA) materials and six coated HA materials. Cu-spiked materials would be used for following evaluations of extraction methods in the next chapter.

4.1 Spectroscopic studies of humic acids

Due to the limited quantities of control soils available for these studies, only twenty grams of each control soil were subjected to the alkaline extraction of HAs as

described in Chapter 2. The commercially available HA obtained from Aldrich Chemical was compared to the extracted humic acids using spectroscopic approaches. IR and NMR spectrometry were employed for analysis of structural functionalities, and UV-VIS spectrometry was used for a diagnostic purpose of the commercial HA.

4.1.1 Infrared spectrometry (IR)

For the first experiment, a solid state IR spectrometer (Perkin Elmer model 1600, Connecticut) was used. The spectrum of the commercial HA in Figure 4.1 was obtained from a solid sample in KBr disc using Diffuse Reflection Infrared Fourier Transform (DRIFT) technique.

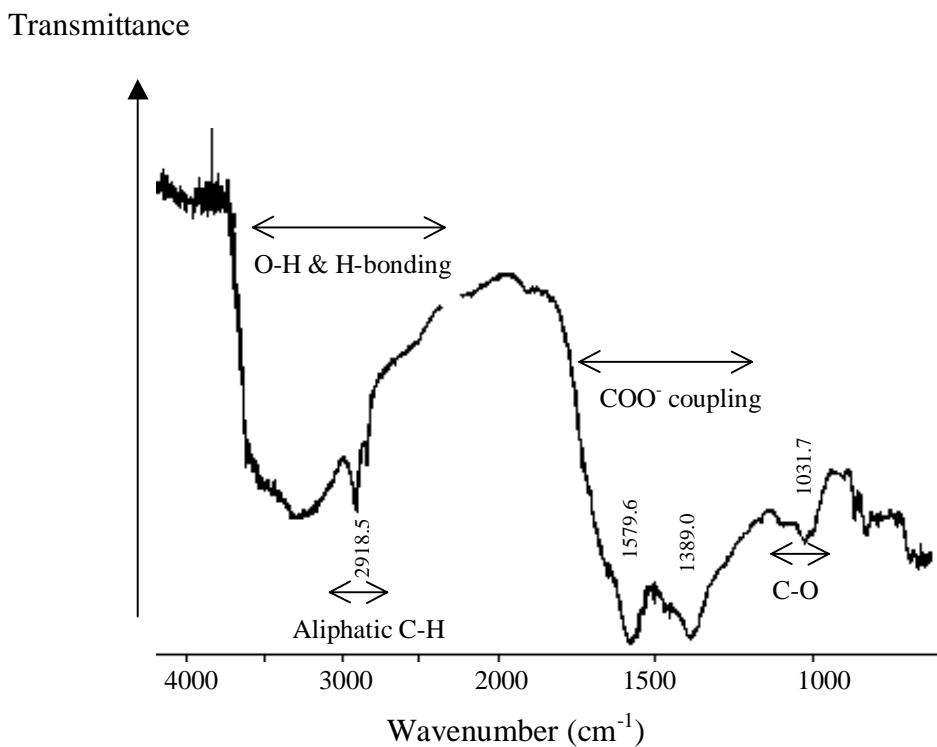


FIGURE 4.1: DRIFT spectrum of the commercial HA using Perkin Elmer model 1600.

As can be seen a broad band characteristic of OH stretching extensively occurred in a range of 2500-3700 cm^{-1} . This behavior could result from the combination of stretching vibrations of non-ionized OH and H-bonded OH sites. A couple of peaks superimposed on the OH stretching region at 2918 cm^{-1} and 2850 cm^{-1} indicated aliphatic C-H groups rather than aromatic C-H groups in this commercial HA. A coupling of asymmetric and symmetric stretching vibrations of carboxylate groups (COO^-) was likely to give rise to a doublet at 1580 cm^{-1} and 1389 cm^{-1} . This should be characteristic of a sodium salt of the commercial HA as originally obtained from the supplier. As part of humic acid polymeric components, C-O stretching was seen at about 1032 cm^{-1} . However, the shift of C-O stretching vibration to a lower energy region of 1000 cm^{-1} may interfere with Si-O vibrations, from the silicates obtained during the extraction soil impurity. Figure 4.1 shows a high background level observed in the fingerprint region possibly due to adsorption by KBr used as a solid medium.

Compared to the commercial HA, the spectra of the extracted HAs in Figure 4.2 present slightly different patterns. Its individually structural characteristic can be seen from these comparable spectra. In addition to OH broadening bands, all three extracted HAs showed N-H stretching vibrations, which were characteristics of amine groups in a range of 3620-3690 cm^{-1} . An interesting point was the spectra of the extracted HAs from Davidson Silty Clay and Davidson Clayey Loam became more dominant in amino substitutes than that of Bertie Sandy Loam. These N-H vibration characteristics could not be seen on the commercial HA spectrum.

As described earlier, the soil background indicated the Davidson Silty Clay and the Davidson Clayey Loam were collected at the same relative time from the same source, Ridge region of Virginia. The IR spectra in Figure 4.2 (b) and 4.2 (c) present similar patterns hence demonstrating the close relationship in geological history of these two soils. Asymmetric stretching vibrations of C=O functional groups can be observed at 1640 cm^{-1} in all extracted HA spectra. A characteristic of C-O stretching of carbohydrate fractions obviously occurred at a low energy region around 1037 cm^{-1} . Once again, it is believed that this vibrational adsorption of C-O has a chance to be obscured by Si-O

vibrational adsorption from an impurity obtained from the extraction processes, which could also be observed as a smaller coupling peak at 915 cm^{-1} on each spectrum. Table 4.1 concludes the typical adsorption regions of HA functionalities by IR methodology.¹⁷

4.1.2 Nuclear magnetic resonance spectrometry (NMR)

To verify functionally structural information obtained from the IR technique and to confirm that HA properties are source dependent, the commercial HA and the extracted HA from the Bertie Sandy Loam were investigated by ^{13}C NMR Cross Polarization Magic Angle Spin (CPMAS) technique. The solid-state NMR technique was preferred to the liquid-state NMR because of the low solubility of HAs in suitable NMR solvents. As shown in Figure 4.3 the commercial HA produces a good spectrum with low noise background. It could be classified into three main regions of 30.416, 128.066, and 175.919-182.863 ppm. From semi-quantitative analysis, it was found that the ratio of peak areas of aliphatic carbon (0-100 ppm), aromatic carbon (100-160 ppm), and carboxyl carbon (160-220 ppm) was approximately 100/51/10. These results indicate that the commercial HA greatly dominated in acyclic carbon at 30.416 ppm, which was two times greater than aromatic carbon at 128.066 ppm, and only 10 % of carbon types were present as carbonyl carbon.

In detail, relatively small peaks could be observed at 53 ppm (methoxy C/C-N), and 73 ppm (carbohydrate/sugar carbons). These types of carbons were also significantly seen in the spectrum of the natural HA from the Bertie Sandy Loam (Figure 4.4). Unlike the commercial HA, the selected extracted HA provided a semi-quantitative ratio of acyclic carbon, aromatic carbon, and carboxyl carbon equaled to 100/85/30. This Bertie soil's HA produced a somewhat noisy spectrum containing acyclic carbon at 30.177 ppm, methoxyl carbon at 53.863 ppm, carbohydrate carbon at 72.423 ppm, aromatic carbon at 128.660 ppm, carboxyl carbon at 171.806 ppm, and aldehyde carbon at around 203-208 ppm. The NMR and IR information suggested that the structural differences might result from HA sources depending upon their formation processes. Typical ^{13}C NMR functional characteristics of humic substances can be seen in Table 4.2.¹⁷

Transmittance

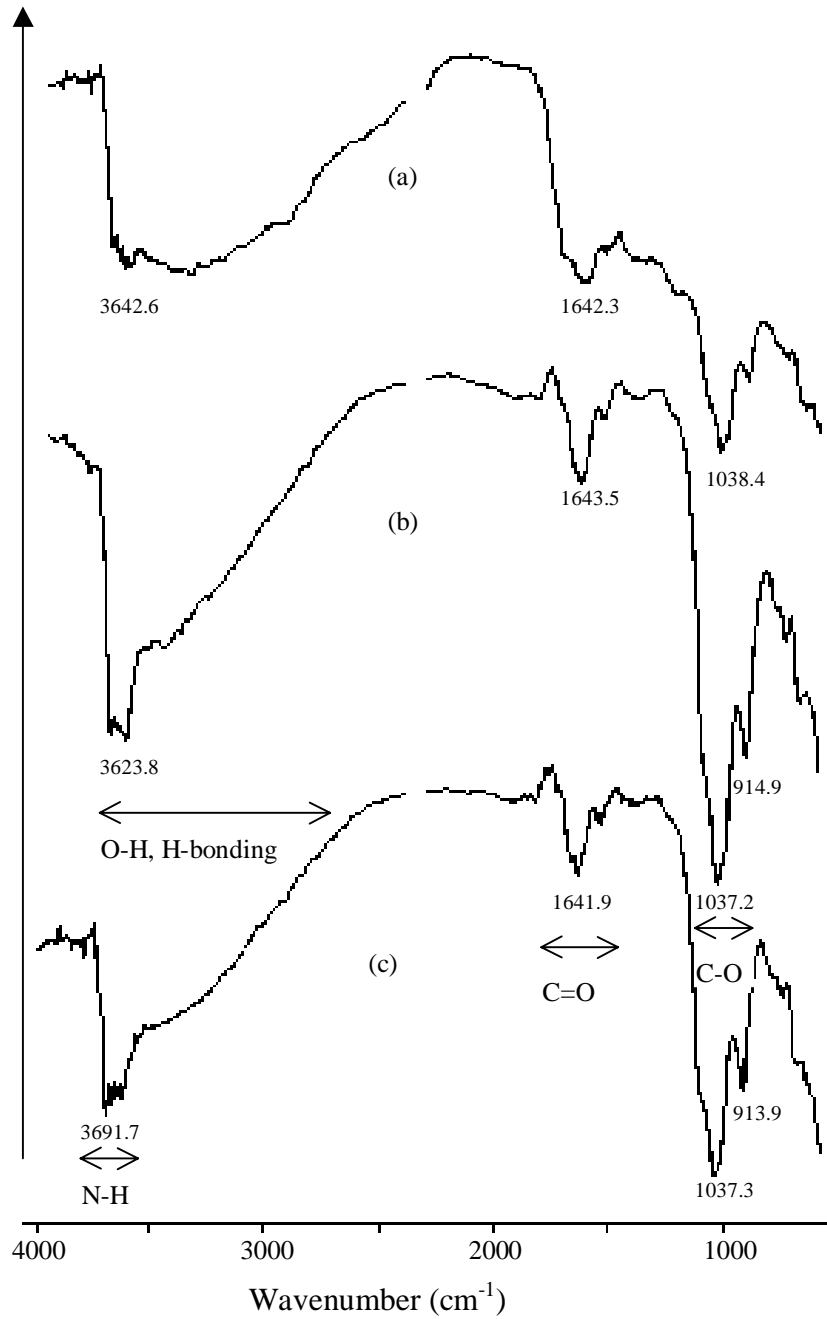


FIGURE 4.2: DRIFT spectra of the extracted HAs from: (a) Bertie Sandy Loam, (b) Davidson Silty Clay, and (c) Davidson Clayey Loam using Perkin Elmer model 1600.

TABLE 4.1: Typical IR absorption characteristics of humic substances extracted from soils.

Functional group	Wavenumber (cm⁻¹)
Si-O of impurities	950-1050
C-O stretching	1150-1250
COO ⁻ symmetric stretching, C=N stretching	1520-1600
C=C stretching	1600-1620
C=O stretching of amide	1630-1660
C=O stretching of COOH and ketones	1700-1725
Aliphatic C-H	2850-2950
Aromatic C-H	3010-3050
H-bonded O-H stretching	3000-3400
N-H stretching	3300-3400
O-H stretching	3600

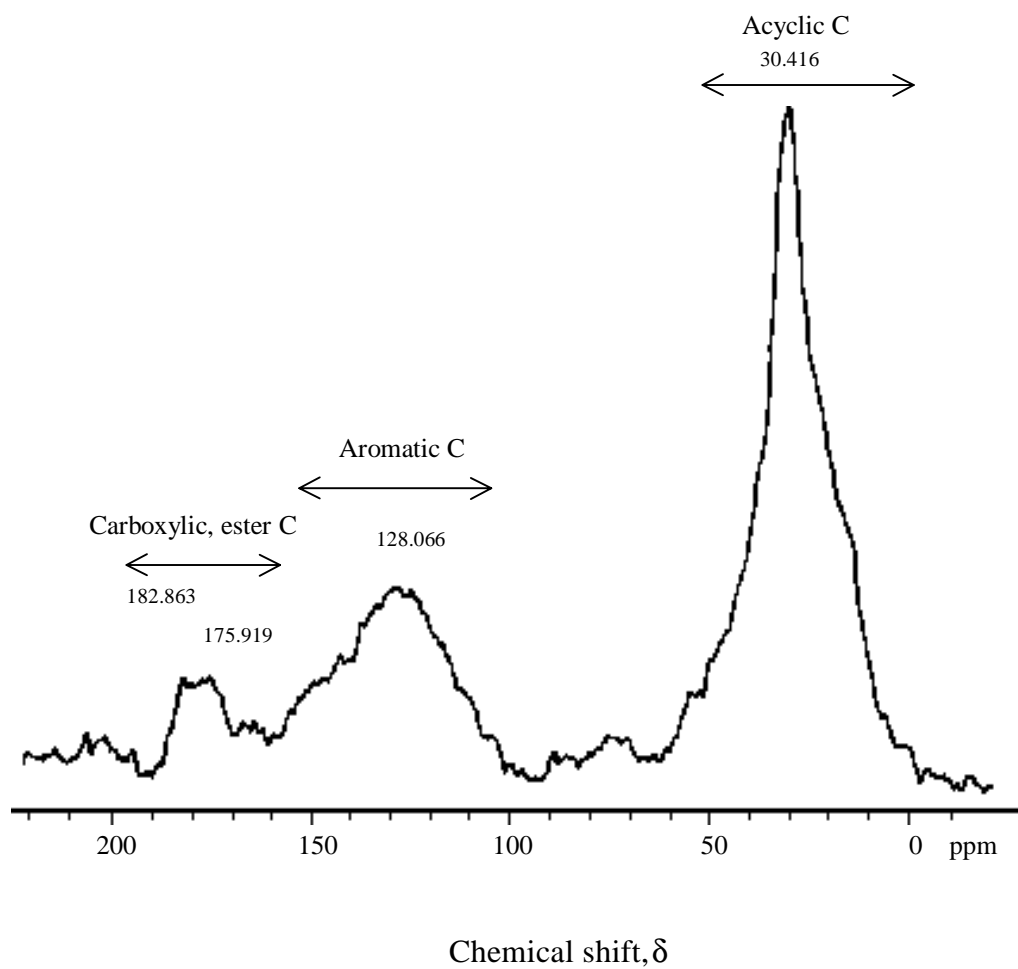


FIGURE 4.3: CPMAS ^{13}C NMR spectrum of the commercial HA using a Bruker model 300/200 solid state NMR instrument.

