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**STUDIES IN CHEMICAL SPECIATION OF Cu(II), Pb(II), Cd(II),
Zn(II), AND Ni(II) IN AQUEOUS ENVIRONMENTAL SAMPLES**

by

Jamaluddin

M.Sc. (Aligarh.M.University, India)

A thesis submitted to the Faculty of Graduate Studies and Research

in partial fulfillment of the requirements for the degree of

Master of Science

Department of Chemistry

Carleton University

Ottawa, Ontario

July 2003

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Jamaluddin

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A handwritten signature in black ink, appearing to be 'M. B.', written over a horizontal line.

Chair, Department of Chemistry

A handwritten signature in black ink, appearing to be 'S. Chakravarti', written over a horizontal line.

Thesis Supervisor

2003, Carleton University

Abstract

Ion-Selective Electrode (ISE) Potentiometry is the only analytical technique that can directly determine the activity of free metal ions. Here, using copper-ion buffers, we have developed an ISE potentiometric method for direct determination of free-copper-ions in soil solutions (pore waters). Using copper-Ion Buffers, the copper ISE was calibrated down to free-copper-ion activities as low as 10^{-15} mol/L, provided that the total concentration of copper in the sample was equal to or above the limit of detection ($\sim 10^{-5}$ mol/L) of the ISE potentiometry of copper. Ion-Selective Electrode Potentiometry using copper-ion buffer was applied for the determination of free copper ions in 17 samples of soil solutions (pore waters) with a wide range of total dissolved copper concentration. All the determinations were done at a constant ionic strength, pH, and temperature (22°C).

Kinetics of metal complexes in model solutions of a well-characterized humic acid (L.J. Evans humic acid) and in freshwater samples from Sudbury, Ontario, were investigated. Competing ligand exchange methods along with Graphite Furnace Atomic Absorption Spectrometry was used to investigate reaction kinetics in the bulk solution. The analytical timescale of measurement was identified as the key factor for defining metal species and predicting bioavailability.

Acknowledgements

I would like to acknowledge and thank all persons who have influenced me in the course of this research. First, I express my gratitude to my supervisor Professor C.L.Chakrabarti for his support, guidance and encouragement during my thesis work.

I would also like to thank all the other members of Chak's research group, past and present, for making my time at Carleton university an experience of a lifetime: Dr.John Murimboh, Nouri M.Hassan, Rupasri Mandal, Professor Idrees Al-Momani, Jeff Guthrie, Cathy Burroughs, Amina L.R.Sekaly, M.Moniruzzaman, Farhad Raoufi, Mohammad Salam, Mufida Ben Younes, Lin Si, Joya(Rong) Wang, Tahir Yapici, K.Hyat; Ismail Al-Fasfous.

I am truly grateful to my family; in particular, to my parents for their consistent support.

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Chapter 1 Introduction

1.1 Scientific Background

Chemical speciation plays an important role in determining transport, fate and bioavailability of metals in the aquatic environment. The knowledge of whether an element exists in or adsorbed on a particulate or colloidal system, or in a soluble state is vital to the understanding of its behaviour. For example, the free cupric ion activity in filtered seawater was reported to show direct correlation with the toxicity to phytoplankton [1]. Trace metals in freshwaters exist in a variety of forms: free metal ions, oxyhydroxides, metal-organic and organometallic complexes-each with their own specific properties. It is now well established that determination of the individual physicochemical forms (chemical speciation) of the metal, is required for understanding the role of trace metals in natural waters in human health. Knowledge of the metal distribution among different chemical forms is, therefore, essential for predicting the potential beneficial or adverse environmental impacts.

Properties of metals such as mobility, bioavailability, and toxicity depend mainly on the chemical form of the metal (i.e. chemical speciation) rather than on their concentrations. In freshwaters, only a small portion of the overall dissolved metal may be present as free hydrated cations, since metal ions form stable complex with a large variety of inorganic and organic complexants, especially, organic complexants such as humic substances which play an important role in the speciation of trace metals in natural waters. It has now been firmly established that the chemical form of the metal (chemical speciation), not the total metal concentration, determines its bioavailability. Therefore, it is important to understand the speciation of metals in freshwaters if we are to understand

the complexity of freshwaters. The overall goal of environmental metal speciation studies is to provide the data required for environmental impact assessment of metals by determining their speciation, distribution, properties, and fluxes. The impact assessment of the trace metals in the natural environment is ultimately concerned with how these metals directly or indirectly affect biota. Some of the trace metals are essential nutrients for organisms in the aquatic environment at very low levels of concentration, but are toxic at slightly elevated levels. Dissolved Organic Carbon (DOC) plays an important role in the regulation of the trace metals in the aquatic environment. The fate and transport of trace metals is partially controlled by the DOC. Because the coordination equilibrium is slow, freshwater systems are usually far removed from chemical equilibrium, and require kinetic techniques for metal speciation. For freshwater systems, kinetic techniques for metal speciation cannot only differentiate chemical species in the system at any time during the biogeochemical processes it may undergo. The results obtained can be used to estimate the metal bioavailability if a kinetic model can be constructed to represent the process of biological uptake, and to evaluate the best predictive parameters.

1.2 Objectives

The objectives of this research were to conduct an experimental determination of the chemical speciation of copper, lead, cadmium, zinc, and nickel in model solutions of well-characterized humic acid and in aqueous environmental samples. Free metal ions were determined by Ion-Selective Electrode Potentiometry, and the kinetic speciation of the metals was determined by Competing Ligand Exchange/Graphite Furnace Atomic Absorption Spectrometry.

1.3 Organization of the Thesis:

This thesis is divided into five chapters. Chapter 1 gives a brief introduction including the scientific background, objectives, and organization of the thesis. Chapter 2 describes metal-complexation properties of humic substances as naturally occurring complexing agents in freshwaters. Chapter 3 deals with the Ion-Selective Electrode Potentiometry for determination of free-copper-ion activity in aqueous environmental samples. Chapter 4 deals with the kinetic speciation of Cu(II), Pb(II), Cd(II), and Ni(II) in model solutions of these metals in a well-characterized humic acid by the Competing Ligand Exchange Method/Graphite Furnace Atomic Absorption Spectrometry. Chapter 5 deals with kinetic study of dissociation of Cu(II), Pb(II), Cd(II), Ni(II), and Zn(II) complexes in freshwater samples using the Competing Ligand Exchange Method/Graphite Furnace Atomic Absorption Spectrometry.

**Chapter 2 Humic Substances As Naturally–
Occurring Complexing Agents For Metals
In Freshwaters**

Natural Organic Matter (NOM) in natural waters is both ubiquitous in the natural environment and extremely variable in its functional, electrolytic, and conformational properties. The NOM composition is highly complex, and because of this, there is still much to discover. NOM constitutes all organic matter in the natural environment, both aquatic and terrestrial, excluding living organisms and man made compounds.

NOM can be classified into different categories[Figure 1].Each type is different in that it is formed from a different process and will therefore have slightly different chemical and physical properties. The most important types of NOM for freshwaters are :

- (1) Pedogenic NOM is due to rain water leaching of soil NOM, which in turn results from the decomposition of higher plants by bacteria and fungi.
- (2) Aquagenic NOM is that formed in the water column itself, essentially by excretion and decomposition of plankton and aquatic bacteria.
- (3) Sedimentary NOM results from accumulation and transformation in sediments of organisms debris and aquatic NOM.

For simplification, natural organic matter divided into two groups: non-humic substances and humic substances. Non-humic substances include compounds that have well known chemical characteristics. These are predominantly polypeptides and polysaccharides but also include common organic acids, aminoacids, carbohydrates, tannins, resins and other low molecular weight organic substances. Humic substances include humic acid and fulvic acid.

Figure 1 The classification of the natural organic matters according to its origin and solubility [2].

Metal complexation with naturally-occurring complexants have been reported for a variety of aquatic environment. The activity of trace metals in aquatic systems and their impact on aquatic life depends on the metal species which are formed. The study of metal binding by naturally organic complexants is of primary importance in understanding their behaviour and fate in aquatic systems [3,4]. One such category of ligands is humic substances (i.e. humic acid and fulvic acid), which arise from the chemical and biological degradation of plant and animal residue.

Humic substances are ubiquitous in freshwaters and contain a mixture of different molecular weights (i.e. polydisperse). Humic substances are complex and are chemically and physically macromolecular, heterogeneous, polyfunctional, polyelectrolytical, organic complexants (Figure 2). Two major types of functional groups are of particular importance: Carboxylic and phenolic groups [5], due to their diverse functional groups, the humic substances behave as heterogeneous ligands. While these are important for metal binding, they may be part of larger moieties such as phthalate and Salicylate groups that are able to form chelate rings with the metal ion. Figure 2 shows a tentative structure of humic acid that contain some of these groups.

Organic substances are ubiquitous in the freshwater environment. The nature and amount of these materials in natural waters arise from a number of complex processes including degradation of terrestrial and aquatic biomasses, leaching of soil by rainfall, chemical and biological activities and entrainment by aerosols [6]. The concentration of

dissolved organic carbon (DOC) in typical streams and rivers ranges from 2 to 10 mg/L but can be as high as 50 mg/L in coloured waters [7].

A number of important properties for humic substances are listed below:

Polyfunctionality: Each molecule can contain a number of different functional groups. Humic and fulvic acids primarily have carboxylic or phenolic groups, but moieties bearing sulphur and nitrogen are also known.

Polydispersity: Humic substances as a class of compounds represent a wide variety of molecules with differing molecular masses.

Polyelectrolytical properties: Each individual molecule carries a large number of functional groups containing ionisable moieties, such as carboxylic acid group (COOH) and phenolic acid group (OH). A tentative structure of humic acid is given in Figure 2.

These factors make metal binding with humic substances highly complex and variable depending on experimental conditions such as ionic strength and pH, and the concentration ratio of the metal ion and the humic ligand. These variables make the selection of binding models difficult.

Two types of binding sites are present on humic substances [8] : minor sites (~1-10% of the total sites present) composed of strongly binding functional groups; and major sites (~90-99% of the total sites present) composed of weakly-binding functional groups. When metals are added to freshwaters, they first occupy the strongly binding (minor) sites of the humic substances, forming strong complexes, which are inert. After all the

Figure 2 A tentative structure of humic acid [9].

strongly binding sites are occupied, the remaining metals then bind to weakly-binding (major) sites, forming weak complexes which are labile.

Although humic substances can bind to virtually all transition metals including the lanthanides and actinides, and the alkaline earth metals, the strength of the binding depends greatly on the identity of the metal. Humic materials have been found to bind more strongly to softer metals such as Pb and Cu rather than hard metals such as Zn and Ca. Ionic strength and pH are also determining factors in the binding of the metal to the humic substances.

In the natural aquatic environment, chemical species of the metal may include [10], simple ionic species, inorganic complexes, organic complexes, lipid -soluble complexes, organometallic species, metals absorbed on colloidal particles, and metals absorbed or assimilated by living organism. The abundance and distribution of metals in the environment, their accumulation by organisms, their bioavailability and their toxicity to terrestrial and aquatic organisms, including humans, depend on the metal speciation.

Two general pathways of assimilation of dissolved metals species by microorganisms can be distinguished. Some metal complexes that are small and hydrophobic may cross cell membranes directly. However, many large, hydrophilic complexes must first dissociate before they are incorporated into the cell by transporter ligands or protein channels. Figure 3 presents a conceptual model of metal-organism interactions in the natural environment.

Figure 3 Schematic diagram of the assimilation of metals (M) by aquatic organisms. L_1 is the natural ligand, L_2 is the transporter ligand, P is a membrane protein channel, and M_a is a metal ion absorbed on a site that will enable transport through the protein channel. Adapted from Wood [11].

Determinations of trace metal speciation in natural waters are based on the non-reactivity of the metal-species in chemical or biological assays; this nonreactivity is generally attributed to metal complexation by dissolved organic ligands [12]. The heterogeneous nature of DOC makes it necessary to fractionate it into a number of operationally defined chemical classes. The fractionation procedure on XAD resin and cation exchange resins is the most common and acceptable method of separating DOC constituents. The humic fraction is a generic term used for the group of complex, high molecular weight organic compounds which are dissimilar to the biopolymers of microorganisms and higher plants (including lignin). The humic substances are fractionated on the basis of their solubility. The fraction which is not soluble in acid or base (called humin) is separated from the dissolved fraction. This dissolved fraction is then acidified to give a precipitate referred to as humic acid (this fraction is base soluble). The fraction that stays in solution is named as fulvic acid (this fraction is acid soluble). These two fractions are the most important in metal ion binding as they contain the most number of functional groups. The fulvic acid fraction is especially important as it typically comprises ~ 50% of the total DOC [13].

Aquatic humic substances are frequently responsible for the regulation of trace metal speciation in freshwaters. The majority of chemical reactions that occur between metal cations and ligands can be classified as complexation reactions. The complexants in the aquatic environment can be divided into two groups: simple and heterogeneous complexants, based on their structural complexity; examples are: citric acid is a simple complexant, whereas, humic and fulvic acids are heterogeneous complexants.

There are two approaches for studying chemical speciation: equilibrium and kinetic. Most of the chemical speciation studies in aquatic systems have been done with the assumption that all chemical reactions in the system have attained equilibria, and the distribution is controlled by equilibrium constants [3,14,15,16]. The distribution of metal ions in the freshwater environment is extremely complex and is governed by a variety of reactions that include complexation with organic and inorganic ligands, ion exchange, adsorption and desorption processes, precipitation and dissolution of solids, and acid-base equilibria [17]. Through adsorption on solid, organic complexants (e.g humic acid), and complex-formation in solutions, multimetals and multiligands in freshwaters constitute a sort of interlocking chemical network, which is a part of chemostasis [18]. Freshwater systems, however, are often far removed from equilibrium and require kinetic approaches to determine their chemical speciation [3]. A quantitative description of chemical speciation of metal ions in freshwaters in terms of various chemical species cannot be considered complete without adequate characterization of the rates of chemical reactions [19,20]. Furthermore, many metal complexes in natural waters dissociate slowly, and equilibrium conditions may not be established during the time of the experiment [21]. Coordination equilibrium of trace metal complexes of humic substances in soils and freshwaters are typically slow, making distributions of trace metals among various thermodynamically possible chemical species kinetically controlled. Slow coordination equilibrium of trace metals in freshwaters may not be attained in analytical time scales, especially the very-short instrumental response time.

The kinetic approach assumes that each species has its own chemical reactivity, which means its own formation and dissociation rate coefficients. Kinetic studies of metal speciation not only can differentiate chemical species according to their kinetic parameters, but also can give information on the distribution of the chemical species in the system at any time during the kinetic process. The results obtained can, therefore, be used to estimate the bioavailability of metal if a kinetic model can be constructed to represent the process of biological uptake. For non-equilibrium systems, kinetic speciation would give a more realistic description of bioavailability of trace metals than metal speciation based on local equilibrium approximations. Freshwaters are generally non-equilibrium systems, in which slow coordination equilibrium results in non-equilibrium distributions of metal species even though some other reactions may reach equilibrium rapidly, providing partial or local equilibrium for these particular reactions.

The importance of kinetic studies is being increasingly realized and in the last few years many studies have been reported. However, there is little information on the kinetics of reactions of metal complexes in freshwaters. This is mainly due to the complexity of naturally-occurring organic complexants in freshwaters. Metal-exchange reactions involving such complexants are difficult to study and very few estimates of dissociation rate coefficients of metal complexes have appeared in the literature. Among the kinetic studies reported is the dissociation of Fe(II)-natural organic matter (NOM) complexes in lake waters without previous NOM fractionation [22]. Different data interpretation models have been reported in the literature: simple dissociation constants [23], discrete multicomponent models [22,24,25,26] discrete kinetic spectra [27],

continuous kinetic spectra [28], and log normal distribution [27,29]. Hering and Morel [30] have studied the rates of ligand exchange reactions of copper-nitrilotriacetic acid complexes and copper-humate complexes. In their work [30], they have found that the rate coefficients of ligand exchange are pH-dependent, and depend not only on the properties of the humic substances but also on the experimental conditions.

In freshwaters, metals are present as either aqueous M-L complexes (where M is the metal ion and L is any ligand except water) or as free metal. Free metal ions; however, do not exist simply as uncomplexed species, since they (as Lewis acids) are always coordinated with the surrounding water molecules (as Lewis base) which can be represented as $M[H_2O]_6^{n+}$, where n is the charge on the metal ion. Because of this coordination, all complexation reactions in water are Double-(Ligand) Exchange Reactions [37], which involve the kinetics of Double-Exchange Reactions.

When metals are bonding to natural organic matter, or other multidentate ligands, the metal may bind to more than one site and form a chelate. For this to occur, the coordination number of the metal must be larger than one, and in the case of transition metals, the common coordination numbers are 4 and 6 corresponding to tetrahedral or square planar geometry for 4 coordinate complexes, and octahedral geometry for 6 coordinate complexes. The more sites the metal is bound to, the stronger the complex. Chelation is also dependent on the metal radius and charge. A metal that is too large or too small will result in a non-ideal bite angle (the separation of the two donor atoms in a chelate ring) which will increase the ring strain and decrease the strength of the bond.

The strengthening of complexes that are chelated is known as the chelate effect, which is essentially an entropy effect for transition metal chelates.

In order to study the interactions of dissolved organic carbon (DOC) with trace metals in freshwater, to predict their impact on water quality, one needs to determine the chemical speciation of metal-DOC complexes using a multiplicity of speciation techniques to cover the entire range dissociation rate coefficients of the metal complexes and the various characteristics of these metal complexes vis-à-vis the analytical techniques used to measure them. Since the entire range covers about 16 orders of magnitude which is far too large to cover by any technique, the practical alternative is to cover the range of dissociations rate coefficients (assuming first-order kinetics) the most-commonly-encountered dissociation rate coefficients (10^3s^{-1} to 10^{-6}s^{-1}) – a span of nine orders of magnitude which requires adoption of several speciation techniques having complementary analytical windows. Results from these techniques should be compared with metal uptake by organisms to assess the relevance of speciation measurements to bioavailability and toxicity and to evaluate the best predictive parameters

Various methods are available for the studies of metal speciation and chemical equilibria in natural and soil waters. The criteria of a successful analytical technique for these studies are: (a) The technique must be sensitive enough to detect metal ions at the concentrations present in natural waters. (b) The techniques must be free from interferences or the interferences be well- defined, readily identifiable and amenable to correction. (c) The results obtained must be easily interpretable in terms of the possible

metal species, such as free metal ion, organic complexes, and colloidal species containing the metal ion etc. Among the more promising, sensitive and commonly used techniques are: Ion-Selective Electrodes Potentiometry, Rotating disk electrode, Competing Ligand Exchange Method using Graphite Furnace Atomic Absorption Spectrometry and Voltammetry with Hanging Mercury Drop Electrodes.

Ion Selective Electrode (ISE) Potentiometry offers a direct electrochemical technique to determine free metal ion activity in natural waters. If the activity can be equated to the free metal ion concentration (by adopting constant ionic strength using buffers), then the ISE can be used to distinguish between free metal ions and complexed species. The usefulness of ISE for chemical speciation and chelation measurements has been widely recognized. Reliable data from ISE measurements have been accumulated by many research groups [31-33].

The problem with the ion selective electrode is inadequate sensitivity. Its lowest limit of detection is about 10^{-5}M , whereas the upper limit of metal concentrations in unpolluted freshwaters is 10^{-7}M . The electrodes are also subject to interferences from sulphide and iron, for example. Adsorption of surfactants onto the membrane may also be a problem in natural waters although Buffle et al [32] did not find any adsorption of HA and FA on Cu, Pb and Cd ion selective electrodes.

In freshwaters, equilibrium assumption for transition metal complexation are always hazardous. In freshwaters, coordination equilibrium of transition metals, especially

involving Double Exchange Reactions, are typically slow in systems containing mixtures of strong and weak ligands and high concentrations of alkaline earth metals or mixtures of competing transition metals, such as are found in freshwaters, especially those receiving inputs from effluents containing nickel, copper (especially copper). Nickel ores are usually associated with copper. The complexation kinetics of d^8 Ni (II) is known to be slow in water because of the unfavourable changes in Ligand Field Stabilization Energy (LFSE) during complex formation [34]. This is in contrast to other divalent 3d transition metals [35,36]. The slow nickel complexation kinetics is likely to influence its speciation in natural systems and to affect interaction of other metal ions with ligands.