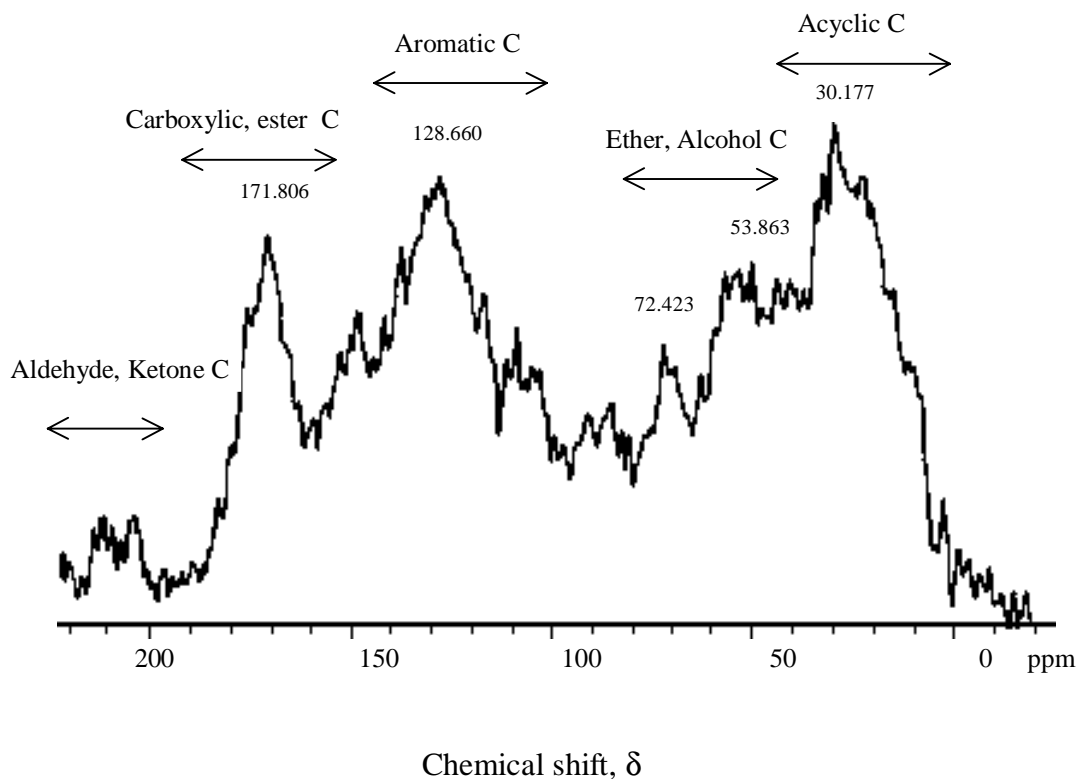


FIGURE 4.4: CPMAS ^{13}C NMR spectrum of the extracted HA from Bertie Sandy Loam using a Bruker model 300/200 solid state NMR instrument.

4.1.3 UV-VIS spectrophotometry

Since the commercial HA would be used for further experiments without a fractionation process, it was helpful to identify whether it had the characteristic of fulvic acids or humic acids. The distribution of molecular weights of humic substances basically controls their adsorption properties in the UV-VIS range. Although UV-VIS absorption spectra of humic materials are not so meaningful because of no obvious maximum peaks, the relative absorption ratio at 465 nm to 665 nm (A_{465}/A_{665} or E_4/E_6)

will be useful for a diagnostic purpose. Fulvic acids having higher polarity and smaller particle sizes normally absorb UV-VIS radiation at lower wavelengths whereas humic acids show the opposite result due to their lower polarity and larger molecular weights. If a humic substance provides that adsorption ratio A_{465}/A_{665} lower than 5, it would represent the overall characteristic of a humic acid. For the commercial HA used here, the measured ratio of A_{465}/A_{665} was 3.68. This confirms a characteristic of humic acids bought from Acro Organic Company.

TABLE 4.2: ^{13}C NMR adsorption regions of different functional carbons.

Functional C	Chemical shift (ppm)
Aliphatic, CH_3CH_2-	0-50
N-alkyl, NH_2-C	40-50
Methoxyl, $\text{CH}_3\text{O}-$	50-70
Carbohydrate/sugar, $\text{C}-\text{O}-$	70-100
Aromatic C, unsaturated $\text{C}=\text{C}$	120-140
Phenolic C	150
Carboxylic, carboxylate C	170-185
Aldehyde C	190-210
Ketonic C	210-230

4.2 Preparation of Cu spiked matrices

As described above, the purpose of this chapter was to obtain suitable matrices selected further for evaluation of the extraction methods. In order that sample morphology would not bias the extraction results, coated HA materials were prepared. The material would represent the sorptive characteristics of organic soils. The prepared organic coated matrices as well as the original uncoated matrices would then be spiked with Cu samples in order to obtain the final contaminated materials.

4.2.1 Preparation of organic coated materials

In Chapter 2, the procedure detail for coating six original solid materials with the HA solution was described. Forty eight-hour equilibration times were chosen to simulate natural HA coating on the matrix surfaces. The comparison of HA adsorption capabilities can be seen in Figure 4.5. Because of no pH adjustment prior to the HA adsorption experiments, the pH changes of equilibrating solutions observed are given in Table 4.3.

Among the selected solid materials, sea sand was most likely to be the least adsorbent. An extremely low level of adsorbed HAs (218 $\mu\text{g/g}$) was found on sea sand. Since the major component of sea sand is quartz, which has a low surface charged density, the minimal adsorption of HA would be expected. Network structures of silica compounds with large particle sizes could be responsible for the adsorption properties of sea sand. Negatively charged surface of alumina also provided low HA adsorption capability (690 $\mu\text{g/g}$). In addition to having large particle sizes, Bertie Sandy Loam mainly contained aluminosilicate compounds that could be classified as similar to sea sand, which provided only HA adsorption capability (365 $\mu\text{g/g}$) as well.

In table 4.3, the pH measurements helped to explain some adsorption behaviors that occurred at the material surfaces. Compared to initial pH at 6, the preparation of HA-alumina resulted in very high pH value of the final solution. This is believed that

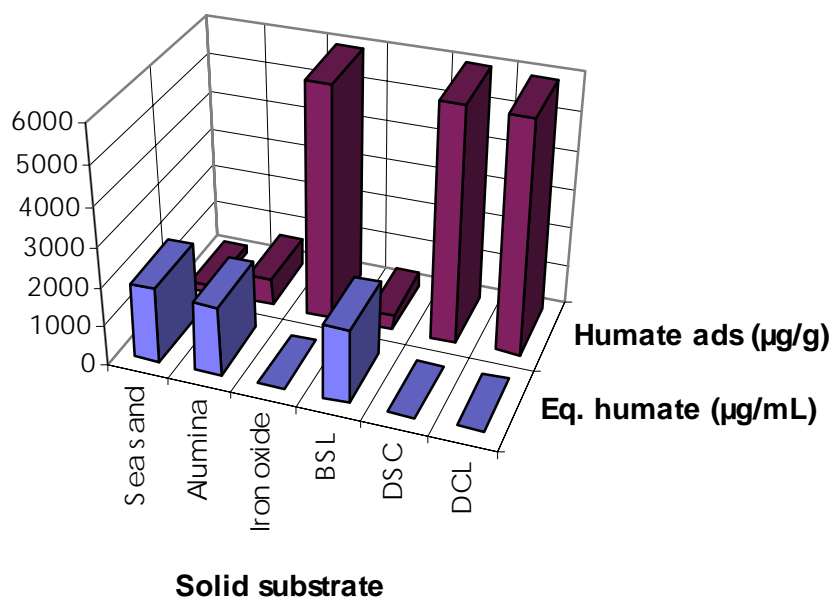
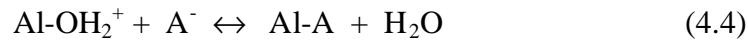
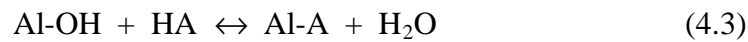
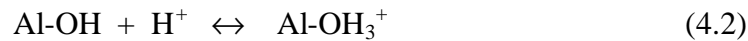


FIGURE 4.5: HA adsorption capabilities of six solid materials (Humate ads = amounts of adsorbed HA on the solid material, and Eq. humate = amounts of dissolved HA left in the solution).

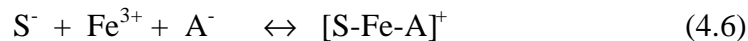
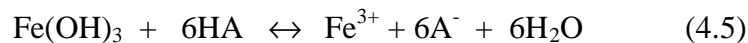
TABLE 4.3: Final pH of the HA equilibrating solutions.

Original material	Final solution pH
Sea sand	6.68
Alumina	9.18
Iron oxide	4.92
Bertie Sandy Loam	7.14
Davidson Silty Clay	7.33
Davidson Clayey Loam	6.20

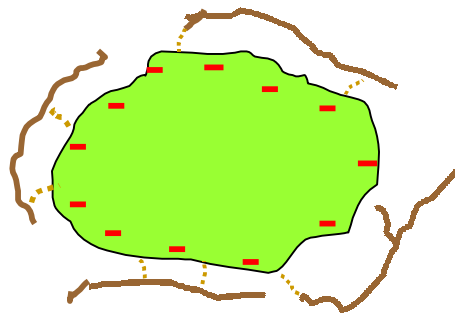
fast protonation may happen once alumina contacts with HA solution as expressed in equations 4.1 and 4.2. The resulting neutral sites (Al-OH) and positively charged sites (Al-OH₂⁺) then rapidly interact with the negatively charged sites of HA molecules as shown in equations 4.3 and 4.4. At higher pH, HA molecules typically uncoil because of the repulsion of negatively charged carboxylate groups within the same polymeric chain. A small number of alumina active sites and the structural behaviors of HA molecules at basic condition could possibly cause low HA adsorption capability as shown schematically in Figure 4.6. Lateral (multiple-site) interactions on alumina are expected to happen in this experiment.



Unlike aluminosilicate compounds, iron oxide and two oxide rich soils provide high adsorption capabilities for HAs. These characteristics could be explained as follows: (1) the HA solution could possibly dissolve iron oxide at the outer layers, and (2) the dissolved iron ions then help to form bridging interactions between negatively charged surfaces (S⁻) and HA molecules as expressed in equations 4.5 and 4.6.



Another interesting point from the pH measurement observed for iron oxide was its acidic property which implied that the iron oxide originally possessed a number of active sites, Fe-OH and Fe-OH₂⁺. These sites preferably reacted with the soluble HAs. Due to the natural porosity of soil particles, some parts of HA structures may probably penetrate through the inner layers and into the pore sites with a resultant change of surface properties. Figure 4.7 shows a possible configuration of interactions between the dissolved HA molecules and a particle of an oxide coated soil.



- Alumina particle
- Surface negative charges
- Extended HA molecules
- Binding interactions

FIGURE 4.6: Adsorption of HA molecules on an alumina particle.



- Soil particle with high porosity
- Oxide component
- HA molecules

FIGURE 4.7: Adsorption of HA molecules on an oxide coated soil.

Among various oxide/hydroxide forms, non-crystalline compounds dissolve easier in HA solutions and hence increasing the number of multiple charged metal ions. This enhances the adsorption capabilities of HAs on the solid surfaces. From soil background, Davidson Silty Clay consisted of smaller average particle sizes than Davidson Clayey Loam. Small particle sizes provided larger surface areas of Davidson Silty Clay. This could generate a high HA adsorption capability. From Figure 4.5, three iron oxide containing materials including Davidson Silty Clay, iron oxide, and Davidson Clayey Loam provided very extremely low HA concentrations (4, 6, and 20 µg/mL, respectively) left after equilibration times had elapsed due to their high adsorption capabilities (5985, 5982, and 5934 µg/g, respectively).

4.2.2 Preparation of Cu contaminated matrices

In addition to the original selected solids, the organic coated matrices (HA-substrates) from previous experiments were subjected to Cu spiking experiments with 24-hour equilibration. The resulting products as Cu-matrices and Cu-HA-matrices were collected for further experiments. Figure 4.8 shows a comparison of Cu adsorption capabilities of two solid sets. It can be proven that sea sand again reacted the least with Cu (II) ions, and the presence of adsorbed HA helped slightly to increase its Cu adsorption capability. These experiments indicated that sea sand alone was not suitable to be a representative of a mineral matrix for metal extraction studies.

Meanwhile, alumina whose surface was much different from those of natural soils provided extremely a high capability of Cu adsorption. This chromatographic alumina preferred positively charged metal ions. Unlike other matrices, after being coated with humic acid, the resulting matrix as HA-Al₂O₃ showed a reduced Cu adsorption capability. This is possibly due to the hindrance of the adsorbed HA molecules and due to the decrease in negatively charged density at the surface as well. The opposite results were observed in iron oxide containing materials. Due to its acidic nature surface, the iron oxide material then provided a lower Cu adsorption capability than alumina.

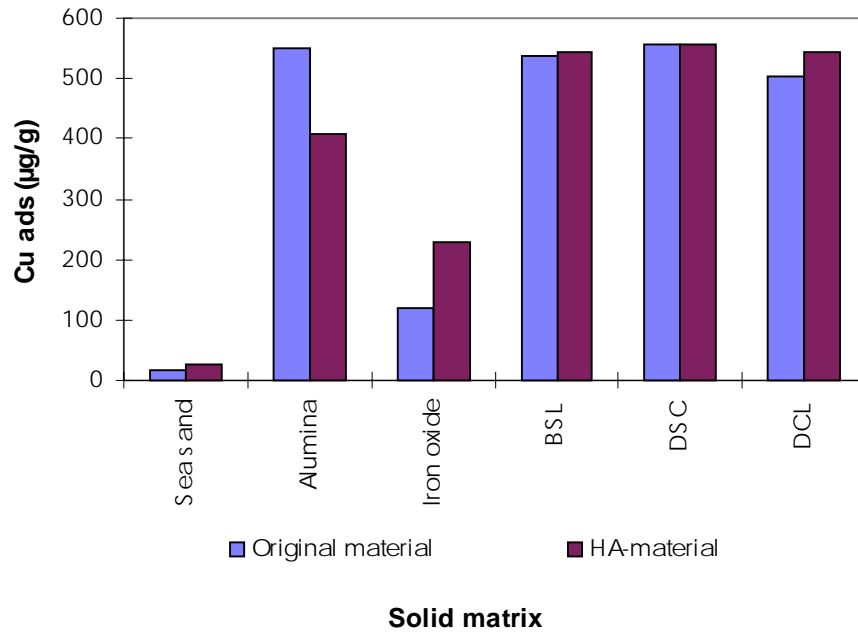


FIGURE 4.8: Cu adsorption capabilities of original and HA-coated materials in the preparation of Cu spiked samples (n=1).

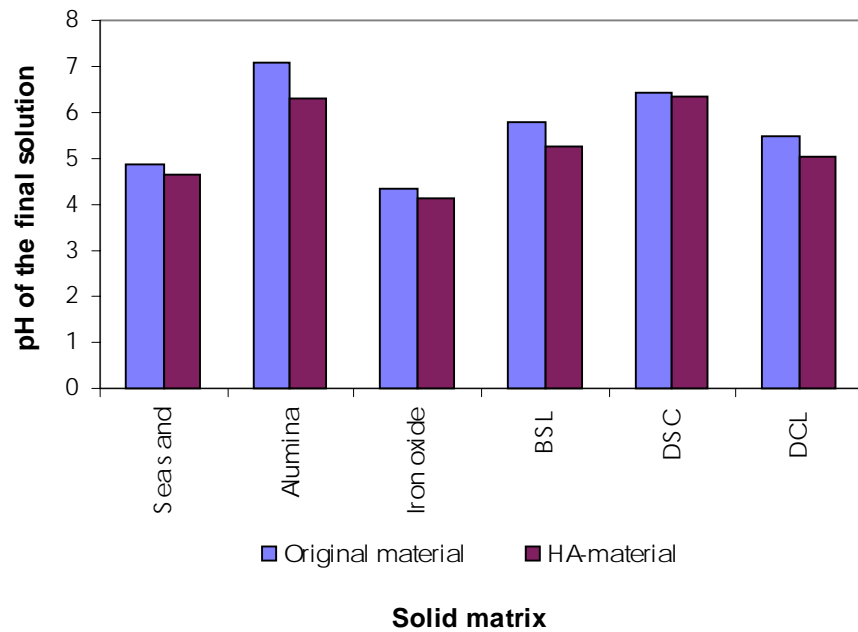


FIGURE 4.9: The effect of coated humic acids on pH of the Cu solutions.

TABLE 4.4: HA and Cu adsorption capabilities of each solid material.

Sample set	Material	Ads. HA (µg/g)	Final HA conc. (µg/mL)	Ads. Cu (µg/g)	Final Cu conc. (µg/mL)
Original	Sea sand	21.8	1927.1	15.6	54.2
	Alumina	690.1	1769.8	551.0	0.6
	Iron oxide	5981.8	5.9	118.6	43.4
	BSL	365.4	1878.0	538.3	1.4
	DSC	5985.2	3.9	556.4	0.1
	DCL	5934.2	19.6	503.5	5.4
Coated with HA	Sea sand	-	-	25.4	53.2
	Alumina	-	-	406.2	14.7
	Iron oxide	-	-	230.0	32.2
	BSL	-	-	544.3	0.8
	DSC	-	-	556.9	0.1
	DCL	-	-	543.7	1.4

It was evident that the presence of HAs positively affected Cu adsorption on HA-iron oxide. In this case, the adsorbed HAs possibly produced more negatively charged surface sites that preferably interacted with Cu (II) ions. This phenomenon can be used to explain for all real oxide soils. Table 4.4 shows that DSC was the best absorbent for both HA adsorption and Cu adsorption. Table 4.4 additionally indicates the ability of each material's surface and the possible limitation of the initial Cu (II) concentration used for real soils. One surface property changed after HA adsorption was acidity. Compared to original (uncoated) HA-materials, all HA-materials provided lower pH values of the final Cu solutions as shown in Figure 4.9.