2.1 Hypothesis

The hypothesis to be tested is that

1. As the $[Cu^{2+}]/[HA]$ mole ratio increases, that is, as the concentration of HA in the model solutions decreases, the strong sites present on the HA are saturated and the remaining copper is then bound to weak site, producing weak complexes that are labile. These labile complexes are comprised of small inorganic complexes as well as free Cu^{2+} ion (the aqua complex, i.e. $[Cu(H_2O)_6]^{2+}$).
2. Using metal ion (cupric ion) buffers the electrode can be calibrated down to cupric ion activities as low as $10^{-13}M$, provided that the total concentration of copper species is maintained at above $10^{-6}M$.

2.2 Theoretical background [37]

Reaction kinetics are important when considering the role of metals in freshwaters since the rates of formation of M-HS complexes and the dissociation of these complexes may determine the speciation of a metal species, which in turn, determines its

bioavailability. Factors that affect the speciation of freshwaters are pH, ionic strength, and major ion concentrations (e.g. Ca^{2+} and Mg^{2+}). Such changes can occur because of natural movement of waters, natural process by biota, and of anthropogenic sources.

The rates of metal-ligand reactions in freshwaters are generally consistent with the following mechanism:

where K_{os} is the outer sphere stability constant, and k_{-w} is the water loss rate coefficient. K_{os} is dependent on the charges of the reacting species and the ionic strength of the medium, and k_{-w} is characteristic of the reacting metal. The rate determining step is the loss of water as the complexation reaction is usually fast. The rate constant for the complex formation, k_f can be written as:

$$\frac{d[\text{ML}^{(2-n)+}]}{dt} = k_f [\text{M}^{2+}] [\text{L}^{n-}] \quad (3)$$

where

$$k_f = K_{\text{os}} k_{-w} \quad (4)$$

The overall reaction for metal and ligand exchange reactions can be expressed as the following:

The rate expression for this reaction can be expressed as

$$\frac{-d[ML]}{dt} = k_{ex} [ML][X] \quad (5)$$

This reaction can either proceed via a disjunctive mechanism, where the complex first dissociates before the second metal or ligand reacts, or by an adjunctive mechanism, where the initial complex goes through a transition state where the second metal or ligand coordinates to the initial complex before dissociation occurs. These two pathways can be summarized by the following equations for a metal exchange reaction, and analogous equations can be written for ligand exchange reactions.

The adjunctive pathway is dependent on the ligand structure since the formation of the M^*LM complex or the dissociation of the transition state can be rate limiting, and the disjunctive pathway is dependent on pH and ligand structure.

Since all exchange reactions are double-exchange reactions (i.e. all metals are initially bound to 6-coordinated water molecules), the kinetics must take this into account. A double exchange reaction which accounts for simultaneous metal- and ligand-exchange reactions can be expressed as

and can be either ligand- or metal-initiated. The reaction is disjunctive with respect the ML complex, and can be either adjunctive or disjunctive with respect to the incoming ligand or metal. This condition will depend on whether the initial M^*L^* complex is strong or weak. If it is strong, the pathway will most likely be adjunctive, and if it is weak, it will be disjunctive. In many cases, a ligand-initiated pathway which results in the replacement of one ligand with another, often involves chain reactions, where the initial ligand reacts further with the initial ML complex. This may strongly affect the rates as these interactions may promote or terminate the mechanism.

2.3 *Metal Speciation Techniques*

Ion-Selective Electrode (ISE) Potentiometry is the only analytical technique that can directly determine free metal ion activity; all other analytical techniques measure concentration, not activity. ISE potentiometry is a promising technique, but the current detection limit of ion-selective electrode potentiometry is about $10^{-5}M$, which is not sufficient for environmentally relevant concentrations. Although ion-selective electrodes have excellent sensitivity for free metal ion activities, the measurements require fairly high total metal concentrations ($\geq 10^{-7}M$) usually in excess of those present in natural waters [38]. Moreover, measurements cannot be made for some metals in high-chloride media such as seawater [39]. The more promising, sensitive and commonly-used techniques in chemical speciation provide results that are operationally-defined; for example-“ASV-labile”, which is the amount of metal that is available by Anodic Stripping Voltammetry[40], and “chelex-labile”, which is the amount of metal that can be taken up by chelex 100 chelating resin. Another example is the operationally-defined fractions obtained by Sequential Solvent Extractions [41].

**Chapter 3 Ion-Selective Electrode Potentiometry for
determination of free-copper-ion activity
in freshwaters and soil solutions (pore
waters).**

3.1 Introduction

Determination of free metal ion activity in soil systems is important for studies of metal solubility, mobility, exchange and retention reactions on soil particles, and also for investigation of metal speciation and “bioavailability”[42]. Metals in soil systems directly affect metals in vegetation and agricultural crops, and also metals in the aquatic environment. Copper is an essential element that is toxic at elevated concentrations. The reactivity and biological uptake of copper are strongly influenced by its free ion activity rather than by total concentration. Solubility, retention and speciation of copper in freshwaters and soil solutions (pore waters) are controlled by the solution: its composition, content of organic matter, dissolved organic ligands, inorganic ligands, ionic strength and pH [43-47]. Ion-Selective Electrode (ISE) Potentiometry is the only analytical technique that can determine free metal ion activity; all other analytical techniques measure concentrations, not activity. ISE Potentiometry is a promising analytical technique and has wide linear dynamic range, typically 4-6 orders of magnitude, and is non-destructive. However, the currently achievable detection limit of ISE is about 10^{-5} M, whereas what is needed for environmental samples is a detection limit which is very much lower, e.g. 10^{-15} M. The cause of the poor detection limit is unknown. However, whatever may be the cause, the detection limit is ultimately set by the solubility of the electrode (sensor), and the only way to extend the detection limit to much lower activity levels is to use insoluble material as the electrode (sensor). Among the insoluble material for sensor are sulphides of some transition elements. If the sensor is made of a mixture of two sulphides of widely different solubilities, such as Ag_2S ($K_{\text{sp}} = 10^{-51}$) and MS (where M is a divalent analyte metal such as Cu^{2+} , and S stands for

sulphide) and, provided $K_{sp}(Ag_2S) \ll K_{sp}(MS)$ [48], then the coupling of dissociation equilibria of Ag_2S and MS will yield a very low M^{2+} activity. The K_{sp} (CuS) will determine the amount of Cu^{2+} that will remain as free Cu^{2+} to satisfy the CuS solubility equilibrium. Since this constant concentration of Cu^{2+} (arising from the solubility of CuS at a given temperature) increases the Cu^{2+} concentration of the sample by this constant concentration of Cu^{2+} , for reasonable accuracy, the ratio of the former to the latter must be as small as possible. This condition sets the lowest limit of Cu^{2+} concentration that can be determined with reasonable accuracy using the mixed sulphides sensor by ISE Potentiometry. Electrode selectivity is related to the solubilities of the metal sulphides. Thus, both Hg^{2+} and Ag^+ (because the K_{sp} of HgS and $Ag_2S \ll CuS$) interfere with the Cu ISE even if present in trace concentrations, whereas lanthanides, alkali metal ions or alkaline earth metal ions hardly interfere at all. The poor detection limit ($10^{-5}M$) of the conventional ISEs is also linked to our inability to prepare accurately-known analytical calibration standards of very low concentrations of metal ions by serial dilution of stock solutions because of adsorption and desorption of cations on and from the container walls. The above shortcomings can be eliminated by using Metal-Ion Buffers as analytical calibration standards. In this case, the parent compound is at such high total concentration that loss or gain of the metal ion by adsorption or desorption on or from the container walls is compensated by equilibrium shifts. However, the lowest metal ion activity that such Metal-Ion Buffer can yield is limited by the strength of metal complex formed, which, for a given metal, is determined by the choice of the complexant (complexing agent) and the pH.

Risk assessment of metal-contaminated soils should take into account the metal bioavailability. Only a small portion of the total metal burden of soils is actually bioavailable [49]. Free copper activity has been shown to be the main bioavailable species of copper in aquatic systems [50-52]. The free copper (II) activity, i.e. the concentration of free cupric ions corrected using Debye-Huckel Theory activity coefficient, is reported as pCu^{2+} , which, for practical purposes, is taken as the negative $\log_{10}$ of the molar concentration of Cu^{2+} ions. pCu^{2+} is unitless.

3.2 Metal-Ion Buffer: *The importance of selection of a complexant that gives high metal-ligand formation constant (stability constant)*

Taking the preparation of Cu^{2+} ion as an example of Metal-Ion Buffer, one can write the following equations using nitrilotriacetic acid (NTA) as the complexant and a pH high enough (≥ 6) for NTA to be in its fully basic form (NTA^{3-}). At a given temperature:

$$K_f = \frac{[CuNTA^-]}{[Cu^{2+}][NTA^{3-}]} \quad (2)$$

where K_f is the formation constant (stability constant) of $CuNTA^-$ complex.

$$[Cu^{2+}] = \frac{[CuNTA^-]}{K_f[NTA^{3-}]} \quad (3)$$

The last equation emphasizes the importance of a high value of K_f in preparing standard solutions containing very low concentrations of the metal ion. The metal-ion buffering effect (analogous to acid-base i.e. pH buffering effect) is exerted by the ratio of the metal complex and the ligand concentrations. As with pH buffering, the best

buffering effect in metal-ion buffering occurs when adding of metal ions to the test solution makes no perceptible change in its pM.

In soils which are highly contaminated with metals, precipitation-dissolution equilibrium relationships may control the activity of free Cu^{2+} in soil solutions. At high copper concentrations, copper activity is also influenced by retention and cation exchange reactions. However, in cases where free Cu^{2+} in soil solutions is very low, the copper activity is probably controlled by specific adsorption mechanisms [53].

In soils, most of the metal is insoluble, but part is bound reversibly to the soil particles. Part is soluble and a portion of the metal ions in solution is bound to various ligands such as Cl^- , SO_4^{2-} , PO_4^{3-} , and naturally-occurring organic complexants. Generally, only a very small fraction of soluble Cu is actually present as the free Cu^{2+} ion species in soil solutions [54]. The potential impact of soil characteristics such as pH and organic matter on the copper speciation in soils must be established in order to correctly evaluate the bioavailability and toxicity of copper.

3.3 Objective

To develop and validate the Ion-Selective Electrode potentiometry for quantitative determination of free copper ions at extremely low activities in soil solutions (pore waters) and freshwaters.

3.4 Metal-Ion Buffers: Quantitative determination of free Cu^{2+} ion by ISE Potentiometry.

For quantitative determination of free Cu^{2+} ion at very low concentrations, standard solutions of Cu^{2+} ions must be prepared to contain accurately-known concentrations of Cu^{2+} ion. This can only be done using a Metal-Ion Buffer. Such a Metal-Ion Buffer must be based on a metal-complex with a large stability constant; the complexing ligand must be present in large excess and the complex must be water soluble and stable at ambient conditions. The limit of detection is then limited by the choice of the metal-ion buffer, the accuracy of the stability constant, and the pH. The Metal-Ion Buffer that has been chosen for copper in this work is EDTA, which for copper, is known to form a 1:1 complex having a large stability constant [55]. Although there are 6 EDTA species in aqueous solutions, the free metal ion concentration can be calculated using EDTA^{4-} as the complexant and an equation which is analogous to the Henderson-Hasselbalch equation (6).

$$K_{\text{CuEDTA}} = \frac{[\text{CuEDTA}^{2-}]}{[\text{Cu}^{2+}][\text{EDTA}^{4-}]} = 7.9 \times 10^{18} \quad (5)$$

$$\text{pM} = \log K_{\text{ML}} + \log \frac{\alpha_{\text{ML}}}{\alpha_{\text{L}}} + \log \frac{[\text{L}]}{[\text{ML}]} \quad (6)$$

where α_{ML} and α_{L} are defined by the equations:

$$\alpha_{\text{ML}} = \frac{[\text{ML}']}{[\text{ML}]}; \quad \alpha_{\text{L}} = \frac{[\text{L}']}{[\text{L}]}$$

Thus, the α coefficients measure the extent to which the species participate in side reactions (which are function of pH).

The mixed, polycrystalline sulphides (CuS/Ag₂S) are pressed into pellets using mechanical pellettizing devices [48], and after polishing and thorough cleaning the pellets, they are used as the electrode surface. The mixed crystals of CuS/Ag₂S contain polycrystalline Ag₂S membrane in which silver ions are sufficiently mobile to conduct electricity through the solid medium of the pellet.

The transmembrane potential is what ISE measures [56]

$$K_{\text{spAg}_2\text{S}} = \frac{[\text{Ag}^+]^2 [\text{S}^{2-}]}{[\text{Ag}_2\text{S}]} = 8 \times 10^{-51} \quad (9)$$

$$K_{\text{spCuS}} = \frac{[\text{Cu}^{2+}][\text{S}^{2-}]}{[\text{CuS}]} = 8 \times 10^{-37} \quad (10)$$

The Cu²⁺ activity controls the S²⁻ activity in solution, which in turn controls the Ag⁺ activity and the electrode response. The Ag⁺ activity level at the sample/membrane electrode interface, assuming that the sample preparation contains no silver ion initially, is determined by the following two, solution- phase equilibria:

$$A_{\text{Ag}^+}^2 A_{\text{S}^{2-}} = K_{\text{spAg}_2\text{S}}$$

$$A_{\text{Cu}^{2+}} A_{\text{S}^{2-}} = K_{\text{sp CuS}}$$

where A is activity of the species shown as its subscripts.

The solubility of CuS must be much larger than that of Ag₂S. If this were not the case, then it would not be possible to have Ag₂S on the electrode surface, as the reaction

would proceed completely to the right. At the same time, the value of CuS must be sufficiently small so that the level of Cu²⁺ in the sample solution produced by the solubility of CuS is small relative to the levels of the Cu²⁺ which are expected in the sample. Since CuS is much more soluble than silver sulphide, the Ag₂S can be considered as a chemically inert matrix through which Ag⁺ ions are free to move, carrying the electric charge. At the membrane-solution interface, the CuS equilibrates with the sample solution, resulting in a silver ion level given by:

$$A_{\text{Ag}^+} = \sqrt{\frac{K_{\text{sp Ag}_2\text{S}}}{A_{\text{S}^{2-}}}} \quad (11)$$

Substitution into the Nernst equation for a silver ion conducting medium gives the result that mixed sulphide crystals of this type behave as though the membrane were a pure sulphide ion conductor.

In addition to the solubility restrictions, it is also necessary that the sulphides of the solid-phase membrane equilibrate rapidly with the ions of the solution-phase sample in order for the electrode to have a reasonable time response. To make the matter even more complex, the two solid-phase (CuS/Ag₂S) equilibria must also be established. Altogether,

four equilibria must be simultaneously established: two solution-phase equilibria and two solid-phase equilibria. Despite the resulting kinetic complications of the solid-phase equilibria, very skilful preparation of the mixed sulphide crystals ($\text{CuS}/\text{Ag}_2\text{S}$) has yielded commercially-available (Orion Research, Inc.) copper electrodes of reasonably fast response and reliable accuracy and precision.

If Cu^{2+} is added to the sample solution, the $[\text{S}^{2-}]$ of Eq.8 is decreased, resulting in a decrease also in the $[\text{S}^{2-}]$ of Eq 7, because Eq 7 and 8 are coupled. This results in an increase in $[\text{Ag}^+]$ concentration in solution and an increase in the $[\text{Ag}^+]$ concentration on the external surface of the electrode. Since Ag^+ ion is the mobile ion, i.e. it carries the electric charge from the external surface a to the internal surface b of the copper electrode, the increase of $[\text{Ag}^+]$ on the external surface results in the potential of the external surface a to be changed with respect to the constant $[\text{Ag}^+]$ value of internal surface b. The trans-membrane potential difference (E_a-E_b) is the analytical parameter in ISE Potentiometry. The question now arises: what is the minimum $[\text{Cu}^{2+}]$ that is required to produce a minimum measurable transmembrane potential difference $[E_a-E_b]$? As explained later in this chapter, the minimum total concentration of copper required in the sample for its satisfactory quantitative determination by ISE potentiometry is equal to or greater than its detection limit.

The solid-phase equilibrium between Ag_2S and CuS solids depends upon proper dispersion of the two solid phases (Ag_2S and CuS), one into the other, to generate an electromotive efficiency ~ 1.0 . The ART takes over from the SCIENCE in the formation

of optimum grain size of the two solids (Ag_2S and CuS), and their kinetically-controlled precipitation (to obtain crystals of desired properties) and optimum dispersion of one solid into the other. The optimum crystalline precipitates of Ag_2S and CuS are prepared individually, and then mixed to form the mixed crystal. Each of Ag_2S and CuS crystal is formed by precipitation, with careful-controlled rates of precipitation — controlling the RELATIVE SUPERSATURATION (the von Weimarn's ratio) which is maintained low throughout the entire process of precipitation, dissolution, re-crystallization, and digestion of the precipitates to yield high-purity (required for highest sensitivity) crystals of the individual sulphide precipitate [57].

3.5 Experimental

3.5.1 Chemicals and reagents

Stock solutions containing 0.1 M of Cu^{2+} (Orion Research, Inc.) was used to prepare the model solutions. Ultrapure water having a resistivity of $18.2 \text{ M}\Omega \text{ cm}^{-1}$ was used to prepare all standards and test solutions. The ultrapure water was obtained direct from a Milli-Q-plus water purification system (Millipore Corporation). All standard solutions of metals were acidified to make them contain 1% (v/v) acid using HNO_3 (ULTREX II, J.T.Baker, Inc. Phillipsburg, NJ, USA). Standard pH buffers (supplied by Fisher Scientific) were used for calibration of the pH-meter. The pH of the test solutions was adjusted to 5.2 ± 0.1 using 2M NaOH.

3.5.2 *Experimental determination of Cu^{2+} and Ca^{2+}*

Copper

An analytical calibration curve for copper was prepared using the earlier-mentioned copper ion selective electrode (ISE) in order to check its response, linearity and detection limit. The instrument calibration was performed using a series of standard solutions of Cu^{2+} ions. At the beginning, the potentiometer was calibrated using 10 $\mu\text{g/ml}$ and 100 $\mu\text{g/ml}$ of Cu^{2+} standard solutions. This procedure measures the slope of the analytical calibration curve that provides the best means of checking the electrode performance. The difference between the first and second potential reading was found to be 29.0 ± 0.1 mV/decade, which means that the electrode response had the Nernstian slope which is $59/2 = 29.5$ mV/decade. All potentiometric measurements were made at the constant, room temperature, 22°C .

The pH of the model solutions were measured using a Fisher Scientific Accumet 925 pH/ion meter. The pH meter was calibrated using two standard pH buffer solutions (pH 4 and pH 7). The pH of the soil solutions was adjusted to 5.0 ± 0.1 . The pCu was determined using an Fisher Scientific Accumet 925 pH/ion meter and the Orion Model 9629 Combination Reference Design copper ion selective electrode (which incorporates a reference electrode in the copper ion selective electrode assembly). The filling solution (3M KCl) of the above Combination Reference Design electrode was changed daily. Between samples, the Combination electrode was rinsed with deionized water and wiped dry with delicate task wiper tissues. When not in use, the Combination electrode was stored immersed in deionized water. The ionic strength of the test solution was adjusted

by adding Orion ionplus Ionic Strength Adjustor (NaNO_3 and deionized water) before any potentiometric measurement was made.

The model solutions for copper were prepared by adding known volumes of the copper standard solutions. The pH of the model solutions and the test solutions was adjusted to 5.0 ± 0.1 using sodium acetate and acetic acid. The Ionic Strength Adjustor was added to the test solutions to make sure that the test solutions and the standards have the same ionic strength and pH. All aqueous solutions of metal ions are unstable and the metal solutions are preserved as soon as prepared by adjusting the pH of the aqueous solutions of metals to 1.0 by adding HNO_3 (ULTREX II, J.T. Baker, Inc; Phillipsburg, NJ, USA).

Calcium

An analytical calibration curve for calcium was obtained using Ca^{2+} ISE in order to check its response, linearity and detection limit. The calibration was performed using a series of calibration standards made of standard solutions of Ca^{2+} ions. At the beginning, the pH/Ion meter was calibrated using $10\mu\text{g/ml}$ and $100\mu\text{g/ml}$ of the Ca^{2+} standard solution. This procedure measures the slope of the analytical calibration curve that provides the best means of checking the electrode performance. The difference between the first and second potential reading was found to be 29.0 ± 0.1 mV/decade, which means that the electrode response had the Nernstian slope. For the determination of Rideau River surface water samples, the pH of the test solutions was adjusted to 5.2 ± 0.1 using sodium acetate and acetic acid.