After equilibrating with Cu (II) solutions, solids were separated from the solutions and allowed to dry without being washed in order to avoid any loss of adsorbed ions. It was expected that Cu concentrations left in solutions would be extremely low. Cu adsorption data were obtained by measuring the difference between the initial Cu and the final Cu concentrations in the equilibrating solutions at 324.75 nm using ICP-AES.

4.3 Summary

Spectroscopic studies previously indicated that soil humic contained several functional groups. In addition to ^{13}C NMR technique, the results from IR spectrometry helped explain details about structural functionalities. The commercially available HA has been shown to consist of a larger fraction of aliphatic carbons rather than aromatic carbons. This was concluded from the semi-quantitative analysis of ^{13}C NMR method and the domination of aliphatic stretching peaks on the IR spectrum. Another interesting point from the IR studies was the strong N-H stretching signals superimposed on O-H stretching bands shown in Figure 4.2 (b) and (c) which implied that the same natural sources of HAs of two Davidson soils obviously got involved in polymerization processes predominating in amino containing compounds.

The UV-VIS technique was helpful for verification of whether FA or HA characteristics and for quantitative analysis of HAs in the matrix preparation experiments.

The difference between the initial HA and the final HA concentrations, measured at 444 nm and pH 5,⁶⁷ hypothetically yielded the amounts of HA adsorbed on each matrix.

HA adsorption information showed that iron oxide containing compounds showed higher HA adsorption capabilities than aluminosilicate compounds. Surface pH and soil components affected adsorption behaviors. The influence of adsorbed HA on Cu adsorption on each substrate was different. It was evident that alumina having basic surface showed adsorption results opposite to that of iron oxide acidic surface. Sea sand should not be used further as a mineral representative for the studies of metal adsorption behaviors in soil systems because it showed much different characteristics than real soils. It was also found that iron oxide containing soils were extremely sensitive to both HA and Cu adsorption. The purpose of using an adsorption procedure as a spiking method was to allow only natural interactions between matrix surfaces and the analyte to occur. Unbound analytes that could cause an overestimation of the extraction efficiency of the developed method would not exist. In the next chapter, a variety of matrices would be evaluated of the extraction capabilities of experimentally adsorbed metals.

CHAPTER 5

CAME & VARIOUS SOIL MATRICES

According to previous studies, it is apparent that adsorption behavior depends upon the properties of both the soil solid and solution phases. In this chapter, the efficiencies of extraction methods dealing with various soil matrices are addressed. For method development of Chelate Assisted Microwave Extraction (CAME), it is important to select suitable sample matrices in order to avoid an incorrect evaluation of metal sorption. Accordingly, sea sand was no longer used to represent a soil matrix because of its very low affinity for the humic acid and Cu (II) ions. These experiments were focused on soil matrices including control soils and metal-spiked soils. Simulated organic materials (described in the previous chapter) were employed to explore the influence of organic matter as compared to oxide/hydroxide components. Highly adsorbed metals were chosen as contaminants in soils.

Also in this chapter, microwave conditions for Cu-spiked samples were investigated. Optimum concentrations of the acceptable extractants in soil science: such as EDTA and DTPA, were also studied. To verify the efficiency of the CAME technique, mass balances were applied to double extractions. The effects of sample size and high Fe content in Davidson Silty Clay (DSC) were investigated as well. Cu extraction capabilities from the organic coated material (Cu-HA-DSC) and from the oxide coated soil (Control DSC) were compared. Both Cu (II) and Cd (II) were examined for the effects of co-extracted species.

Lastly, the choice of extracting solutions for CAME was explored using a variety of complexing agents including citric acid, oxalic acid, nitrilotriacetic acid (NTA), ethylenediaminetetraacetic acid (EDTA), 1,2-cyclohexylenedinitrilotetraacetic acid (CyDTA), ethylenebis (oxyethylenenitrilo) tetraacetic acid (EGTA), diethylenetriaminepentaacetic acid (DTPA), triethanolamine (TEA), diethylenetriamine

(DETA), and triethylenetetraamine (TETA). Using the control samples of BSL and DSC, trace metals such as Cd, Cr, Cu, Ni, Pb, and Zn, were determined. Selectivity and extractability of each selected chelating agent were discussed.

5.1 Microwave conditions for oxide based materials

This experiment was performed in order to modify the conditions used for the sandy soils in Chapter 3. Since it is known that sandy soils generally provide low adsorption affinity for metal ions, it was necessary to find out the optimum condition applicable to all types of soils. Oxides and humic substances in soils can have a significant impact on metal-soil reactions due to potentially strong interactions. For the first experiment, alumina and iron oxide based materials including Cu-alumina, Cu-HA-alumina, Cu-iron oxide, and Cu-HA-iron oxide were extracted by EDTA-CAME using a 2-step microwave profile. Microwave intensities were set at higher levels than those used in Chapter 3 in order to reduce extraction times for strong adsorption sites, which were expected to occur in oxide soils. At the initial stage, 120 W of microwave power were applied for 5 min and then reduced to 60 W for 15 or 20 or 25 or 30 or 35 min. Each extraction employed a 0.025 M EDTA solution at pH 7 in $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$ the same as used in Chapter 3. A ratio of 0.5 g-sample/10.0 mL-extraction solution was used.

Figure 5.1 shows Cu recoveries from the four matrices extracted by CAME. It was evident that the increase in extraction times from 15 to 35 min resulted in no significant improvement of extraction recoveries. Probably, the microwave condition of 120 W for 5 min followed by 60 W for 10 min may be efficient in removing all adsorbed Cu contents. Cu extraction capabilities were found in the range of 96 to 103% for all four Cu-spiked materials. It can be seen that alumina showed slightly lower extraction efficiencies than HA-alumina. HA-iron oxide yielded higher extraction efficiencies than the original iron oxide. Among them, it is thought that Cu-alumina may have the strongest interaction between Cu (II) ions and solid matrix. The Cu recovery range of 96 to 98% Cu was achieved for Cu-alumina.

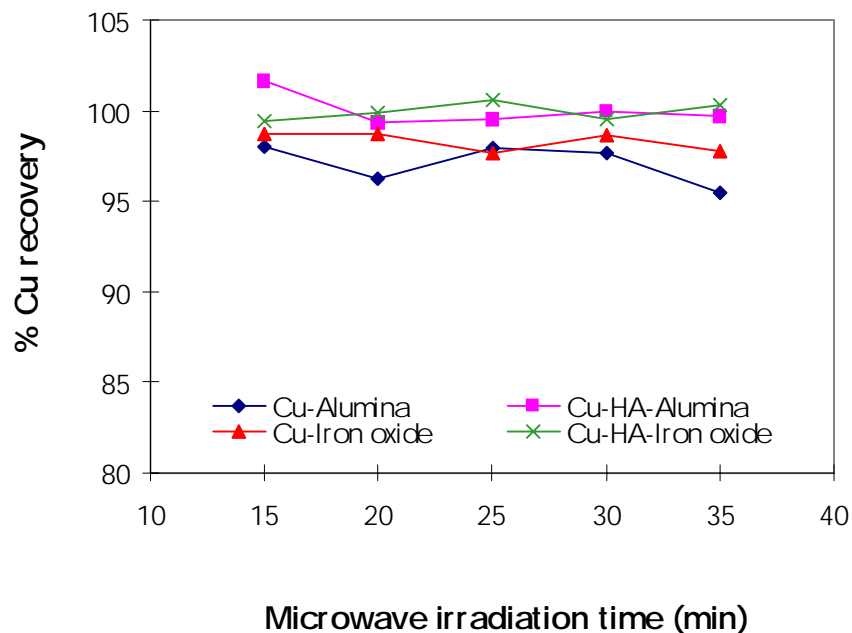


FIGURE 5.1: Extraction recoveries of adsorbed Cu from the selected oxide matrices based on amounts of Cu adsorbed (n=1).

In reality, pure oxide matrices act quite differently from real soils. To obtain suitable extraction conditions for real soil matrices, two spiked samples of Davidson Clayey Loam including Cu-DCL and Cu-HA-DCL, were chosen for testing CAME conditions studies. Eight extraction times of 3, 10, 15, 20, 25, 30, and 35 min were also explored. Triplicate extractions were conducted for each time. The results of using DCL matrices are presented in Figure 5.2. Extraction recoveries were calculated based on the data of the Cu (II) spikes prepared in Chapter 4.

There was only a small difference between the two profiles observed at extraction times from 0 to 10 min. This probably resulted from the minimal differences in binding strengths due to sample surfaces. Cu extractable levels obtained from Cu-HA-DSL were

slightly higher than those from Cu-DSL after a short period of irradiation times. The steady-state levels, slightly higher than 100%, tended to be achieved after 10 min-extraction for both matrices.

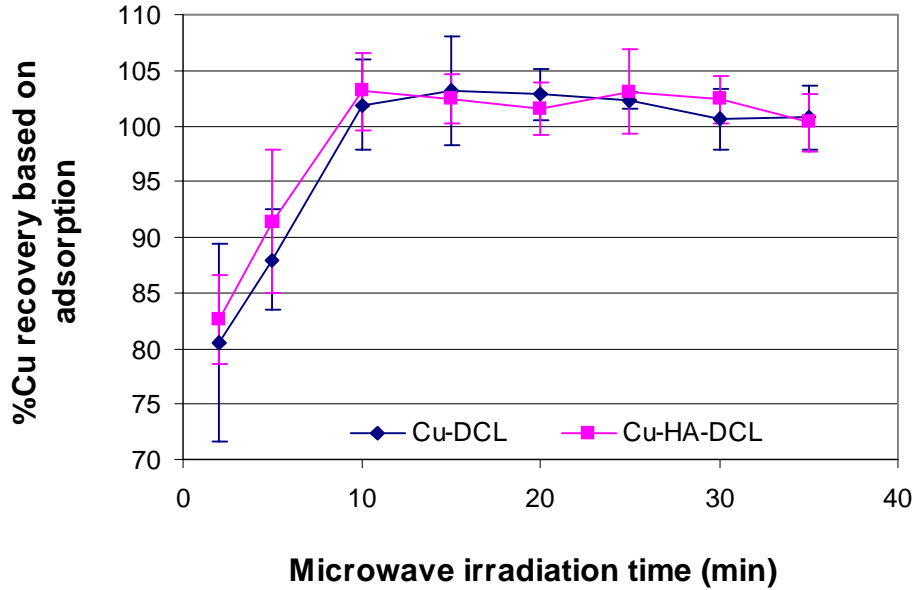


FIGURE 5.2: Extraction profiles of Cu-DCL and Cu-HA-DCL using 120 W for 5 min followed by 60 W for longer times (n=3).

From this study, the two-step extraction of 15 minutes was selected for the remaining experiments. Compared to the condition used for the sandy soils in Chapter 3, the higher microwave intensities used here may be capable of removing all adsorbed metals in a shorter extraction time. It was observed that applying microwave power exceeding 120 W generated strong sputtering inside the extraction vessel. The sample loss due to aerosol production and non-uniform heating may occur.

5.2 The effect of solution composition

EDTA solutions used in the previous experiments were modified to pH 7 with Na₂CO₃/NaHCO₃ at an ionic strength of about 0.04. A carbonate buffer was used to explore the possibility of buffering capacity remaining after metal extraction. In the following experiment, we would like to explore extraction efficiencies using EDTA solutions containing different pH adjusting substances (buffers): such as NaOH, NH₄OH, and Na₂CO₃/NaHCO₃. The control solution was a 0.025 M EDTA solution without pH adjustment. Extraction investigations were then applied to Cu-alumina and Cu-iron oxide. The selected conditions were the two step-extraction with 15 min- exposure time and an extraction ratio of 0.5 g-sample/10.0 mL-solution. Extraction recoveries as well as pH changes of the solutions were observed. Results are shown in Figures 5.3 and 5.4, respectively.

These results indicate that the pH adjusting substances provided no significant influence on Cu recoveries, but affected the final pH of the extracts. Unlike the others, the EDTA solution containing Na₂CO₃/NaHCO₃ species gave rise to high pH values of the final extracts (pH of 10.4). This could be the result of anion exchange mechanisms that predominated at the surfaces. The bicarbonate species (HCO₃⁻) probably compete with other anions in the solution for the surface hydroxyl groups. Consequently, the surface hydroxyl groups were replaced with the bicarbonate species as expressed in equation 5.1. This phenomenon was observed for all solid matrices used in this experiment.



Generally, higher pH not only affects the dissociation of EDTA but also causes the competition between hydroxyl groups and EDTA species for metal complexation. The control EDTA solution having a pH of 4 showed extraction capability similar to EDTA solution of pH of 7. This indicated that the initial pH range from 4 to 7 had no significant impact on extraction capabilities of the adsorbed Cu fractions by the CAME.

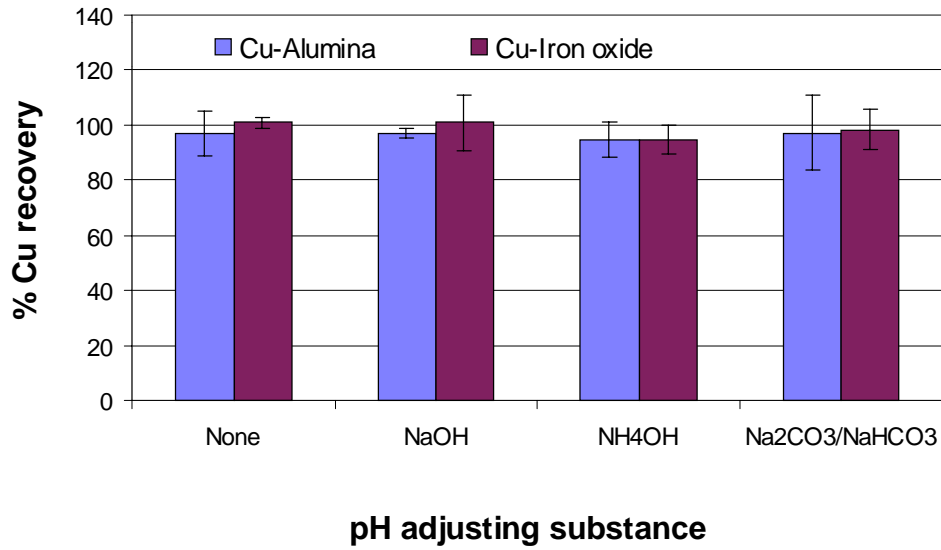


FIGURE 5.3: The effect of pH adjusting substances on % extraction recoveries using microwave exposure of 120 W for 5 min followed by 60 W for 10 min (n=2).

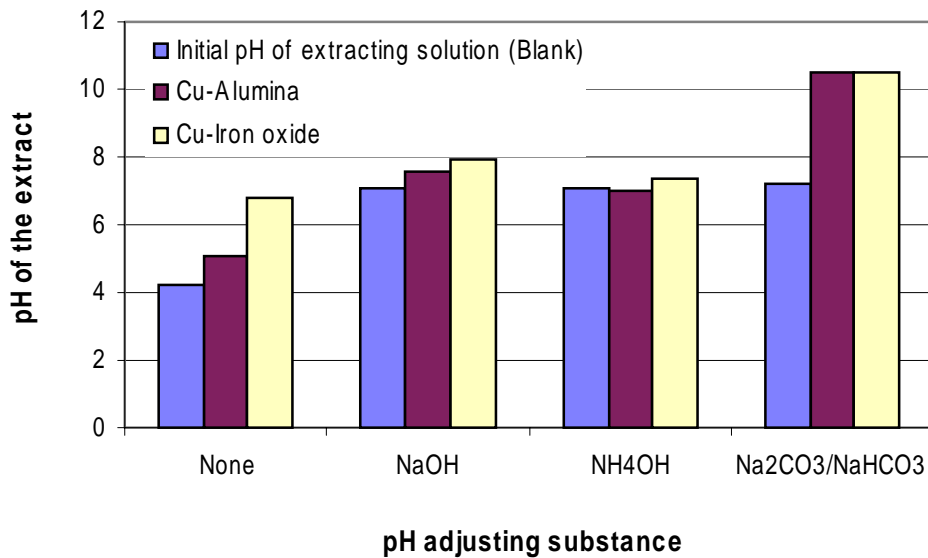


FIGURE 5.4: The pH changes after microwave extraction.