3.6 Ion-Selective Electrode Potentiometric Determination of $p\text{Cu}$ in Soil Solutions (Pore Waters)

3.6.1 Materials and Methods

Ion-Selective Electrode Potentiometric method for determination of free copper ions in soil solutions (pore waters) using Metal-Ion Buffers and the above-mentioned copper electrode was applied to 17 samples of soil solution (pore waters) having wide-ranging copper activity levels. All the determinations were done at a constant ionic strength, pH, and temperature (22°C).

3.7 Results and Discussion:

Metal-Ion Buffers are essential for determining free-copper-ion activity at extremely low levels because only Metal-Ion Buffers can be prepared to contain precisely known concentrations of metal ions at extremely low levels of concentrations. Metal standards lower than 10^{-5}M must be prepared in metal-ion-buffered solutions because otherwise the presence of impurities or complexants, uncontrolled hydrolysis, or loss by adsorption onto the container walls would make the measurement very uncertain.

In this section of the thesis which presents the results of the applications of ISE Potentiometry for determination of $[\text{Cu}^{2+}]$, it is necessary to re-emphasize that although ISE Potentiometry measures the activity of the metal ion, the results are here reported as molar concentrations (which are readily measurable) instead of activity which is ‘thermodynamic’ concentration (the molar concentration multiplied by activity coefficient – the latter is mostly not available). In the laboratory, the unknown sample and standards are prepared to contain the same ionic strength by adding the Ionic Strength Adjustor and the same pH. By treating the unknown sample and the test solution

in same manner, i.e. by swamping out the ionic strength of test solution, one analytical calibration curve serves to determine the concentration of a given ion regardless of its original environment.

3.7.1 *pCu values*

The copper ion (Cu^{2+}) activity, A_{Cu} , is related to the Cu^{2+} concentration by the equation:

$$A_{\text{Cu}^{2+}} = [\text{Cu}^{2+}] \cdot f_{\text{Cu}^{2+}} \quad (12)$$

where $f_{\text{Cu}^{2+}}$ is the activity coefficient of Cu^{2+} . A p_{Cu} (i.e. $-\log A_{\text{Cu}^{2+}}$) value calculated from the electrode potential is a function of ionic strength through its influence on Liquid Junction Potential and $f_{\text{Cu}^{2+}}$. At constant ionic strength, p_{Cu} is directly proportional to $-\log [\text{Cu}^{2+}]$. The p_{Cu} -scale used in this Thesis does not pretend to be thermodynamic one, but rather an operational scale, but yields valid and satisfactory value of the copper ion activity in the samples.

Figure 4 shows an analytical calibration curve for p_{Cu} (the linear regression line), which was used to test the electrode response to Cu^{2+} activity in an aqueous test solution. The $[\text{Cu}^{2+}]$ concentration used in the test solution was $6.35 \times 10^{-5} \text{M}$; the $[\text{Cu}^{2+}]$ concentration found was $6.20 \times 10^{-5} \text{M}$, yielding a recovery 97.6%, which was satisfactory. The above determination was repeated three times and close reproducibility in the three results was obtained.

Figure 5 shows an analytical calibration curve for pCu (the linear regression line), which was used to test the electrode response to Cu^{2+} activity in an aqueous test solution. The slope found was 37.0mV/decade which was somewhat greater than the theoretical (Nernstian) slope of 29.58 mV/decade, and was therefore less than satisfactory. The $[\text{Cu}^{2+}]$ concentration used in the test solution was $1.38 \times 10^{-7}\text{M}$; the $[\text{Cu}^{2+}]$ concentration found was $1.34 \times 10^{-7}\text{M}$, yielding a recovery 97.1%, which was satisfactory. The above test was repeated three times and similar results were obtained.

3.8 Effect of total copper concentration in a sample on the electrode response to copper ion activity

It has been experimentally established that the detection limit of this electrode for copper ion is $\sim 10^{-5}\text{M}$. The Cu^{2+} of the test solution controls the sulphide-ion activity in solution, which in turn controls the Ag^+ (which is the mobile ion inside the electrode) activity and the electrode response. The detection limit for copper, by its definition, is the minimum concentration of copper required to obtain an unambiguous experimental detection. For Ion-Selective Electrode (ISE) Potentiometry, the Cu^{2+} in the test solution must be in equilibrium with the total copper in the test solution, and the former must be $\geq 10^{-5}\text{M}$ in order to be detectable. The Cu^{2+} in the test solution must be also in equilibrium with the CuS of the mixed crystals of CuS/Ag₂S on the electrode surface. Besides the solution-phase equilibrium mentioned above, there must be also a solid-phase equilibrium between the above two sulphides, finally linking the solution-phase equilibrium to the solid-phase equilibrium. To satisfy that the above equilibria are established and to define the satisfactory conditions for the working of the electrode, it is necessary to establish the minimum total concentration of copper in the sample required

Figure 4 The electrode response as a function of copper ion activity (pCu). The solid line represents the linear regression line for the experimental data points shown by filled circles. Temperature 295 K.

Figure 5 The electrode response to copper ion activity (pCu) in a soil solution (site#3, lessivzge, parc Aiguebelle), collected from the Rouyn-Noranda area, pH 3.8 ± 0.1 , Temp. 295K. The quantification was made using the Standard Addition Method.

to satisfy the above-mentioned equilibria. This required the following experimental determination using Metal-Ion-Buffers.

3.9 Experimental Determination of Cu^{2+} using Metal-Ion-Buffers

Figures 6, 7, and 8 present the results: electrode response in model solutions containing accurately known copper-ion-activities, prepared by the Metal-Ion-Buffering technique using ethylenediaminetetracetic acid (EDTA) as the complexing agent. Only by using a complexing agent which forms strong complexes with the analyte metal, can one prepare accurately known quantities of the analyte metal of extremely low concentrations. Figures 6 and 7 which represent $[\text{Cu}^{2+}]$ concentrations of 10^{-5}M and 10^{-6}M , respectively, show satisfactory electrode response as a function of copper ion activity, whereas Fig.8 which represents $[\text{Cu}^{2+}]$ concentration of 10^{-7}M , shows unsatisfactory electrode response. This experiment was repeated three times and satisfactory agreement in the results was obtained. The conclusion is that for satisfactory performance of the electrode, the total copper concentration of the sample must be equal to or above the detection limit for copper ($\sim 10^{-5}\text{M}$ - 10^{-6}M) by ISE potentiometry using the $\text{CuS}/\text{Ag}_2\text{S}$ electrode system.

3.10 Limitations on the lowest concentrations of Cu^{2+} that can be satisfactorily determined by ISE potentiometry.

Detection limit (for Cu^{2+} by ISE potentiometry), by its definition, means that at the for Cu^{2+} concentration equal to the detection limit, a Nernstian slope ($\sim 29\text{ mV/decade}$) is observed in the analytical calibration curve for Cu^{2+} ; in other words, the detection limit is the lowest concentration of Cu^{2+} that yields a Nernstian slope in the analytical calibration curve for Cu^{2+} . At the ambient total copper concentration in the sample lower than its

Figure 6 The electrode response as a function of copper ion activity (pCu) in a solution containing total copper concentration 10^{-5}M and Ionic Strength Adjustor. pH 4.3 Temp. 295K.

Figure 7 The electrode response as a function of copper ion activity (pCu), in a solution containing total copper concentration 10^{-6} M and Ionic Strength Adjustor. pH 4.3, Temp .295 K.

Figure 8 The electrode response as a function of copper ion activity (pCu) in a solution containing total copper concentration 10^{-7} M and Ionic Strength Adjustor. pH 4.3 Temp.295 K.

detection limit, non-Nernstian slope is observed because lack of equilibration between the electrode membrane and the bulk solution results in a non-ideal electrode behaviour. This observation has serious implications for the application of ISE potentiometric determination of very low Cu^{2+} concentrations. Since this limitation arises from a fundamental problem of equilibration between the electrode membrane and the bulk solution, it can be confidently predicted that such limitation will apply to all elements that can be determined by ISE potentiometry, not just to copper alone.

3.11 Calcium Ion-Selective Electrode: Testing of the performance of a calcium ion selective electrode for ISE Potentiometry

The following experiments were done using a commercially available calcium ion selective electrode, No 93-20 (purchased from Orion). Calcium is a major cation and can compete with the trace metal ions for binding by dissolved organic matter present in freshwaters. Ca^{2+} ions are generally present in freshwaters at several orders of magnitude greater concentrations (than the ions of trace metals) which outweigh their weaker complexing power. Calcium forms complexes with some inorganic anions such as EDTA. The extent of complexation by Ca^{2+} increases with increasing calcium concentrations and with more alkaline pH and depends on the ionic strength of the medium.

Figure 9 presents an analytical calibration curve (ACC) for Ca^{2+} ion by ISE Potentiometry using a model solution as an 'unknown' solution to determine the performance characteristics of the calcium electrode. The slope of ACC is

31.5mV/decade against the Nernstian slope of $59/2=29.5$ mV/decade and is considered satisfactory. The recovery of Ca^{2+} was found to be 98.8%, which was satisfactory.

Now that satisfactory performance of the calcium electrode in model solutions has been experimentally established, it was decided to test the analytical performance of the electrode in unknown samples of soil solutions (pore waters).

3.12 Effect of Mg^{2+} ions on the calcium electrode response to calcium ion activity

Ion-selective electrodes are subject to a number of interferences including inter-element interferences. Their precision and accuracy can be reduced by presence of interfering ions. Since Ca^{2+} and Mg^{2+} , as major cations are ubiquitous in the freshwater environment, both originating from carbonate (e.g. dolomite) bedrock, and are almost always present together in freshwaters, the quantitative determination of Ca^{2+} by ISE Potentiometry requires a knowledge of the probable interferences of Mg^{2+} with the quantitative determination of Ca^{2+} in freshwaters. Hence, this experiment was done in the laboratory.

Figure 10 shows the effect of addition of 10 ug/mL concentrations of Mg^{2+} to an analyte, 10ug/mL of Ca^{2+} ; no change was observed i.e there was no interference. Similar experiments were done using separately 5ug/mL, 15ug/mL, and 20 ug/mL of Mg^{2+} to an analyte, 10ug/mL of Ca^{2+} ; no interference was observed.

Figure 9 Analytical calibration curve for calcium by Ion-Selective Electrode Potentiometry. pH 5.0 ± 0.1 , Temp. 295 K.

Figure 10 Effect of addition of Mg^{2+} to 10 $\mu g/mL$ Ca^{2+} .

Figure 11 shows an analytical calibration curve (the linear regression line) of Ca^{2+} , which was used to test the electrode response to Ca^{2+} activity in an unknown solution: a through-fall precipitation sample. The $[\text{Ca}^{2+}]$ concentration added in the test sample was $1.15 \times 10^{-5}\text{M}$; the $[\text{Cu}^{2+}]$ concentration was found to be $1.1 \times 10^{-5}\text{M}$, yielding a recovery 95.6 %, which was satisfactory. The above test was repeated three times and satisfactory reproducibility was observed in the results.

Figure 12 shows an analytical calibration curve (the linear regression line), which was used to test the electrode response to Ca^{2+} activity in natural sample of Rideau River surface water. A model solution was prepared by adding $2.0 \times 10^{-3}\text{M}$ of River water to ultrapure water; the concentration by ISE potentiometry was $1.5 \times 10^{-3}\text{M}$, i.e. the recovery was 75%, which was not satisfactory. The above test was repeated three times and the same results were obtained in all the tests. The conclusion was that the interference made the results unacceptable.

Figure 13 shows an analytical calibration curve obtained using the usual laboratory standard solutions of Cu^{2+} for calibration (i.e. without using a Metal-Ion Buffer). The electrode exhibits a Nernstian response, but suffers from very poor limit of detection (pCu 4.0). Figure 14 shows an analytical calibration curve obtained using a Metal-Ion Buffer (EDTA as the complexing agent). The electrode exhibits a Nernstian response, and has a much lower limit of detection (pCu 14).

Figure 11 Determination of calcium ion activity in a throughfall precipitation sample (Abitibi, site#3, Aiguebelle) from Rouyn-Noranda area by Standard Addition Method using Ion-Selective Electrode Potentiometry, pH 3.5 ± 0.1 , Temp. 295 K.

Figure 12 Determination of calcium ion using an analytical calibration curve made of aqueous standards of calcium ions. The test sample was Rideau River surface water.

Figure 13 Analytical calibration curve for copper without the Metal-Ion Buffer, using ISE potentiometry.

Figure 14 Analytical calibration curve for copper with the Metal-Ion Buffer, using ISE potentiometry.

3.13 Determination of copper in soil solutions (soil pore waters) and through-fall precipitation using the CuS/Ag₂S mixed sulphide crystals as copper electrode

Dissolved Organic Carbon (DOC) in soil solutions (soil pore waters) and through-fall precipitation samples are naturally-occurring organic complexants which are mostly interferences in ISE Potentiometry of Cu²⁺. It was therefore necessary to investigate the effect of [Cu]/[DOC] mole ratios on pCu of natural samples. Figure 15 shows a fair correlation ($r^2 = 0.77$) between [Cu] / [DOC] mole ratios and pCu values, considering that several types of aqueous samples have been analyzed (such as, soil solutions and through-fall rain water samples). Table 1 shows the numerical values of the results presented in Figure 15. Table 2 presents the results of Cu²⁺ determination in soil solutions (pore waters) and through-fall precipitation samples.

Although Metal-Ion Buffer must be used in order to prepare accurately known, very low concentrations of a metal ion, its applications to ISE potentiometry requires prior knowledge of the lowest concentrations of the analyte metal ions that are correctly determinable by ISE potentiometry. To determine the above limit of ISE potentiometry we investigated the effect of total analyte metal concentrations on the linearity and the slope of the analytical calibration curve for Cu using Metal-Ion buffer.

Figure 15 Effect of [Cu]/ [DOC] mole ratios on copper ion activity for soil solution samples from Rouyn-Noranda and the Sudbury area.

Table 1 Results: Soil Solutions (soil pore waters) from Rouyn-Noranda and Sudbury area.

*Copper, M	[Cu] / [DOC]	pCu
5.32×10^{-7}	8.35×10^{-4}	10.19 ± 0.01
1.16×10^{-8}	7.45×10^{-4}	10.05 ± 0.04
5.79×10^{-8}	3.83×10^{-4}	11.43 ± 0.03
1.33×10^{-7}	2.92×10^{-4}	11.26 ± 0.05
6.29×10^{-8}	2.87×10^{-4}	11.03 ± 0.01
5.03×10^{-8}	2.62×10^{-4}	12.36 ± 0.02
5.00×10^{-8}	9.34×10^{-5}	12.01 ± 0.03

*Determined by Graphite Furnace Atomic Absorption Spectrometry.

Table 2 Concentrations of copper ion activity in soil solutions pore waters and through- fall precipitation samples, determined by Ion Selective Electrode Potentiometry using Metal-Ion Buffers. Samples collected in June-September 2002.

Sample	Experimental	Total Cu*	Free Cu ²⁺	pCu
	pH	mol L ⁻¹	mol L ⁻¹	
Organic site # 2 (Sudbury)	3.9	4.00×10^{-5}	6.8×10^{-10}	9.2

Experimental pH is the pH of the test solution set by the added reagents.

The above sample was filtered through 0.45 µm filter (Gelman Material No.63077)

* Determined by Graphite Furnace Atomic Absorbtion Spectrometry

The metal ion buffers were made up to have approximately the same ionic strength as the soil solutions (pore waters) so the ISE could be calibrated in terms of concentration. Since the response of the ISE is logarithmic, the results are expressed in pCu units [58,59]. Provided the electrode is not used in a static environment, there is negligible interference by naturally occurring organic matter.

Cu has been studied far more extensively than the other metals, probably because it is the only metal for which an ISE can be used at environmentally relevant free- Cu^{2+} -ion concentrations [60]. Citations for the other metals decrease in the order $\text{Cu} > \text{Cd} > \text{Zn} >>$ all others; references to trivalent metals of environmental importance (e.g; Al^{3+} or Fe^{3+}) are conspicuously absent [60].

3.14 Conclusions

Using Metal-Ion Buffers the copper electrode can be calibrated down to cupric ion activities as low as 10^{-15}M , provided that the total concentration of copper in the sample is equal to or above its limit of detection ($\sim 10^{-5}\text{M}$).

3.15 Environmental Significance

Ion Selective Electrode Potentiometry using mixed sulphides ($\text{CuS}/\text{Ag}_2\text{S}$) crystals as the sensing electrode and Metal-Ion Buffers has enabled satisfactory determination of copper-ion activity in freshwaters at many orders of magnitude lower concentrations than it has been possible before. Since free-copper-ion activity has been found to be a reliable predictor of bioavailability this is a significant advance for the protection of the aquatic environment. An added advantage of ISE technique, is the field portability for environmental application where sample preservation and transport may be a problem.

**Chapter 4 Kinetic speciation of Cu(II), Pb(II), Cd(II),
Ni(II), and Zn(II) in model solutions of
these metals in a well-characterized humic
acid by the Competing Ligand Exchange
Method (CLEM)/Graphite Furnace Atomic
Absorption Spectrometry (GFAAS).**

4.1 Introduction

Site heterogeneity of the complexants influences both the equilibrium and the kinetic properties of these metal-complexes. For heterogeneous complexants such as humic substances, the nature of the complexing site changes with the metal to ligand mole ratio. Hence, study of the metal/ligand mole-ratio dependence of kinetics is of primary importance in understanding the behaviour and fate of these metals in aquatic system [30,61]. However, useful information on rates of chemical reactions with the change of metal/ligand mole ratio in freshwater systems is scarce [61]. Although some studies have been reported in the literature [54-56], the results are not environmentally significant because the ligand and metal concentrations and the mole ratios are far from freshwater conditions, which reveal only the characteristics of the more abundant and weaker complexing sites. Some studies were conducted on only one metal/ligand ratio [53] which did not unveil the whole scenerio. The strongest binding sites cannot be identified if the metal-complexation experiments are not done at very low metal/ligand mole ratio. In assessing the extent of trace metal complexation by humic compounds in natural waters having very low metal concentrations, the possible existence of high affinity humate sites is an important point to consider. That is why the experimental conditions need to be as close as possible to those prevailing in the aquatic environment.

Sunda and Hanson for the first time reported the existence of strong sites in humic acid (HA) [62]. It is noteworthy that they used an initial metal concentration of 10^{-7} M that was one order of magnitude lower than most of the other workers. Morel [63] reported that the use of the low metal concentration was probably the reason why these

authors found these strong binding sites. Nevertheless, most of the results confirm the existence of a distribution of dissociation rates of humic complexes, with dissociation increasing with increasing metal to ligand mole ratio [24,30,64].

The specific objective of the study presented in this Chapter is to investigate the progressive occupation of strong and weak binding sites of a well-characterized humic acid by selected metals. This study shows how kinetic data lead to differentiation between strong and weak binding sites in HA. The metals selected for the study were Cu(II), Cd(II), Pb(II), and Ni(II).

4.2 The Kinetic Model

4.2.1 Competing Ligand Exchange Method (CLEM)

Adapting the kinetic model proposed by Olson *et al.* [65], the following kinetic model has been developed further [66,67] to study the dissociation kinetics of complex ML, where M is a metal ion and L is a macromolecular, polyfunctional, multisite, heterogeneous, organic complexant such as a humic acid.

Consider an aqueous mixture of n components in which each component, designated ML_i , undergoes a first-order or pseudo-first-order reaction simultaneously with all other components

where k_1 and k_{-1} are the rate coefficients for the forward and the backward reaction, respectively. The charges on M and L have been omitted for simplicity in this general statement of the reaction.

In the Competing Ligand Exchange Method (CLEM) with solid Chelex-100 cation-exchange resin, the latter reacts with M as follows:

The model assumes that reaction (2) is much faster than reaction (1), M-Chelex complex is thermodynamically much stronger than ML complex, $C_{\text{Chelex}} \gg C_{\text{M}}$, and Chelex does not bind with the ML.