However, it is believed that low pH solutions have more chances to alter the surface properties beyond that of the extraction of sorbed species; it may produce more interferences for trace metal analysis by the liberation of non-contaminant (geological) metals. From microwave theory,¹²⁻¹³ it is thought that the addition of pH adjusting substances may give rise to higher heat transfer rates due to the contribution of ionic conduction, and hence a quicker boiling of the extraction mixtures. Under these conditions, the ionic conductive heating mechanism may dominate the dipolar heating mechanism. However, these combined mechanisms are also likely to promote metal leaching from the surface bound fractions. Evaporation of volatile species such as NH₃ and CO₂ from the extracting solutions could partially occur during the microwave irradiation due to superheating spots inside the vessel. This study suggested that the buffer capacity of the carbonate solution no longer existed in the soil extract probably because of a great extent of anion exchange reactions, which released a large amounts of OH⁻ group from soil surfaces. To avoid the possible competition between the excess released OH⁻ groups from anion exchange reactions and EDTA species, the next experiments would employ NaOH to adjust the pH of the EDTA extracting solution to 7.

5.3 Concentrations of EDTA/DTPA

Soil studies normally use 0.005 M DTPA or 0.05 M EDTA at pH 7 as an extracting solution. In this section, the capability of CAME to liberate the adsorbed Cu fractions was explored using two spiked samples: Cu-BSL and Cu-DSC. Due to very high Cu contents in both spiked matrices, a range of EDTA concentrations from 0.005 to 0.100 M was investigated. The effect of EDTA concentrations on extraction recoveries is given in Figure 5.5. It can be seen that EDTA concentrations affected the extraction efficiencies for highly Cu contaminated samples. It was most likely that two selected matrices showed different maximum extraction levels. For Cu-BSL, 0.025 M EDTA might be sufficient to stabilize all extracted metals, but the extraction of Cu-DSC obviously required a slightly higher EDTA concentration. Extractability obtained from Cu-BSL was approximately 97% while that from Cu-DSC was about 104%. The difference between these two extraction recoveries might result from the difference in

naturally adsorbed Cu contents in the control matrices used to prepare Cu spiking samples.⁶¹⁻⁶³ Since the natural soils have been previously used for the Cu spiking methods, it is important to consider soil background. This study implied that the optimum concentration of EDTA depended upon soil nature and natural occurring metal contents in soils. As described in Chapter 2 and the results obtained from Chapter 3, it indicated that the control BSL contained low Cu concentration less than 10 µg/g. Therefore, this Cu level should not have a significant effect on the extraction recoveries obtained from the calculations based on the spiking/adsorption data. If it could, the maximum recovery would not be lower than 100% when no sample loss was assumed. The steady-state recoveries of Cu-BSL were found in a range of 96 to 98%. This could possibly result from the distribution of particle sizes of BSL that contained about 60% of sandy fraction. During the spiking method, smaller particle sizes of the aluminosilicate compounds of BSL were expected to govern adsorption capability of Cu-BSL, due to higher surface charged density, rather than the larger particle sizes as quartz and mica. The filtering step used to separate the Cu-spiked BSL from the filtrate could cause some sample loss of small particles; subsequently, the average adsorbed Cu content in µg-Cu/g-soil left after filtration would be lower than the calculated value from the spiking experiment. Compared to BSL, DSC consisted of 50% clay fraction but only 10% sandy fraction and most particles were coated with oxides. The loss of small particle sizes might have much less effect on the change of adsorbed Cu density in the final Cu-DSC matrix due to more uniform of the particle size distribution.

The following experiment was performed in order to verify the abilities of two traditional chelating agents, EDTA and DTPA, which provide high formation constants with Cu (II): $10^{18.8}$ and $10^{19.5}$ for Cu-EDTA and Cu-DTPA, respectively. Again, two matrices of Cu-BSL and Cu-DSC, and only two concentrations of 0.005 and 0.100 M were selected for this study. The comparable results are shown in Figure 5.6. A statistical test using two-way ANOVA analysis proved that there was no significant difference for extraction recoveries due to the difference in the selected chelating agents, but there was significant difference due to sample types. Up to this point, 0.100 M

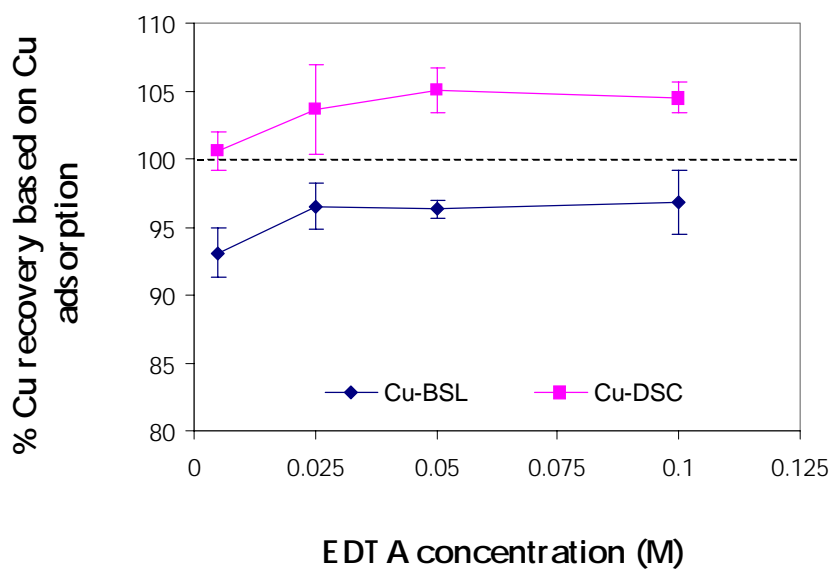


FIGURE 5.5: Extraction profiles as a function of EDTA concentration and soil nature using microwave exposure of 120 W for 5 min followed by 60 W for 10 min (n=3).

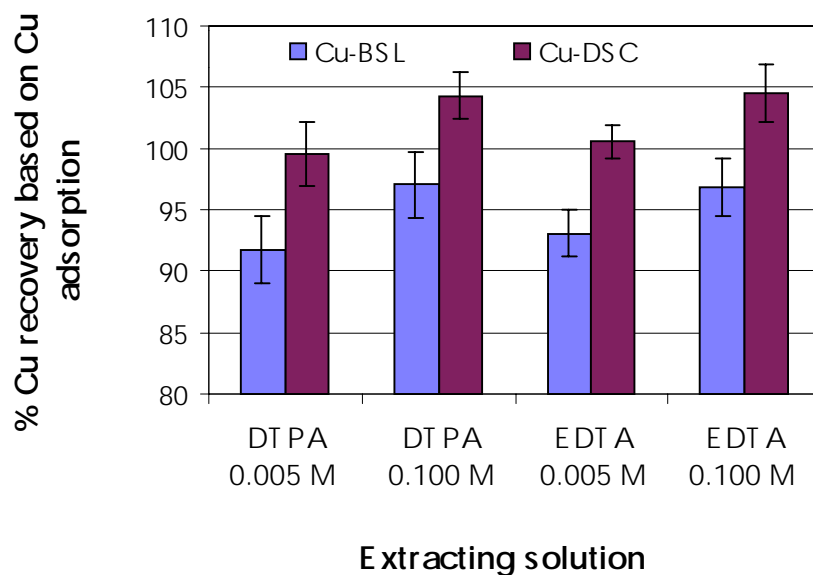


FIGURE 5.6: Comparison of EDTA and DTPA at different concentrations using microwave exposure of 120 W for 5 min followed by 60 W for 10 min (n=3).

EDTA solution would be used further for the next experiments in order to make sure that there were enough EDTA species available for other extractable metals.

5.4 Mass balances of Cu contents in the control DSC and Cu-DSC

In order to explore what caused the average Cu recovery obtained from Cu-DSC to be larger than 100%, double extractions were performed for mass balances of the control DSC and Cu-DSC. Initially, the samples were extracted by CAME using 0.5 g-sample/10.0 mL of 0.100 M EDTA at pH of 7 with the optimized two step-microwave condition obtained from the previous section. Once filtration/centrifugation step was complete, the residues from CAME were dried and then subjected to the hot acid extraction, as described in Chapter 2. Two sets of standard solutions were prepared to compensate the solvent effect for each extraction method. Single extraction using the hot acid extraction was also applied to the control Davison Silty Clay. The combined Cu concentration from double extractions was then compared with the total Cu concentration obtained from the single acid extraction. The results of mass balances are illustrated in Figure 5.7. It was considered that approximately 21 ppm ($\mu\text{g/g-soil}$) of naturally adsorbed Cu in the core of Cu-DSC were leached out, hence caused the observed recovery achieved 4% larger than the adsorbed amount, 556 ppm, obtained from the Cu spiking method. For Cu-DSC, the hot acid digestion after CAME provided slightly higher Cu level to the amount expectedly left in the residue compared to the control DSC.

These comparisons implied that the control DSC contained approximately 40-45 ppm of total Cu content which included 20 ppm of adsorbed Cu available for CAME and 20 ppm of the residual Cu. Accordingly, about 20 ppm of naturally adsorbed Cu fractions in the control DSC might cause the overestimated evaluation of the extraction efficiency of the CAME technique when Cu-DSC was studied.

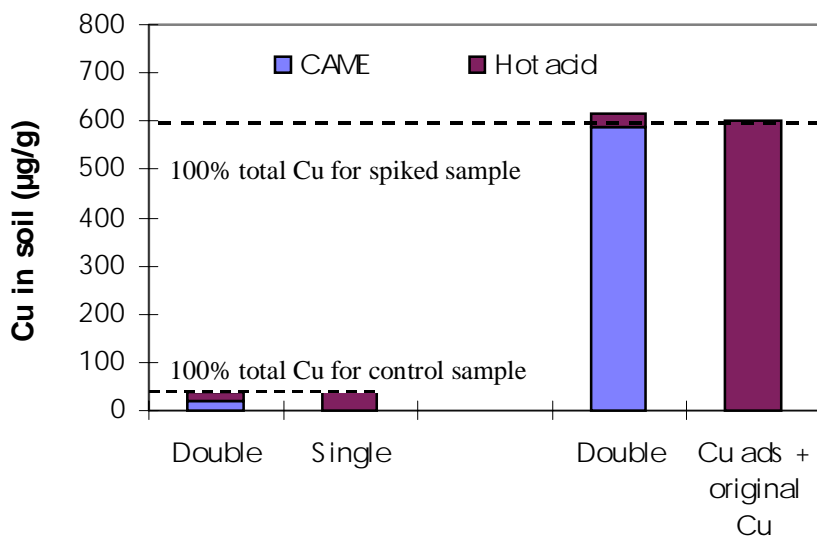


FIGURE 5.7: Mass balances by a combination of double extractions compared to total single acid extraction.

5.5 The optimum sample size and the effect of Fe content in DSC

According to an extremely high content of Fe oxides/hydroxides in DSC, we further investigated if there had some effect of very high Fe content on Cu extractability using CAME. It was considered those large complexation constants of Fe (II)-EDTA ($10^{14.3}$) and Fe (III)-EDTA ($10^{25.1}$) might affect the determination of adsorbed Cu fractions. From soil background,⁶² free Fe content was about 5.5% in DSC. This Fe level was approximately 100 times larger than the Cu spiked content. In order to explore how much Fe and Cu concentrations could be extracted from soils, two sample sizes, 0.5 and 1.0 g, were subjected to CAME using 0.100 M EDTA at pH 7. Again, double extractions compared to the single acid extraction for the determinations of Cu and Fe in Cu-DSC were performed to verify mass conservation and the capability of CAME as well.

TABLE 5.1: Mass balances of Cu and Fe contents in the control-DSC and Cu-DSC (95% confidence limits, n=3).

Sample	Extracted Cu ($\mu\text{g/g}$)				Extracted Fe (mg/g)			
	Double			Single	Double			Single
	I CAME	II ACID	TOTAL	ACID	I CAME	II ACID	TOTAL	ACID
Control				43.4\pm3.5^b				64.37\pm4.57^d
0.5 g	20.0 \pm 3.5	20.0 \pm 9.7	40.0 \pm 10.2 ^a		6.32 \pm 0.74	58.22 \pm 5.64	64.54 \pm 5.68 ^c	
1.0 g	19.0 \pm 1.5	19.6 \pm 8.7	38.6 \pm 8.9		5.39 \pm 0.25	59.67 \pm 7.37	65.06 \pm 7.37	
Cu-DSC								
0.5 g	586.8 \pm 29.2	30.3 \pm 4.0	617.1 \pm 13.9		6.13 \pm 1.04	62.20 \pm 2.93	68.33 \pm 3.10	
1.0 g	589.1 \pm 2.0	35.9 \pm 1.0	625.0 \pm 2.2		5.50 \pm 0.32	59.95 \pm 8.42	65.45 \pm 8.42	

- Spiked Cu + Cu in the control-DSC = 556.4 + 43.4 = 599.8 $\mu\text{g/g}$
- The total extracted Cu of step I (CAME) combined with step II was compared to one step extraction by hot acid method.
- Comparison of a (extracted Cu amounts obtained from 2 steps) and b (extracted Cu obtained from one step of hot acid) shows no significant different of means ($t_{df} = 0.98 < t_{crit} = 2.78$).
- Comparison of c (extracted Fe amounts obtained from 2 steps) and d (extracted Fe obtained from one step-hot acid) shows no significant different of means ($t_{cal} = 0.05 < t_{crit} = 2.78$).

The extraction results are given in Table 5.1. It can be seen that approximately 20 $\mu\text{Cu/g}$ -soil were unable to be extracted by CAME. This Cu content was close to 21 $\mu\text{g/g}$ -soil in the residue of the control DSC as reported in literature.⁶² For both samples, Fe extractable levels were approximately 6.5% that were quite close to the early reported content of free iron oxide. Probably, about 94% of Fe content were parts of geological-lattice structures. Even some fractions of EDTA preferably reacting with Fe, CAME was still efficient in leaching all adsorbed Cu fractions. However, the remarkable point was quite high Cu concentrations were obtained from the second extractions of Cu-DSC from both sample sizes. These high Cu levels left in the residues after CAME might result from the entrained solutions of very high Cu concentrations in the CAME extracts. The

residues after CAME were not rinsed before being dried, so the ability of DSC to retain some liquid volume could be a major factor. This effect did not dominate in the determination of Fe concentration in Cu-DSC since the Fe concentration available for CAME was much lower than the Fe concentration present in the geological form. Eventually, 100% extraction capabilities of adsorbed Cu fractions from Cu-DSC were still achieved, regardless of sample size.

From Table 5.1, the statistical comparisons indicated that for 0.5 g of the control DSC there was no significant difference between means of the combined Cu concentration (from 2 extraction steps) and total Cu concentration. Also there was no significant difference between means of the combined Fe concentration (c) and total Fe concentration (d).

For the rest of this research, the experimental conditions of CAME would be used as follows: microwave exposure of 120 W for 5 min followed by 60 W for 10 min, 1.0 g of a control sample or 0.5 g of a spiked sample, and 10.0 mL of 0.100 M EDTA at pH 7.

5.6 Influence of oxide and organic coatings

For following investigation, Cu-DSC and Cu-HA-DSC were used to evaluate the effect of two major components in soils: oxides and organic matter. DSC previously known as an oxide coated clay was employed to prepare HA-DSC as described in Chapter 4. Both Cu-DSC and Cu-HA-DSC were extracted for Cu by CAME. The extractabilities obtained from both matrices are shown in Table 5.2. At 95% confidence with triplicate extractions, there was no significant difference resulting from oxide or humate coatings. It was most likely that CAME was very powerful to liberate all spiked and naturally adsorbed Cu fractions. The outer layers of simulated humate coating on the natural oxide fraction could not be able to sustain the naturally adsorbed Cu at the inner layer of the control DSC. This naturally adsorbed Cu fraction in the control DSC was considered to be the cause for Cu recoveries to be higher than 100% that are calculated on the spiking data in Chapter 4.