Because $[\text{Chelex}] \gg [\text{M}]$, reaction (2) is pseudo-first-order. Since k_2 is large, as has been determined by inductively-coupled plasma mass spectrometry [68,69], and Chelex-100 is in sufficiently large excess, the condition $k_2 [\text{Chelex}] \gg k_{-1}[\text{L}]$ is valid. Hence, the overall reaction (3) is irreversible.

The rate of formation of the M-Chelex complex is hence determined by the rate of dissociation of ML_i in reaction (1), and the rate expression is simply as follows:

$$\frac{d[\text{Chelex} - \text{M}]}{dt} = -\frac{d[\text{ML}_i]}{dt} = k_1 [\text{ML}_i] \quad (4)$$

If each complex, ML_i , independently and simultaneously, undergoes a first-order or pseudo-first-order dissociation reaction, the sum of the concentrations of all copper complexes remaining undissociated at time t , $C_{ML}(t)$, decreases exponentially as:

$$C_{ML}(t) = \sum_{i=1}^n C_i^0 \exp(-k_i t) \quad (5)$$

where C_i^0 is the initial concentration of ML_i , the i^{th} component. The concentration of the metal complexes remaining undissociated at time t , $C_{ML}(t)$, is measured as a function of time. The time taken for completion of reaction (2), i.e. its rate will establish the theoretical upper limit of the dissociation reaction that can be observed with this technique.

4.3 Experimental

4.3.1 Reagents and materials

Stock solutions (1000 $\mu\text{g/mL}$) of Cu(II), Cd(II), Ni(II) and Pb(II) were prepared separately for each metal by dissolving an appropriate quantity of copper metal (SPEX 99.999%), cadmium metal (SPEX 99.999%), and $\text{Pb}(\text{NO}_3)_2$ (Fisher, ACS reagent) in ultrapure nitric acid (ULTREX II, J.T. Baker, Phillipsburg, NJ, USA) with heating, and diluting to the appropriate volume with ultrapure water; the final solutions contained 1% (v/v) ultrapure nitric acid. Ultrapure water of resistivity 18.2 $\text{M}\Omega\cdot\text{cm}$ was obtained direct from a Milli-Q-Plus water purification system (Millipore Corporation).

Chelex-100 cation exchange resin (Bio-Rad, 100-200 mesh, sodium form) was soaked and stored in ultrapure water, and then filtered just before use. The pH of the solution was adjusted to 5.0 ± 0.5 using ultrapure nitric acid (ULTREX II, J. T. Baker Inc., Phillipsburg, NJ, USA).

4.4 Humic Acid

The soil HA was supplied by Professor L.J Evans (University of Guelph, Guelph, Ontario), who characterized and purified the HA according to the procedure recommended by the International Humic Substances Society [70]. A stock solution of approximately 1.00 g L^{-1} HA was prepared by dissolving approximately 0.100 g of the soil HA in 100 mL of ultrapure water and electrolytically pure NaOH – the solution pH was 10. The pH of the HA solution was then adjusted to 7 with HNO_3 using an Accumet 20 pH/conductivity meter (Fisher Scientific), fitted with an Accuplast combination glass electrode. The HA solution was stored in the dark at 4°C .

4.5 Analytical Methods

4.5.1 CLEM/GFAAS

Three test solutions for the CLEM/GFAAS experiment were prepared by adding known amounts of the HA solution to a calculated volume of a $5.0 \times 10^{-7} \text{ M}$ copper solution to make the $[\text{Cu}^{2+}]/[\text{HA}]$ mole ratios 0.1, 0.01, and 0.001. The molar mass of the HA was estimated by using its bidentate complexing capacity [70] since the actual molar mass was not directly ascertainable for such naturally-occurring, heterogeneous, polydisperse complexants. The mole ratio of $[\text{Cu}^{2+}]/[\text{HA}]$ for saturation of the strong sites was estimated to be 0.01; but to be on the safe side, a mole ratio of 0.001 was used as the

starting point. The pH of the three model, test solutions were adjusted to 7 by adding NaOH, and then the test solutions were equilibrated for 24 hours in the dark at room temperature before the kinetic experiment. The pH of the model test, solutions was again determined prior to analysis.

All measurements were done in triplicate. The variation between each replicate was less than 5% and the daily variation was less than 8%. The changes in the pH of the test solutions between the beginning and the end of the experiment were found to be $< \pm 0.5$ pH units.

4.6 QA/QC

Quality control for metal analyses included repeated injections and periodic (two in every two-hour operation of the equipment) analysis of Certified Reference Standard, NIST 1643d. A prior set of samples was re-run if the analysed value differed from the Reference Standard's certified value by $>20\%$. The relative standard deviation among replicate determinations was typically $\leq 5\%$.

4.7 Apparatus

Screw-capped Teflon cylindrical bottles (500 mL capacity) were used to fabricate the Reactors for the kinetic studies of the samples using Graphite Furnace Atomic Absorption Spectrometry (GFAAS) [71], for which a Zeeman GFAAS Varian model Spectra AA 880 equipped with an AS-70 autosampler was used. The instrumental parameters used in the GFAAS determination of the metals Ni(II), Cu(II), Pb(II) and Cd(II)) are shown in Table 3. An Accumet 20 pH/mV/conductivity meter (Fisher Scientific) was used for the pH and conductivity determinations.

4.8 Cleaning procedure

All containers were made of Teflon, and were pre-cleaned by soaking in 10% HNO₃ (AR grade) for one week at room temperature in order to leach out trace metals and other impurities. The containers were then rinsed five-times with ultrapure water. Finally, they were kept soaked in ultrapure water until used.

4.9 Test solutions

The model solutions were prepared to contain a constant concentration (10^{-7} M) of one of the following metals, Ni (II), Cu (II), Pb (II) and Cd (II), and varying concentrations of the L. J. Evans humic acid to give the [metal] / [HA] mole ratios of 0.001, 0.01 and 0.1. It should be noted that for a naturally-occurring, heterogeneous, polydisperse complexant, such as the HA the molar mass is not directly accessible. However, a molar concentration was calculated, based on the bidentate complexing capacity, 4.88 mmol g⁻¹, of the L. J. Evans humic acid [70]. A stock solution (1.01 g / L) of the L. J. Evans HA was prepared by dissolving appropriate amount of the freeze-dried HA in ultrapure water. The stock solution was stored in the dark at 4⁰C until use. The stock solution of the L. J. Evans humic acid 1.01 g/L corresponded to 4.9×10^{-3} mol / L solution of the HA, calculated on the basis of the bidentate complexing capacity of the HA. The pH of the model solutions containing HA was adjusted to 7.0 ± 0.2 using 1 % (g/v) NaOH solution. The NaOH solution was purified by electrodeposition at -1.2 V (vs. E_{SCE}) for at least 48 hours immediately prior to use.

Table 3 Analysis lines and instrumental conditions for Zeeman GFAAS Varian model Spectra AA 880.

Elements	Wavelength (nm)	Ashing temperature (°C)	Atomization parameters	
			Temperature (°C)	Duration (s)
Ni	232.0	800	2400	3.1
Pb	283.3	400	2100	3
Cu	327.4	800	2300	3.1
Cd	326.1	250	1800	4

4.10 Method for kinetic measurement

The kinetics of uptake by Chelex-100 of free metal ions plus other small labile metal species arising from the dissociation of metal-HA complexes as a function of time was determined by GFAAS as follows. One percent (w/v) of pre-treated Chelex-100 resin was added to a 100 mL of the above samples placed in the Reactor, and the mixture was stirred with a Teflon-coated magnetic stirring bar (Figure 16). Test solutions for the kinetic run were withdrawn from the Reactor through a nylon membrane filter placed at the end of the Reactor tube, which filtered out the Chelex-100 resin and injected a clear filtrate into the graphite furnace. The zero time for the kinetic measurement was taken as the time at which the Chelex-100 resin first came in contact with the test solution in the Reactor. The concentrations of the metal complexes remaining in the test solution, $C_{ML}(t)$, (eq. 5) was determined by GFAAS as a function of time. For the determination of metal concentration, 20 μL of the test solution was injected into the graphite furnace, where it was dried, ashed and atomized. The signal was measured in the peak area mode. Each completed determination was followed by a 4 s clean-up cycle of the graphite furnace at 2400°C . During the drying, ashing and clean-up cycles, the internal argon gas was passed through the graphite furnace at 250 mL / min. The internal argon gas flow was interrupted during the atomization cycle, but was restored for the clean-up cycle. The changes in the pH of the test solutions between the beginning and the end of the kinetic experiment were found to be <0.5 pH unit.

Figure 16 The Teflon Reactor used for the kinetic experiment.

4.11 Results and Discussion

4.11.1 Effect of [metal]/ [HA] mole ratios on the kinetic lability of the metal –HA complexes

Figures 17-20 show the effect of [metal]/ [HA] mole ratios on the rates of the dissociation of the HA complexes of Cu (II), Cd (II) and Pb (II). The [metal]/[HA] mole ratios were varied by keeping the metal concentration constant ($5 \times 10^{-7} \text{M}$), and varying the HA concentration. In Figures 17-20, the [metal]/[HA] mole ratio, the HA was assumed to have ~1% strong binding sites, and ~ 99% weak binding sites. If the HA had ~1% strong binding sites, then the mole ratio of the metal to HA for occupation of only the strong binding sites of the HA would be 0.01. To be on the safe side, the experiments were started with a [metal]/ [HA] mole ratio of 0.001.

In Figures 17-20, the top curves represent the [metal]/ [HA] mole ratio of 0.001. At this mole ratio, the rates of the dissociation of the metal-HA complexes were very slow and hardly detectable over the entire period of measurement. When the [metal]/ [HA] mole ratios were increased by one order of magnitude (middle curves in Figures 17-20, the rate of dissociation increased initially, but became slow again at longer time. When the [metal]/ [HA] ratios were further increased by one order of magnitude to 0.1 (the bottom curves in Figures 17-20), a rapid initial decrease was observed, which levelled off to a slower rate of dissociation at longer time.

These observations can be interpreted as follows. At the [metal]/ [HA] mole ratio of 0.001, the metal concentrations were much lower ($5 \times 10^{-7} \text{M}$) than that of HA (10^{-4}M), and

the HA had enough strong binding sites to occupy all the metal ions, resulting in the formation of strong metal-HA complexes, which were inert. With further increase in the [metal]/[HA] mole ratios to 0.01, and still further to 0.1, all the strong sites (~1%) were completely occupied, leaving the remaining metals to bind to the weak binding sites (~99%) of the HA, resulting in the formation of weak metal-HA complexes, which were labile.