TABLE 5.2: Extraction recoveries of Cu from oxide and organic coated matrices based on amounts of Cu adsorbed in spiking procedure (95% confidence limits, n= 3).

Sample	%Cu recovery
Cu-DSC	104.5 ± 2.5
Cu-HA-DSC	104.2 ± 2.7

There was no significant difference due to $t_{cal} = 0.28 < t_{crit} = 2.78$

5.7 Co-extracted Cu and Cd in spiked soils

At some contaminated sites, various kinds of metals could possibly be involved. In order to have mixed Cu, Cd contaminated samples, adsorption procedures were again carried out for 24 hour-equilibration using a mixed solution containing 0.001 M Cu (II) and 0.001 M Cd (II) at pH 5. Two sets of six materials, original-substrates and HA-substrates, were again subjected to spiking experiments at the ratio of 10 g-material/200 mL-solution. Adsorption capabilities of Cu and Cd in a mixed system are given in Table 5.3 and Figure 5.8. The molar adsorption capabilities helped explain that at the same condition Cu (II) ions generally possessed higher affinity to these matrices than Cd (II) ions. The influences of HA coating on Cd adsorption were quite similar to those on Cu adsorption as described in Chapter 4. Afterwards, real soil based matrices containing very high concentrations of co-extracted Cu and Cd were used further for extraction studies by CAME, whose results are shown in Figure 5.9. It was likely that most recoveries, based on adsorption behaviors, were nearly 100%. The interesting point can be observed for DCL based matrices which provided quite high Cd recoveries, up to 107%. To ensure there was no interference from naturally occurring Cu and Cd contents

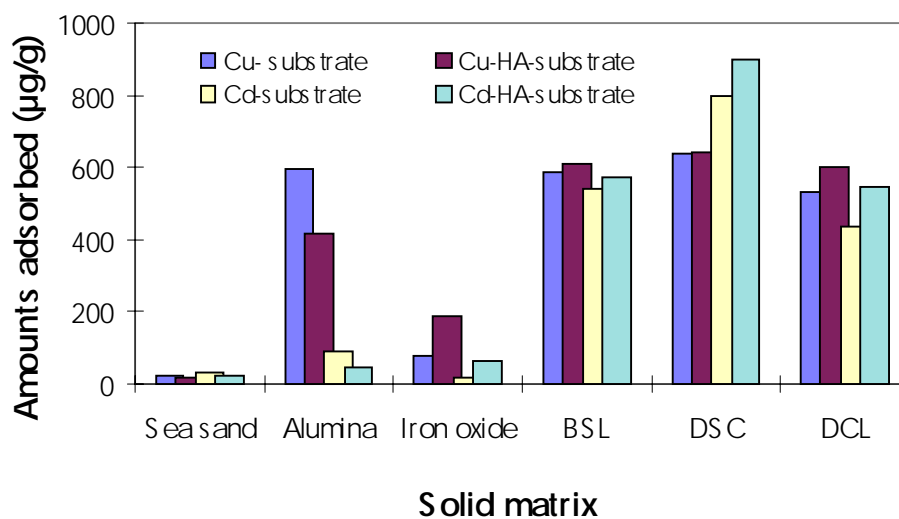


FIGURE 5.8: Competitive adsorption reactions between Cu and Cd occurring on the same material (n=1).

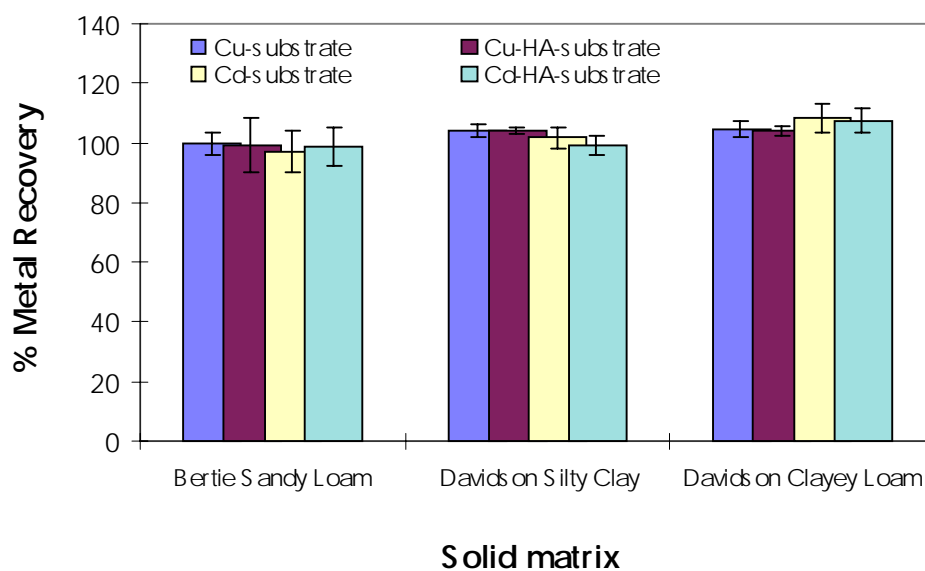


FIGURE 5.9: Extraction recoveries of Cd and Cu based on amounts adsorbed (spiked) from different spiked soil matrices using CAME technique (95% CI, n=3).

in all control soils, the hot acid extraction method was applied to the determinations of total Cu and Cd concentrations in each soil while CAME was utilized to evaluate the surface bound Cu and Cd contents.

The comparison data of the control soils are shown in Table 5.4. Generally, most non-contaminated soils contain very low Cd concentrations. As found here, all three soils possessed total Cd contents in a range of 0.3 to 6.8 ppm. Only 0.7 ppm Cd from DCL could be extracted by CAME. Because of this, the naturally adsorbed Cd content in the control DCL should not significantly cause very high Cd recoveries as obtained from Cu, Cd-DCL and Cu, Cd-HA-DCL. It is thought that the major factor for the overestimated evaluation in this case could possibly result from the entrained solutions left after the spiking time has elapsed which still contained quite high Cd concentrations up to 69 and 58 $\mu\text{g/mL}$. Without further interactions, a small volume retained, 0.6 mL/g-DCL, probably caused approximately 7% overestimated Cd recovery. The effect of the entrained solution possibly slightly impacted on DSC because the Cd concentrations left in the final equilibrating solutions were two times lower than those of DCL matrices. Oxide coated soils could retain larger volumes of aqueous solutions much more than BSL materials. So, this factor had no significant influence on recoveries of both metals for BSL matrices. Finally, this study proved that CAME was useful for accessing several kinds of adsorbed metals in contaminated soils.

5.8 The choice of extractants

One of characteristics of an extraction method is selectivity. Here, it was described to be able to observe the extraction ability related to the selectivity of CAME by using various extractants. As may be known, the selectivity of the conventional chelation depends upon the identity of the selected extracting solution. With Chelate-Assisted Microwave Extraction (CAME), a short extraction time has been expected from the application of microwave radiation while it has been thought that the selectivity may still be governed by not only the selected extractant characteristic but the detection technique also. In this task, seven acidic complexing agents including citric acid, oxalic

TABLE 5.3: Comparison of adsorption capabilities in the mixed metal solution.

Sample	Cu			Cd		
	Adsorption		Final solution µg/mL	Adsorption		Final solution µg/mL
	µg/g	µmol/g		µg/g	µmol/g	
Original materials						
Sea sand	20.7	0.32	62.4	33.4	0.30	109.6
Alumina	596.3	9.38	4.7	93.1	0.83	103.6
Iron oxide	77.2	1.22	56.8	16.7	0.15	111.2
BSL	558.8	8.79	5.5	539.2	4.80	58.9
DSC	637.8	10.04	0.6	798.0	7.10	33.0
DCL	532.1	8.37	11.2	435.4	3.87	69.3
HA-materials						
HA-Sea sand	20.5	0.32	62.4	21.6	0.19	110.7
HA-Alumina	416.3	6.55	22.8	44.1	0.39	108.5
HA-Iron oxide	187.7	2.95	45.7	62.7	0.56	106.6
HA-BSL	608.9	9.58	3.4	574.3	5.11	55.3
HA-DSC	641.4	10.09	0.2	898.6	7.99	22.8
HA-DCL	600.0	9.44	4.4	547.9	4.87	58.0

TABLE 5.4: Extracted Cu and Cd concentrations from the control soils (95% confidence limits, n = 3).

Control soil	Total (µg/g)		Available (µg/g)	
	Cu	Cd	Cu	Cd
Bertie Sandy Loam	9.0 ± 6.0	0.3 ± 0.2	6.0 ± 3.0	ND
Davidson Silty Clay	45.3 ± 7.7	4.1 ± 1.2	18.8 ± 4.2	ND
Davidson Clayey Loam	65.3 ± 7.0	6.8 ± 1.0	17.0 ± 3.5	0.7 ± 0.7

acid, NTA, EDTA, CyDTA, EGTA, and DTPA were explored. Also three basic complexing agents such as TEA, DETA, and TETA were investigated. All extracting solutions were prepared at 0.100 M and adjusted to pH 7 with NaOH or HCl. Hot acid extraction method was initially employed to evaluate the total concentrations of Cd, Cr, Cu, Ni, Pb, and Zn in control BSL and DSC. As can be seen in Figure 5.10, BSL and DSC contained quite high levels of Pb, but very low levels of Cd. Because of this, only five heavy metals of Cr, Cu, Ni, Pb, and Zn, were analyzed by ICP-AES after CAME applications. These selected extractants were applied to both control soils, BSL and DSC. With the 2-step microwave application with CAME, metal extractabilities were calculated as % recovery based on each total content.

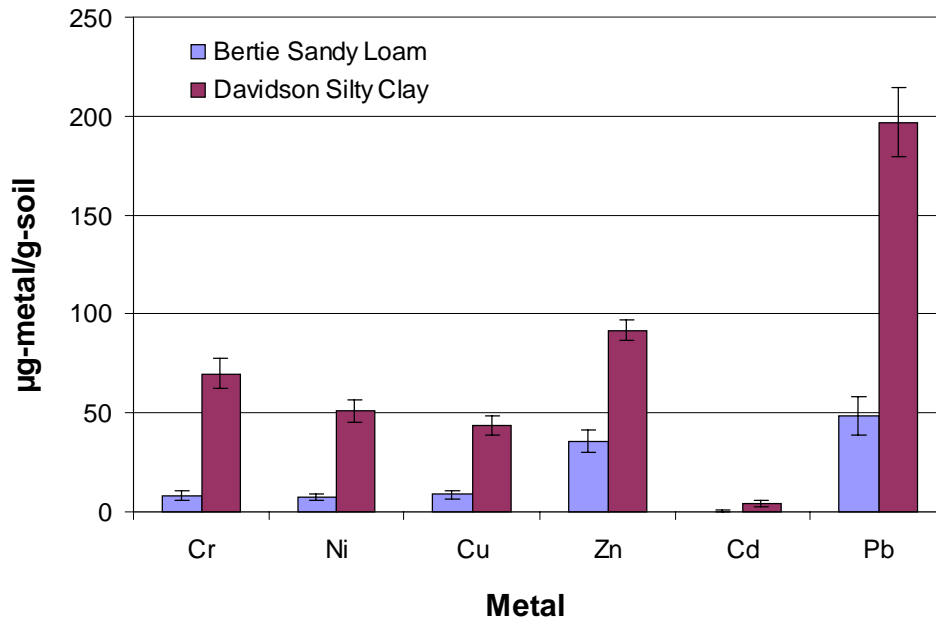


FIGURE 5.10: Total heavy metals in the control BSL and DSC by hot acid extraction method (95%CI, n=3).

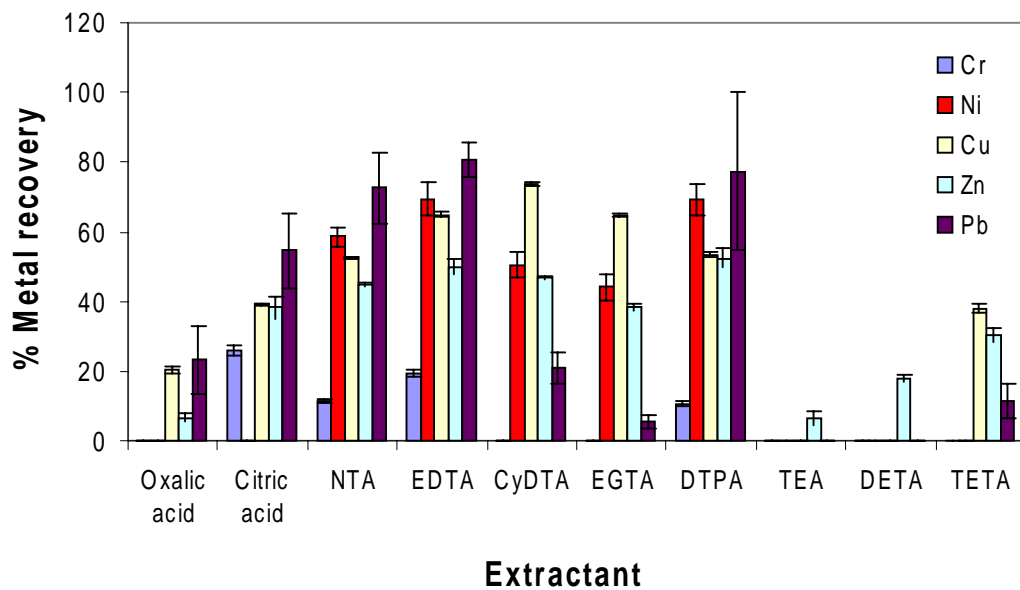


FIGURE 5.11: The effect of the selected extractants using CAME with control Bertie Sandy Loam (95% CI, n=3).

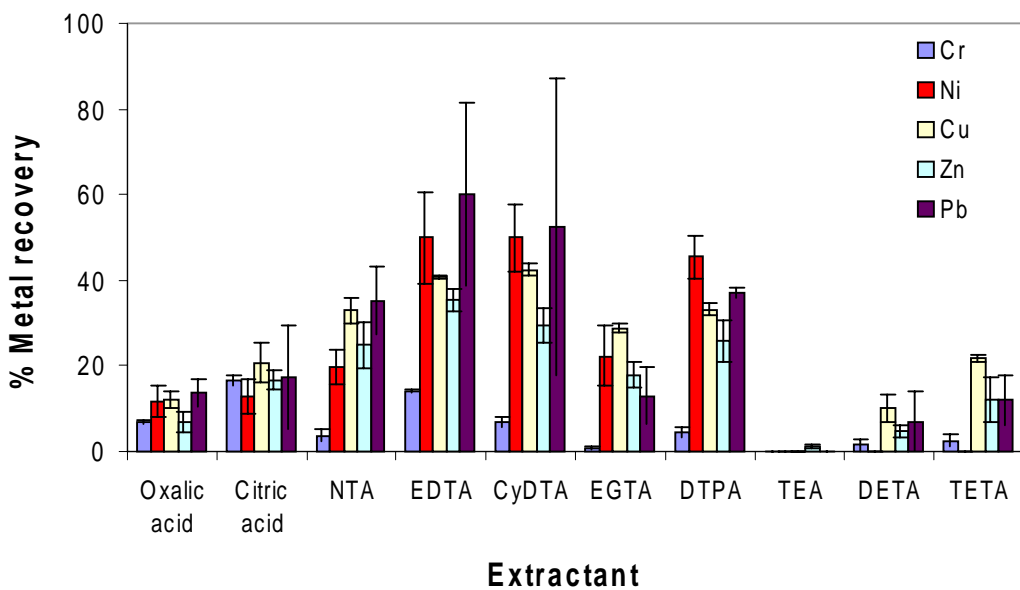


FIGURE 5.12: The effect of the selected extractants using CAME with control Davidson Silty Clay (95% CI, n=3).

Figure 5.11 and Figure 5.12 show that chromium was the most difficult to be extracted. Of different oxidation states, Cr (III), which is thought to be available for chelation, has high possibility to form a stable solid of CrOH_3 ($K_{sp} = 10^{-31}$) and thus may make some difficulty for extraction even with the most acceptable chelating agents like EDTA or DTPA. Among 4-carboxylic group containing compounds: such as EDTA, CyDTA, and EGTA, the results indicated that EDTA provided the best recoveries of Cu, Ni, Pb, and Zn for both soils. Compared to EDTA, CyDTA produced comparable recoveries for Cu and Zn from BSL, and Cu and Ni from DSC. On the other hand, EGTA was likely to provide the worst metal recoveries compared EDTA and CyDTA. These three chelating agents are expected to form four or six coordinated metal complexes and normally could work well with transition metals.

NTA, consisting of 3 carboxylic groups, resulted in lower extraction abilities than EDTA. This might concern about the stabilization ability of each metal chelate in a mixed system. In a pure solution system, it has been found that DTPA with five carboxylic groups and three amine groups can have higher complex formation constants (K_{ML}) with most heavy metals than EDTA. However, this study suggests that a soil mixture undergoing CAME can give rise to different results. It may be true that DTPA can stabilize bigger metals ions which need to form eight coordinated complexes, but the steric hindrance between DTPA and dissolved metals might play an important role in the CAME system. Citric acid provided higher extraction efficiencies than oxalic acid for all metal interests, and also they resulted in much lower extraction efficiencies compared to those obtained from using some selected complexanes (EDTA derivatives) as seen in Figures 5.11 and 5.12. Having different structure from TEA and DETA, TETA containing four amine groups yielded higher metal recoveries due to more stable complexes possibly formed.

In order to better understand extractant behaviors in the CAME system, these data were evaluated in light of K_{ML} values of metal chelates and ionic radii. However, it was found that there was no correlation between metal recoveries and $\log K_{ML}$ of EDTA

derivatives neither the correlation between metal recoveries and ionic radii. This probably results from heterogeneous properties of soils.

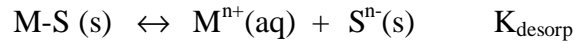
The difference in size of the central metal may also play an important role in forming a chelate complex. This packing effect can be explained that small ions can be repelled from the formation of the complex while very big ions may be unable to fit into the central cavity of the complex. All described factors involving the geometric structure of a metal chelate primarily control the stability of the complex in the extract and indicate the selectivity of the extraction method. Eventually, metal-EDTA complexes were likely to be the most stable forms under microwave extraction possibly due to their suitable chelating structures.

5.9 Summary

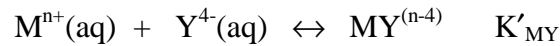
In this chapter, extraction chemistry of CAME has been described. The 2-step microwave extraction using a power of 120 W for 5 min followed by 60 W for 10 min proved to be useful for the estimation of adsorbed metal fractions in soils. In the microwave extraction system, side-reactions could occur under microwave irradiation that accelerates all possible reactions in the vessel. Working on the open focused microwave system, it became apparent that the power control was important for the achievement of high extraction efficiency and reproducibility without the risk of sample loss. Besides metals incorporated into the lattice structure of the soil, all adsorbed metals could be completely extracted regardless of soil components, such as oxide or organic coated soils. According to several metal levels in soil matrices, concentrations of the selected extractant have to be considered. At the same concentration, DTPA has not shown better extraction efficiency than EDTA. So, EDTA should be a better choice for CAME because its ease in dissolving and low cost. For normal aluminosilicate soils, 0.025 -0.050 M EDTA should be sufficient to stabilize all extracted metal species. Some metal contaminated samples may require higher concentrations of EDTA up to 0.100 M. The choice of extractants was evident that EDTA was the most suitable chelating agent for evaluating the levels of trace metals available in soils systems.

Up to this point, the extraction mechanism in the microwave extractor using CAME is proposed as follows:

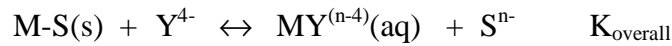
First metal dissolution rate at soil surface increases after microwave irradiation that probably results from direct interaction between microwave radiation and soil surfaces, and also the higher temperature of the aqueous mixture enhancing rates of exchanges of chemisorbed metals.



Once in the aqueous phase, the recently liberated contaminant metals react with EDTA.



This chelation reaction effectively depletes the $M^{n+}(aq)$ concentration in the aqueous phase and serves to drive the exchange reaction to completion. Hence, the overall reaction can be summarized as follow:



$$\text{Where} \quad K_{\text{overall}} = K_{\text{desorb}} * K'_{MY}$$

It can be seen that the larger desorption constant (K_{desorb}) and formation constant (K'_{MY}) the extraction reaction will be more favorable.

In this work, the metal spiked samples were prepared by adsorption procedures. In the next chapter, an investigation of the effect of equilibration/aging time used for the spiking method and its influence on the evaluation of extraction efficiencies of different methods would be pursued. Also, included would be an exploration of the effect of extracting temperature on metal removal from soil surfaces by using the hotplate heating method and compare the results to those obtained from CAME.

CHAPTER 6

AGING TIME & EXTRACTION TEMPERATURE EFFECTS

As described in Chapter 5, the metal-spiked samples that were extracted by CAME represented metal contaminated matrices. A 24-hour equilibration procedure was employed in order to allow the soil sample to have enough time to interact with the added metal ions. As demonstrated in the literature,⁶ researchers have attempted to evaluate the efficiencies of developed methods using spiked materials. It is possible that the interaction time may play a key role for method development. In order to elucidate the effect of the aging times for the preparation of metal-spiked samples, efficiencies of different extraction methods were investigated as a function of equilibration/aging times. The first part of this chapter describes how aging times influence the extraction recovery of each extraction method.

Due to the lack of a temperature-monitoring device available for the Soxwave 100 microwave extractor, it is ambiguous to relate the actual temperatures inside the vessel and % metal recoveries. Since the microwave extractor operates at atmospheric pressure, it is certain the average maximum temperature is near or at the normal boiling point of water for an aqueous solution. Accordingly, the experiment performed in the second part of this chapter involved monitoring extraction efficiencies of a conventional heating method as a function of heating times and temperatures. Extraction capabilities of the hotplate heating method and the optimized EDTA-CAME method, selected from the previous chapter using the 2-step microwave application of 20 W for 5 min followed by 60 W for 10 min, were compared.

6.1 The effect of equilibration/aging time

6.1.1 Determination of adsorption capability of Cu (II)

This investigation was applied to all three control soils: BSL, DSC, and DCL. An equilibration system was prepared in a 50 mL screwed-cap polyethylene using 2 g of soil with 25.0 mL of 0.001 M Cu (II) where the initial pH was at 5.4. For each soil, five equilibration times of 2, 8, 24, 72, and 240 hours were observed. Two replicate determinations were performed at each equilibration time. After being equilibrated for a certain time, the final solution was separated from the soil solid by filtration/centrifugation. The resulting solid residue, as a Cu-spiked matrix, was allowed to dry, then ground, and finally kept for next extraction studies. The clear solution was used for the pH measurement and the determination of Cu concentration. The adsorbed Cu concentrations at different aging times were obtained from the differences between the initial and final Cu concentrations, measured by ICP-AES.

Without pH pre-equilibration of the solid materials, the final pH values were monitored as shown in Figure 6.1. This observation indicated that the pH change occurred within the first few hours of the equilibration period. All final pH values were lower than the original pH of 5.4. This may imply that the first mechanism of Cu adsorption on soil surfaces involves rapid replacement of acidic sites by Cu (II) ions. Due to buffer properties of soil surfaces, there was no significant change of the solution pH at longer times. As shown in Figure 6.2, DSC achieved the maximum adsorption capability for Cu (II) ions within 8 hours, whereas DCL and BSL required more time up to 72 hours to reach their adsorption equilibria.

It has been explained that there are several possible rates of reactions at soil-solution interfaces.⁶⁸ Metal adsorption of an oxide material is possibly a rapid reaction. However, a mixture of secondary minerals in soil can provide the adsorption equilibrium in a wide time scale from microseconds to months. In the case of multi-layer minerals, different adsorption rates can occur and are dependent of the available surface sites.

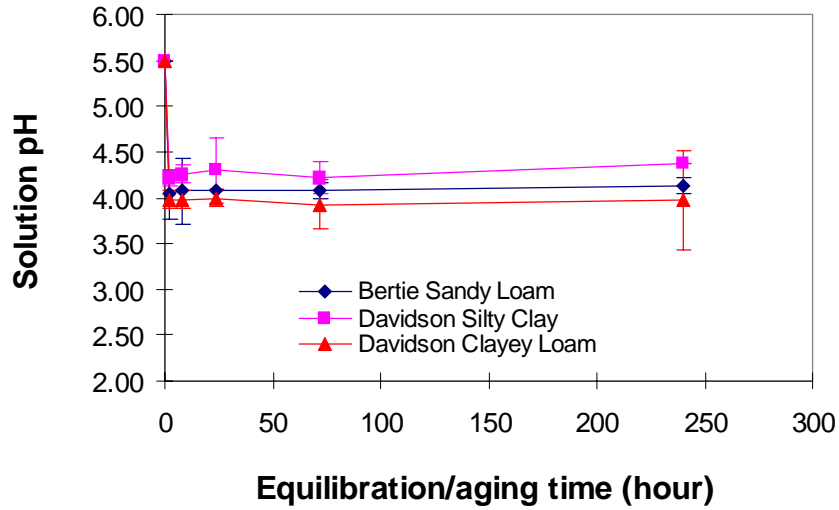


FIGURE 6.1: Final pH values of equilibrating solutions using three different control soils at different aging times (n=2).

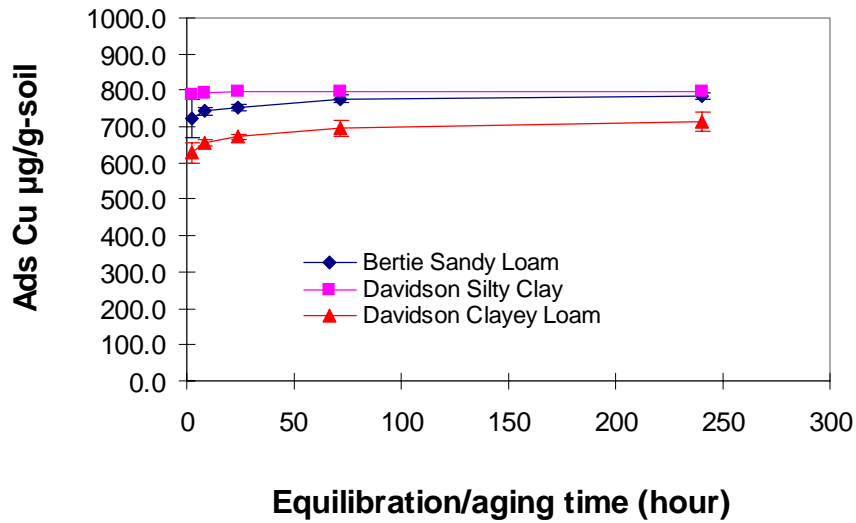


FIGURE 6.2: Cu adsorption capabilities of three control soils at different aging times. Error bars shown on the graph are so small (n=2).

Adsorption at outer layers mostly tends to be faster than at the intermediate and inner layers. Besides the surface properties, the initial Cu concentration may be a major factor to control the maximum adsorption level for each soil. This study indicated that the Cu adsorption capability of each soil was in the order of DSC>BSL>DCL as given in Table 6.1. For the effect of soil pH, it seemed likely that the final pH values were consistent with the Cu adsorption capabilities. At higher pH, the adsorption capabilities of Cu (II) ions increased. As can be seen in Figure 6.1, DSC produced higher pH value of Cu solution than BSL and DCL, but all final pH values dropped below the initial pH value.

TABLE 6.1: Cu adsorption capabilities and Cu concentrations remained in the solutions after equilibration at different aging times using ICP-AES analysis of the initial and the final Cu solutions (95% confidence limits, n = 2).

Soil	BSL		DSC		DCL	
	Ads Cu ($\mu\text{g/g}$)	Cu left ($\mu\text{g/mL}$)	Ads Cu ($\mu\text{g/g}$)	Cu left ($\mu\text{g/mL}$)	Ads Cu ($\mu\text{g/g}$)	Cu left ($\mu\text{g/mL}$)
2	722.8 \pm 53.0	6.1 \pm 4.5	787.5 \pm 9.9	1.0 \pm 0.9	628.6 \pm 27.9	13.7 \pm 1.8
8	742.4 \pm 12.6	4.6 \pm 0.9	794.1 \pm 0.9	0.4 \pm 0.0	655.1 \pm 9.0	11.6 \pm 0.9
24	753.3 \pm 6.3	3.7 \pm 0.9	795.8 \pm 1.8	0.3 \pm 0.0	672.7 \pm 6.3	10.1 \pm 0.9
72	776.7 \pm 12.6	1.8 \pm 0.9	798.2 \pm 1.8	0.1 \pm 0.0	696.4 \pm 21.6	8.3 \pm 1.8
240	783.0 \pm 8.1	1.3 \pm 0.9	798.4 \pm 2.7	0.1 \pm 0.0	712.7 \pm 27.0	6.9 \pm 1.8

6.1.2 Comparison of the EDTA-CAME method and the EDTA- shaking method

The Cu-spiked samples obtained from the previous section were employed to explore the effect of equilibration times on the extraction efficiency of each method: EDTA-shaking and EDTA-CAME. Both extraction methods used the same EDTA

concentration and the same extraction ratio, but different extraction times. The EDTA-shaking method was performed at room temperature for 1 hour-extraction while the EDTA-CAME method was conducted using the total 15-min microwave exposure of 120 w for 5 min followed by 60 W for 10 min. Extraction efficiencies were calculated based on the concentration of Cu adsorbed on each soil at each aging time. The comparison results are shown in Figure 6.3-6.5.

For all soils, the aging times were likely to have no impact on extraction efficiencies of the EDTA-CAME method. The EDTA-CAME method was able to provide 100% Cu recoveries for all soils. The extraction recoveries were comparable to some of those obtained from Cu-BSL and Cu-DSC in Chapter 5. On the other hand, the EDTA-shaking method provided different efficiencies depending upon the aging time. As aging times increased, the decrease in Cu recoveries from 81 to 75% occurred. The steep decrease was observed when the aging times were increased from 2 to 24 hours. This could be explained that the conventional shaking method might not be able to completely leach out all adsorbed Cu contents.

It has been considered that longer equilibration times may allow adsorbed Cu ions form stronger interactions with soil components that are more difficult to be extracted by the EDTA strength at room temperature with 1 hour extraction. A variety of the interactions between Cu ions and surface active sites can be possible. This study indicated that the aging times from 2 to 240 hours had no effect on the EDTA-CAME technique for all soil matrices. It was evident that the direct adsorption of microwave radiant of a soil-solution system could provide high energy enough to liberate all adsorbed Cu contents using a short microwave exposure time of 15 min. Therefore, the aging time used to prepare spiked samples can measure the leaching strength of each extraction method. The aging times should be closely considered when the spiked samples are employed to evaluate an extraction method.

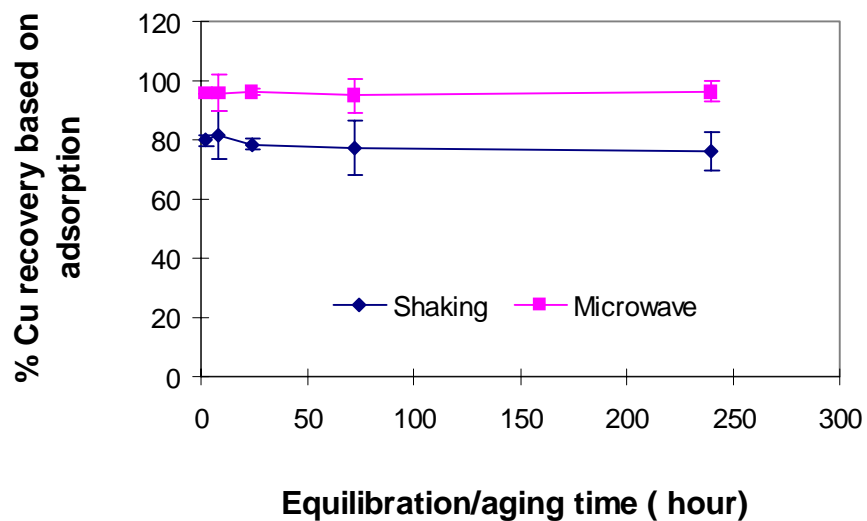


FIGURE 6.3: Extraction efficiencies of EDTA-shaking method and EDTA-CAME method at different aging times of Cu-BSL (n=3).

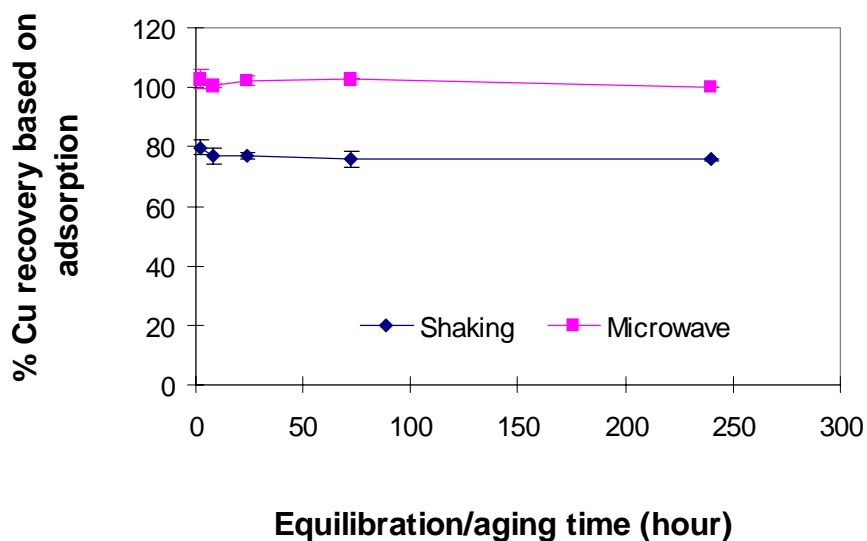


FIGURE 6.4: Extraction efficiencies of EDTA-shaking method and EDTA-CAME method at different aging times of Cu-DSC (n=3).

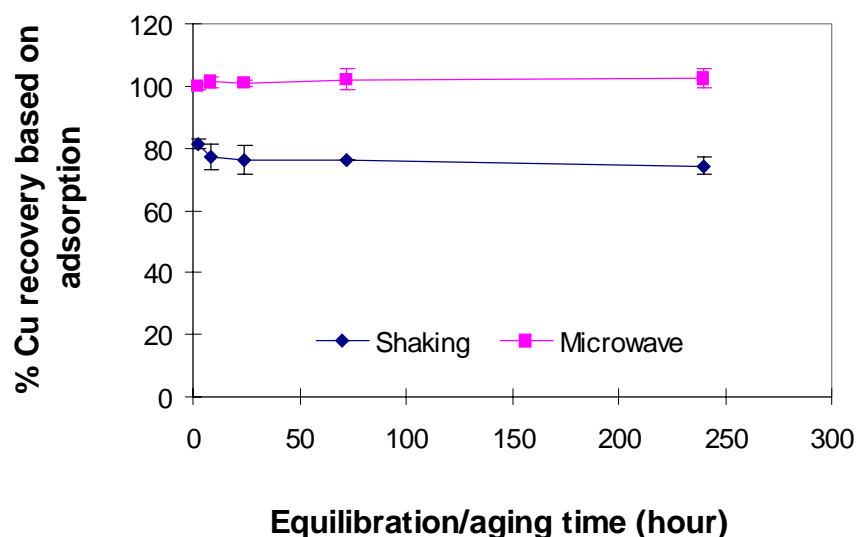


FIGURE 6.5: Extraction efficiencies of EDTA-shaking method and EDTA-CAME method at different aging times of Cu-DCL (n=3).

6.2 Extraction temperature effect

In order to understand the chemistry occurring inside the microwave extractor, several kinds of samples were used to evaluate whether the temperature would be a major effect on metal removal from soil surfaces. No temperature-monitoring device was available for the Soxwave 100 system; therefore, the temperature effect was observed using a conventional heating method. With a hotplate as a heat source, temperatures were recorded during extractions from 5 to 60 min using a thermometer. The heating rate was initially adjusted to a high level until the extracting mixture was boiled, and then was lower to an intermediate level to maintain the boiling condition and avoid strong bumping that can cause possible sample lost.

6.2.1 Control DSC and amended BSL

Each extraction was performed using the ratio of 0.5 g-sample/10.0 mL of 0.100 EDTA solution in a 125 mL erlenmeyer flask. Triplicate sets were performed for each extraction time. Extracted Cu concentrations were reported as μg -extracted Cu/g-soil. The extraction results are shown in Figure 6.6 and 6.7. For this experiment, most extraction mixtures were boiled at about 10 min of heating and the maximum temperatures were close to 100 °C. As temperatures and heating times increased, the concentrations of extracted Cu from the control DSC and the amended BSL increased apparently. Table 6.2 shows that the increases in extractabilities from both samples after 20 min-heating were very small. Control DSC showed the increase in the extracted Cu concentrations from 1.7 to 17.5 $\mu\text{g}/\text{g}$, whereas the amended BSL indicated the increase in the extracted Cu concentrations from 23.2 to 34.5 $\mu\text{g}/\text{g}$. Considering for a 5 min-extraction, only 1.7 ppm Cu was removed from the control DSC. This implies that the Cu ions probably interact with DSC more strongly than BSL. This characteristic may result from the domination of the oxide fractions in DSC.^{61, 62}

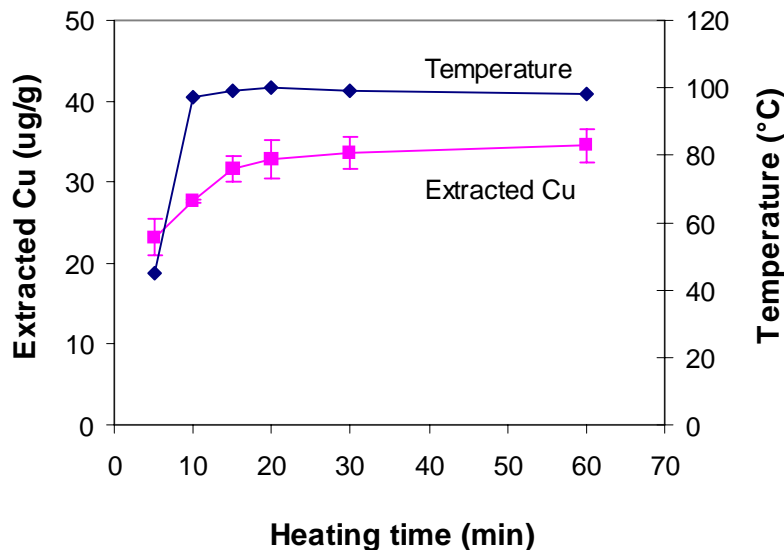


FIGURE 6.6: Cu removal from amended BSL using EDTA- hotplate heating method (n=3).

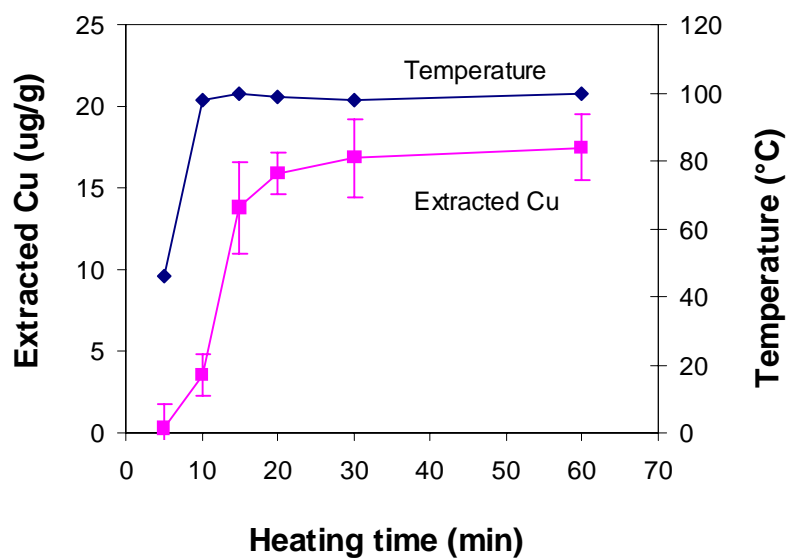


FIGURE 6.7: Cu removal from control DSC using EDTA- hotplate heating method (n=3).

TABLE 6.2: Comparison of Cu removal from the control DSC and the amended BSL as a function of heating times using EDTA-hotplate heating method (95% confidence limits, n=3).

Heating time (min)	Extracted Cu ($\mu\text{g/g-soil}$)	
	Control DSC	Amended BSL
5	1.7 ± 7.2	23.2 ± 2.2
10	3.5 ± 1.2	27.7 ± 2.7
15	13.8 ± 2.7	31.7 ± 1.5
20	15.9 ± 1.2	32.8 ± 2.5
30	16.8 ± 2.5	33.6 ± 2.0
60	17.5 ± 2.0	34.5 ± 2.0

Besides the naturally occurring (control) DSC and the long-term Cu amended BSL, metal spiked samples including Cu, Cd-BSL, Cu, Cd-DSC, Cu, CD-HA-BSL, and Cu, Cd-HA-DSC were also subjected to the study of extraction temperature effect by the conventional heating method.

6.2.2 Cu, Cd-BSL and Cu, Cd-DSC

Two spiked samples investigated here contained different matrices due to the original components in control soils. Cu and Cd recoveries were determined as functions of heating times and temperatures. Table 6.3 shows the comparison of extraction recoveries for each metal obtained from these two matrices. It was found that all extracting mixtures could achieve the boiling conditions in about 10-min heating. Cu recoveries varied from 78.6 to 93.1% for Cu, Cd-BSL and from 69.2 to 92.9% for Cu, Cd-DSC, whereas Cd recoveries varied from 83.3 to 94.9% for Cu, Cd-BSL and from 86.4 to 99.7% for Cu, Cd-DSC. These observations can be seen in Figure 6.8. It implies that the spiked Cu (II) ions may form stronger interactions with the oxide components than the coated humic acids in DSC. Considering at 60-min heating, maximum Cu recovery from Cu, Cd-DSC was only 93%. In case of Cd, it was likely that Cd (II) ions weakly interacted with both spiked samples. Almost 100% Cd recovery from Cu, Cd-DSC could be achieved within 20-min heating; however, only 95% Cd recovery seemed to be the maximum level available from Cu, Cd-BSL. For Cu-BSL, the hotplate heating method produced the maximum Cu recovery approximately 4% lower than the EDTA-CAME reported in Chapter 5. This showed the limitation of the hotplate heating method, which required more lengthy extraction time and provided lower recoveries.

6.2.3 Cu, Cd-HA-BSL and Cu, Cd-HA-DSC

In order to confirm the extraction capabilities of the hotplate heating method, other samples such as Cu, Cd-HA BSL and Cu, Cd-HA-DSC that were prepared in the previous chapter were then subjected to the EDTA-hotplate heating method. Extraction conditions were the same as used in Section 6.2.2. Table 6.4 and Figure 6.9 show the

TABLE 6.3: Cu and Cd recoveries obtained from Cu, Cd-BSL and Cu, Cd-DSC as a function of heating time using EDTA-hotplate heating method (95% confidence limits, n=3).

Heating time (min)	% Cu recovery		% Cd recovery	
	Cu, Cd-BSL	Cu, Cd-DSC	Cu, Cd-BSL	Cu, Cd-DSC
5	78.6 ± 8.9	69.2 ± 3.7	83.3 ± 5.0	86.4 ± 5.0
10	89.8 ± 5.2	86.5 ± 4.0	87.1 ± 3.2	97.4 ± 2.2
20	91.0 ± 3.5	89.7 ± 2.2	94.2 ± 5.0	99.7 ± 4.7
30	92.8 ± 2.7	91.4 ± 0.5	94.3 ± 4.7	100.0 ± 1.5
60	93.1 ± 3.0	92.9 ± 3.2	94.9 ± 4.2	99.7 ± 2.2

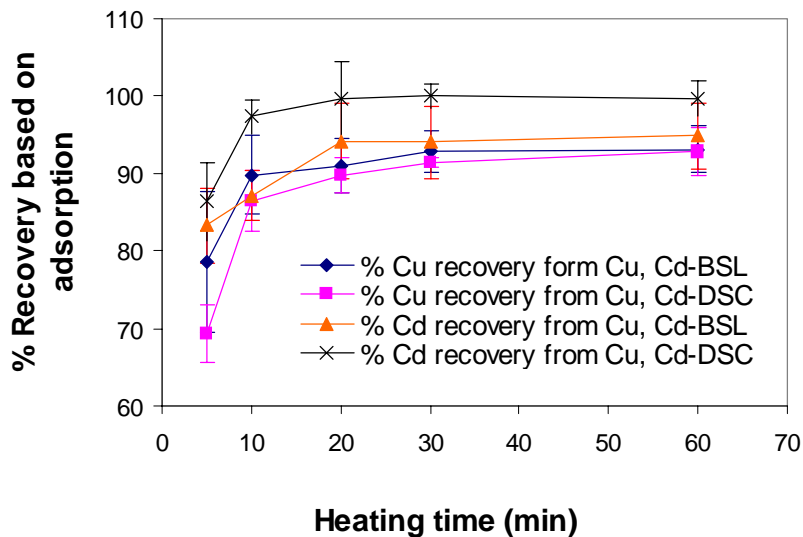


FIGURE 6.8: The effect of heating times on Cu and Cd recoveries from Cu, Cd-BSL and Cu, Cd-DSC using EDTA-hotplate heating method (n=3).

TABEL 6.4: Cu and Cd recoveries obtained from Cu, Cd-HA-BSL and Cu, Cd-HA-DSC as a function of heating time using EDTA-hotplate heating method (95% confidence limits, n=3).

Heating time (min)	% Cu recovery		% Cd recovery	
	Cu, Cd-HA-BSL	Cu, Cd-HA-DSC	Cu, Cd-HA-BSL	Cu, Cd-HA-DSC
5	88.5 ± 4.2	74.6 ± 1.0	93.5 ± 2.7	89.8 ± 2.2
10	94.5 ± 2.0	90.6 ± 2.7	95.9 ± 5.0	95.7 ± 4.0
20	97.4 ± 5.0	96.3 ± 5.7	99.9 ± 1.7	99.7 ± 3.2
30	98.7 ± 2.0	98.6 ± 3.5	100.8 ± 1.7	100.2 ± 5.0
60	97.2 ± 4.7	100.0 ± 1.5	101.8 ± 2.7	100.1 ± 4.7

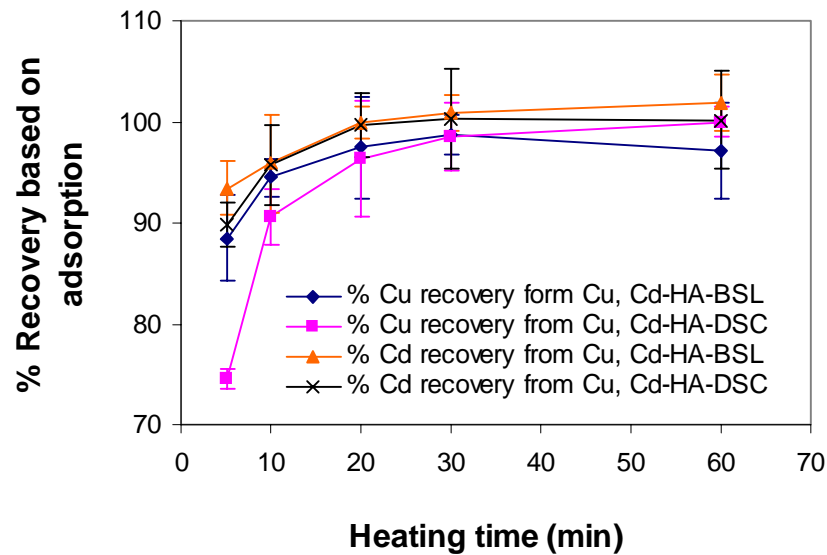


FIGURE 6.9 The effect of the heating time on Cu and Cd recoveries from Cu, Cd-HA-BSL and Cu, Cd-HA-DSC using EDTA-hotplate heating method (n=3).

increase in capabilities to remove both adsorbed Cu and Cd as the heating times and temperatures were increased. Metal recoveries show that both Cu and Cd ions form weaker interaction with humic acids than with oxide components in soils. Compared to the results in Table 6.3, Cu recoveries found were in higher levels from 88.5 to 98.7% for Cu, Cd-HA-BSL and from 74.6 to 100.0% for Cu, Cd-HA-DSC. In addition, the extraction results of Cd determinations indicated the same tendency. Cd recoveries were found in a range of 93.5 to 101.8% for Cu, Cd-HA-BSL and in a range of 89.8 to 100.1% for Cu, Cd-HA-DSC. It was likely that only complete removal of adsorbed Cd from organic rich soils might be possible using a heating time scale of 1 to 2 hours.

Statistical analysis using 2-way ANOVA without replication was utilized to test whether there were significant differences due to matrix and heating time factors. Comparing two extraction profiles from Figure 6.8 and 6.9, the results are expressed in Table 6.5. From the calculated F statistic, it was clear that different heating times gave significantly different results. For Cu extraction, the matrix effect resulted in significantly different efficiencies of the conventional heating method. For Cd extraction, there was a significant difference due to matrix effect of Cu, Cd-BSL and Cu, Cd-HA-BSL. This experiment proved that Cu ions provided a wide range of interaction strengths depending upon soil matrix.

6.3 Summary

Equilibration or aging time proved to be an important factor for evaluating the efficiency of an extraction method. Cu adsorption equilibrium on the selected soil surfaces occurred in 8-72 hours. DSC, which mainly contained small particles, achieved the fastest equilibrium within 8 hours. The increase in aging times exceeding 48 hours did not significantly show the increase in adsorption capability, but did affect the metal removal mechanisms of thermal desorption. The increase of aging times from 2 to 240 hours resulted in the decrease of extraction recoveries of adsorbed Cu from 81 to 75%. It may be explained that at longer equilibration times some fractions of diffused and outer-sphere complexes may have some chances to move to the stronger attraction sites of the

TABLE 6.5: Two-way ANOVA applied to different extraction profiles at 95 % CI.

Metal	Matrices compared	Factor	F_{crit}	F_{calc}	Data interpretation
Cu	Cu, Cd-BSL vs. Cu, Cd-HA-BSL	Matrix	7.71	37.11	Different
		Time	6.39	19.31	Different
	Cu, Cd-DSC vs. Cu, Cd-HA-DSC	Matrix	7.71	105.46	Different
		Time	6.39	229.90	Different
Cd	Cu, Cd-BSL vs. Cu, Cd-HA-BSL	Matrix	7.71	93.69	Different
		Time	6.39	24.32	Different
	Cu, Cd-DSC vs. Cu, Cd-HA-DSC	Matrix	7.71	0.37	Same
		Time	6.39	32.57	Different

soil. This results in more difficulty to remove all adsorbed metals using the conventional heating method. These conventional methods only generate sample heating by the convection heating mechanism providing the maximum temperature inside an extracting reactor at the boiling point of the mixture. However, longer equilibration times seemed to have no effect on the EDTA-CAME technique. This probably resulted from strong energy of the microwave radiation that can directly interact with the metal bound sites at soil surfaces and provide uniform heating. Even through the Soxwave 100 system has been designed to operate at the atmospheric pressure, superheating possibly providing temperature exceeding the boiling point about 5 to 10 °C, has been expected to occur inside the vessel. The EDTA-CAME method clearly showed better recoveries than the EDTA-hotplate heating method.

CHAPTER 7

CONCLUSIONS

The goal of this research was to develop a new extraction method for metals in soil using a combination of chelation and microwave energy. Appropriately, this technique was named “Chelate Assisted Microwave Extraction (CAME).” Throughout this research, the optimized conditions of CAME have been compared to the conventional extraction methods such as EDTA-shaking, hot acid extraction, and EDTA-hotplate heating extraction. It has been found that CAME was efficient to liberate all sorbed metals studied regardless of oxide or organic components in soils. With a significant reduction in extraction time, CAME allows the complete extraction of contaminated metals from soil. Hence, the CAME method is feasible for evaluation of some sorbed metals found in soil samples.

Initial studies focused on the application of CAME to two sets of Bertie Sandy Loam (BSL): control (untreated) and amended samples. It was found that the extraction solution pH had an impact on the efficiency of the EDTA-shaking method, but had no effect on the CAME technique. The increase in pH from 7.8 to 10.1 of the EDTA solution resulted in an improvement of the extraction efficiency of the EDTA-shaking method, whereas the CAME method was pH-independent in this pH range. Using different solution compositions could liberate different adsorbed metal fractions in the soil sample. It was indicated that along with the microwave application the extracting strengths of the selected solutions were in the order of EDTA > Na₂CO₃/NaHCO₃ > H₂O when coupled with microwave energy. Water exchangeable reactions were apparently accelerated by microwave energy. The Na⁺ ions in the carbonate buffer solution can rapidly replace exchangeable metals. The presence of EDTA evidently helped in the leaching of metals from stronger binding sites in the soils. Extraction equilibrium of each fraction was obtained within 15-20 min using a microwave application at 90 W for 3 min followed by 30 W for longer times. From the method comparisons, the CAME method

produced lower Cu concentrations than the hot acid extraction method, but higher than the EDTA-shaking method. It was found that using EDTA provided better precision than using high concentrations of strong inorganic acids (HNO₃/HCl). This finding implies the extracting solvent associated with the detection method, which is solvent-dependent, plays an important role in controlling the precision of the analytical method. The hot acid extraction method used in this work was EPA method 200-A, which normally provides total concentrations of trace metals in soil samples by atomic spectrometry.^{10,41} From data obtained in this study, it is suggested that the difference of the extracted Cu concentrations obtained from the hot acid method and from the CAME method was due to the Cu fraction as geologically lattice structures in the control soil.

Since the extraction of adsorbed Cu from sandy soils was accomplished by CAME with relative ease, other soil matrices (oxide and organic soils) that were capable of significant chemisorption processes were studied. IR and NMR spectroscopic studies of soil humic acids indicated their chemical properties were source-dependent. The commercially available HA was successfully employed to prepare organic coated materials. Adsorption procedures were used not only for the preparation of simulated organic matrices but also for the preparation of metal spiked samples. This provided useful information about surface properties of soils. Adsorption data used for coating methods showed that sea sand was not a suitable matrix for the study of metal extraction from soils. The heterogeneous surface properties of real soils were proven to be much different from synthetic and pure materials.

Optimization of microwave conditions was verified on Cu-spiked samples including Cu-Alumina, Cu-HA-Alumina, Cu-Iron oxide, Cu-HA-Iron oxide, Cu-DCL, and Cu-HA-DCL. The optimized condition obtained from studying these matrices was a 2-step microwave application of 120 W for 5 min followed by 60 W for 10 min. This 15-min exposure time was sufficient to completely remove adsorbed metals from all soil surfaces. High Cu-spiked concentrations as well as natural Cu compositions of soils indicated the effect of EDTA and DTPA concentrations on the extraction efficiency of CAME. There was no significant difference of extraction efficiencies between EDTA

and DTPA at the same concentration by testing on Cu-BSL and Cu-DSC. High oxide containing soils, especially Fe oxide/hydroxide, may be a major factor in the determination of the minimal concentration of a chelating agent essential for CAME. For general aluminosilicate soils, 0.025 M EDTA is sufficient for extracting adsorbed metals, whereas for oxide rich soils, a minimal 0.050 M EDTA is necessary. Sample sizes should be in a range of 0.5 to 1.0 g. The extraction ratio of 0.5 g/10.0 mL of 0.100 M EDTA solution was successfully employed on Cu spiked-oxide soils. It was also found that there was no significant difference in Cu recoveries between oxide and organic coatings. For metal-spiked samples, both Cu and Cd were studied as co-extracted metals. The EDTA-CAME technique achieved 100% recoveries of both adsorbed metals. Metal recoveries from metal-spiked samples were calculated using adsorbed concentrations obtained from the spiking procedures as references. Among different extractants coupled with CAME, EDTA was the most suitable to determine several trace metals such as Cu, Ni, Pb, and Zn. This study was performed on two control soils: BSL and DSC. For this investigation, extraction recoveries of these metals were calculated based upon the total concentrations obtained from the hot acid extraction method. With EDTA-CAME, the maximum extraction capabilities of Cr, Cu, Ni, Pb, and Zn obtained when the control BSL were studied were 20, 65, 70, 82, and 50%, respectively, whereas those obtained when the control DSC were studied were 15, 41, 50, 60, and 35%, respectively.

Working with Cu-spiked samples, the effect of aging/adsorption times was also investigated. A variation of adsorption times from 2 to 240 hours showed significant decrease in the extraction efficiency of the EDTA-shaking method, which provided Cu recoveries in a range of 81 to 75% as adsorption times increased. It was found that the adsorption time had no effect on the EDTA-CAME method, by which complete extraction (96-104%) could be achieved.

To investigate the extraction temperature effect, the conventional EDTA-heating method was studied on different matrices by varying heating times. A hotplate was used as a heat source. With this experimental condition, the solution was boiling after about 10 min. A small increase in metal recoveries could be observed after the solution boiled

longer than 30 min.. At atmospheric pressure (720 mmHg in Blacksburg), the highest temperature was the boiling point of the mixture (about 95-97 °C). It is likely that the EDTA-hotplate heating method may require extraction times longer than 1 hour to quantitatively extract all adsorbed fractions. As evident, temperature is a major factor for chelation extraction.

From the studies conducted in this work, the chemistry of CAME can be explained as follows. First, the heating process starts from the center or inner-layers of the aqueous solution. Working at 2450 MHz, this microwave radiation penetrates into the aqueous target; subsequently, heat is controlled by dipolar molecules. Superheating at temperatures exceeding the normal boiling point by 5 to 10 °C probably occurs. Second, the presence of ionic species such as Na^+ and EDTA species help to induce conductive heating as well as promote the exchange of loosely associated metals in the soils. Lastly, it is due to a function of the properties of soil samples. The extraction system is a mixture of solid and liquid phases. It is thought that the fixed wavelength microwave energy could penetrate into the inner-layers of the soils. The direct interaction between soil surfaces and the applied microwave is possible. It has been discussed that solid samples can provide higher amounts of energy at the surface than the original energy generated from the microwave source.¹² This information indicates that microwave energy can directly promote metal leaching from the target surface.

Studies where soil was heated with $\text{Y}^{4+}(\text{aq})$ only on a hot plate did not promote good recoveries of the sorbed metals. Neither does heating the soil in a Na^+ solution without chelate. It is the interaction of the microwave radiative with the soil that promotes the kinetically controlled exchange of the sorbed metals. This coupled with the chelation reaction allows CAME to achieve a complete recovery of sorbed metals.

It can be concluded that CAME is an effective technique and is an alternative to the conventional EDTA-shaking method for monitoring adsorbed metals in soil samples. The open microwave system is easy to use and is not expensive. Moreover, it can

provide high sample throughput due to reduced extraction time, high precision and is safer than using strong inorganic acids.

APPENDIX A

REDUCTION OF METAL CONCENTRATIONS IN NITRIC ACIDS BY SUB-BOILING DISTILLATION

TABLE A.1: Conditions for Cd, Cr, and Fe determination by GFAAS.

Parameter	Cd	Cr	Fe
wavelength (nm)	228.8	357.9	243.8
Background correction	No	No	No
Modifier	Pd	-	-
Temperature (°C)/Time (sec)			
Drying	120/20	120/20	120/20
Ashing	180/20	1000/30	900/70
Atomizing	1500/1.5	2400/6.0	2300/6.0
Cleaning	2500/2	2500/2	2500/2

TABLE A.2: Metal concentrations (ppb) in nitric acids. Data are reported as mean value \pm standard error.

Acid/Metal	Cd ^(a)	Cr ^(b)	Fe ^(c)
Starting HNO ₃	6.5 \pm 0.2	13.34 \pm 0.09	1400 \pm 120
Redistilled HNO ₃	4.5 \pm 0.3	0.75 \pm 0.03	210 \pm 120

^(a) Determination performed using standard addition ($n = 3$) with a 1:100 dilution of the acid.

^(b) Determination performed using working curves ($n = 3$). No dilution of acid required.

^(c) Determination performed using standard addition ($n = 4$) with a 1:200 dilution of the acid.

APPENDIX B

CALCULATIONS OF METAL CONCENTRATIONS

B.1 Metal extractability from natural soils

$$\mu\text{g-extracted metal/g-soil} = \frac{CV}{W}$$

where

- C = metal concentration in the final extract ($\mu\text{g/L}$)
- V = volume of the final extract (L)
- W = weight of a soil sample (g)

B.2 Percent recovery based on adsorption/spiking method

$$\% \text{ Extraction recovery} = \frac{CV[1 + (A \times 10^{-6})]}{AW} \times 100$$

where

- C = metal concentration in the final extract ($\mu\text{g/L}$)
- V = volume of the final extract (L)
- W = weight of a metal-spiked sample (g)
- A = metal adsorption capability ($\mu\text{g/g}$)

APPENDIX C

ADSORPTION CHARACTERISTICS OF HUMIC ACID

C.1 Application of Langmuir isotherm model for HA adsorption

$$\frac{C_{eq}}{X_a} = \frac{C_{eq}}{X_m} + \frac{1}{AX_m}$$

- where
- C_{eq} = an equilibrium concentration ($\mu\text{g-HA/g-substrate}$)
 - A = the binding constant depending upon surface properties
 - X_a = the adsorption density as a function of initial concentration ($\mu\text{g-HA/g-substrate}$)
 - X_m = monolayer or maximum adsorption capability of HA on the surface

With a series of different concentrations of HA, each solid matrix provide HA adsorption characteristics as shown in Table C.1.

TABLE C.1: Adsorption characteristics obtained from the plot of C_{eq}/X_a vs. C_{eq} .

Substrate	Y= mx + b $C_{eq}/X_a = C_{eq}/X_m + 1/AX_m$	R^2	X_m ($\mu\text{g-HA/g-substrate}$)	A
Sea sand	Y= 0.0043x + 0.0718	0.9924	232.2	0.060
Alumina	Y= 0.0009x + 0.0050	0.9987	2071.1	0.361
Bertie sandy loam	Y= 0.0027x + 0.0275	0.9980	361.3	0.143
Iron oxide	Y= 0.0001x + 0.0017	0.9993	8208.6	0.073
Davidson silty clay	Y= 9E-05x + 0.0027	0.9922	13154.7	0.100
Davidson clayey loam	Y= 8E-05x + 0.0008	0.9997	11012.7	0.034

APPENDIX D

DIELECTRIC PROPERTIES OF SOME SOLVENTS

TABLE D.1: Dielectric properties of common solvents at 3×10^9 Hz.^{12, 14}

Solvent	Dielectric constant ϵ'	Dielectric loss ϵ''	Dissipation factor $Tan\delta = \frac{\epsilon''}{\epsilon'}$
Ice	3.2	0.0029	0.0009
Water	76.7	12.04	0.157
0.10 M NaCl	75.5	18.12	0.240
Methanol	23.9	15.30	0.640
Ethanol	6.5	1.62	0.250
<i>n</i> -Propanol	3.7	2.48	0.670
<i>n</i> -Butanol	3.5	1.6	0.457
Ethylene glycol	12.0	12	1
CCl ₄	2.2	0.0009	0.0004
Heptane	1.9	0.0002	0.0001

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VITA

Urai Chatreewongsin was born in Nakhon Nayok, Thailand. In 1987 she received a B.S. (Hons) degree in chemistry from Chiang Mai University located at the northern part of Thailand. She began her industrial career as a chief of quality control at Siam Algae Co. in May 1987. In 1989, she returned to Chiang Mai University and received a M.S. degree in analytical chemistry on April 1992. Afterwards, she worked for TOA Chemical Industrial Co. as a R & D supervisor about five months before she started her teaching career at King Mongkut's Institute Technology Thonburi (King Mongkut's University of Technology Thonburi), in Bangkok, on November 1992. She transferred to Chiang Mai University in November 1994. In 1995 she got a good opportunity to pursue a Ph.D. degree in chemistry at Virginia Polytechnic Institute and State University. The requirements for her Ph.D. were completed at Virginia Tech in March 2000 under the direction of Dr. Gary L. Long and Dr. Harold M. McNair.