The experimental data presented in Figures 17-20 were fitted to the kinetic model (eq14), and analyzed using a non-linear regression method to obtain the numerical values of the kinetically distinguishable components and the corresponding dissociation rate coefficients. The values for kinetic parameters of the metal-HA complexes are presented in Tables 4-7. The points in Figures 17-20 are the experimental data, and the solid lines are results from the non-linear regression analysis. At the [metal] / [HA] mole ratio of 0.001, only one dissociation rate coefficient was estimated, whereas two kinetically distinguishable components and two rates of dissociation were involved for the mole ratios of 0.01 and 0.1. This is consistent with the presence of two distinct classes of binding sites in the L.J. Evans HA.

Figures 17-20 and Tables 4-7 show that the fraction of the free metal ions plus other small labile species and the corresponding dissociation rate coefficients increase with increasing [metal]/ [HA] mole ratios. This observation was common for all the metal studied. The results are in agreement with the hypothesis that as the [metal] / [HA] mole ratio is increased (by keeping the metal concentration constant and varying the HA

concentration), the strong binding sites become saturated and the excess metal then binds to the weaker sites of the HA. These weak metal complexes are labile, releasing free metal ions, which are probably bioavailable.

The kinetic lability of the metal species varies in a reasonable way with the [metal] / [HA] mole ratio, suggesting that the kinetic speciation model gives chemically significant metal species. The change in the dissociation rate coefficient measured for each component of the metal-HA complexes provide experimental evidence of the polyfunctionality of the humic acid. The results show how kinetic data can detect the progressive occupation of strong and weak binding sites of a well-characterized HA and the existence of strong and weak binding sites in humic acids.

Figure 17 Effect of $[\text{Cu(II)}]/[\text{HA}]$ mole ratio on the kinetic lability of the Cu(II)-HA complexes in model systems containing varying concentrations of the L.J. Evans HA, determined by Zeeman GFAAS Varian model SpectrAA 880. Concentrations of copper were same for all curves. pH 7.0 ± 0.1 , ionic strength ~ 0 . The error bars represent 95.5 % confidence limit.

(O): $[\text{Cu (II)}] / [\text{HA}] = 5 \times 10^{-7}\text{M} / 5 \times 10^{-4}\text{M} = 0.001$;

(□), $[\text{Cu (II)}] / [\text{HA}] = 5 \times 10^{-7}\text{M} / 5 \times 10^{-5}\text{M} = 0.01$;

(Δ), $[\text{Cu (II)}] / [\text{HA}] = 5 \times 10^{-7}\text{M} / 5 \times 10^{-6}\text{M} = 0.1$

Figure 18 Effect of $[\text{Cd(II)}]/[\text{HA}]$ mole ratio on the kinetic lability of the Cd(II)-HA complexes in model systems containing varying concentrations of the L.J. Evans HA, determined by Zeeman GFAAS Varian model SpectrAA 880. Concentrations of cadmium were same for all curves. pH 7.0 ± 0.1 , ionic strength ~ 0 . The error bars represent 95.5 % confidence limit.

(○): $[\text{Cd(II)}]/[\text{HA}] = 5 \times 10^{-7} \text{ M} / 5 \times 10^{-4} \text{ M} = 0.001$;

(□): $[\text{Cd(II)}]/[\text{HA}] = 5 \times 10^{-7} \text{ M} / 5 \times 10^{-5} \text{ M} = 0.01$;

(△): $[\text{Cd(II)}]/[\text{HA}] = 5 \times 10^{-7} \text{ M} / 5 \times 10^{-6} \text{ M} = 0.1$

Figure 19 Effect of $[\text{Pb(II)}]/[\text{HA}]$ mole ratio on the kinetic lability of the Pb(II) -HA complexes in model systems containing varying concentrations of the L. J. Evans HA, determined by Zeeman GFAAS Varian model Spectr AA 880. Concentrations of lead were same for all curves. $\text{pH } 7.0 \pm 0.1$, ionic strength ~ 0 . The error bars represent 95.5 % confidence limit.

(○): $[\text{Pb(II)}]/[\text{HA}] = 1.6 \times 10^{-7} \text{ M} / 5 \times 10^{-4} \text{ M} = 0.001$;

(□): $[\text{Pb(II)}]/[\text{HA}] = 1.6 \times 10^{-7} \text{ M} / 5 \times 10^{-5} \text{ M} = 0.01$;

(△): $[\text{Pb(II)}]/[\text{HA}] = 1.6 \times 10^{-7} \text{ M} / 5 \times 10^{-6} \text{ M} = 0.1$

Figure 20 Effect of $[\text{Ni(II)}]/[\text{HA}]$ mole ratio on the kinetic lability of the Ni(II)-HA complexes in model systems containing varying concentrations of the L. J. Evans HA, determined by Zeeman GFAAS Varian model SpectrAA 880. Concentrations of nickel were same for all curves. $\text{pH } 7.0 \pm 0.1$, ionic strength ~ 0 . The error bars represent 95.5% confidence limit.

(○): $[\text{Ni(II)}]/[\text{HA}] = 5 \times 10^{-7} \text{ M} / 5 \times 10^{-4} \text{ M} = 0.001$;

(□): $[\text{Ni (II)}]/[\text{HA}] = 5 \times 10^{-7} \text{ M} / 5 \times 10^{-5} \text{ M} = 0.01$;

(△): $[\text{Ni (II)}]/[\text{HA}] = 5 \times 10^{-7} \text{ M} / 5 \times 10^{-6} \text{ M} = 0.1$

Table 4 Kinetic parameters of the Cu (II)-HA complexes in model systems, as a function of [Cu]/[HA] mole ratio, at a constant concentration of Cu(II) and varying concentrations of the HA. pH 7.0 ± 0.1 , ionic strength ~ 0 . [Cu(II)] = 5×10^{-7} M.

[HA] M	[Cu(II)]/[HA]	Kinetically distinguishable components		Dissociation rate coefficients of	
				C ₁	C ₂
		C ₁ (%)	C ₂ (%)	$k_1 \times 10^3 \text{ (s}^{-1}\text{)}$	$k_2 \times 10^5 \text{ (s}^{-1}\text{)}$
10^{-4}	0.001	—	98.2 ± 0.4	—	0.14 ± 0.04
10^{-5}	0.01	6.7 ± 0.9	94.1 ± 0.5	5.9 ± 1.6	0.8 ± 0.1
10^{-6}	0.1	55.2 ± 0.8	45.2 ± 0.8	0.4 ± 0.01	$<10^{-6}\text{s}^{-1}\text{(inert)}$

The uncertainty represents 95.5 % confidence limit in the non-linear regression analysis.

Table 5 Kinetic parameters of the Cd(II)-HA complexes in model systems, as a function of [Cd]/[HA] mole ratio, at a constant concentration of Cd(II) and varying concentrations of the HA. pH = 7.0 ± 0.1, ionic strength ~ 0. [Cd(II)] = 5 × 10⁻⁷ M.

[HA] M	[Cd(II)]/[HA]	Kinetically distinguishable components		Dissociation rate coefficients of	
				C ₁	C ₂
		C ₁ (%)	C ₂ (%)	k ₁ × 10 ³ (s ⁻¹)	k ₂ × 10 ⁵ (s ⁻¹)
10 ⁻⁴	0.001	—	103 ± 0.7	—	0.4±0.16
10 ⁻⁵	0.01	46.8 ± 14.4	59.5 ± 14.8	11.6 ± 5.3	<10 ⁻⁶ s ⁻¹ (inert)
10 ⁻⁶	0.1	62 ± 2.2	42.9 ± 1.4	92.8 ± 9.3	<10 ⁻⁶ s ⁻¹ (inert)

The uncertainty represents 95.5% confidence limit in the non-linear regression analysis.

Table 6 Kinetic parameters of the Pb (II)-HA complexes in model systems, as a function of [Pb]/[HA] mole ratios, at a constant concentration of Pb(II) and varying concentrations of the HA. pH = 7.0 ± 0.1, ionic strength ~ 0. [Pb(II)] = 1.6 × 10⁻⁷ M.

[HA] M	[Pb(II)]/[HA]	Kinetically distinguishable components		Dissociation rate coefficients of	
				C ₁	C ₂
		C ₁ (%)	C ₂ (%)	k ₁ × 10 ³ (s ⁻¹)	k ₂ × 10 ⁵ (s ⁻¹)
10 ⁻⁴	0.001	—	105 ± 0.9	—	0.6 ± 0.2
10 ⁻⁵	0.01	74.8 ± 15.0	25.2 ± 1.5	0.13 ± 0.04	<10 ⁻⁶ s ⁻¹ (inert)
10 ⁻⁶	0.1	58.2 ± 3.3	43.5 ± 2.6	4.8 ± 0.5	8.7 ± 1.5

The uncertainty represents 95.5% confidence limit in the non-linear regression analysis.

Table 7 Kinetic parameters of the Ni (II)-HA complexes in model systems, as a function of [Ni]/[HA] mole ratios, at a constant concentration of Ni(II) and varying concentrations of the HA. pH = 7.0 ± 0.1, ionic strength ~ 0. [Ni(II)] = 5.0 × 10⁻⁷ M.

[HA] M	[Ni(II)]/[HA]	Kinetically distinguishable components		Dissociation rate coefficients of	
				C ₁	C ₂
		C ₁ (%)	C ₂ (%)	k ₁ × 10 ³ (s ⁻¹)	k ₂ × 10 ⁵ (s ⁻¹)
10 ⁻⁴	0.001	9.0 ± 1.5	90.4 ± 0.6	8.7 ± 2.8	1.2 ± 0.08
10 ⁻⁵	0.01	23 ± 0.8	77.4 ± 0.4	8.3 ± 0.6	1.8 ± 0.1
10 ⁻⁶	0.1	42.9 ± 4.3	57.6 ± 4.6	7.2 ± 1.2	2.5 ± 1.3

The uncertainty represents 95.5% confidence limit in the non-linear regression analysis.

4.12 Conclusions:

Kinetic studies of metal speciation can provide valuable information on the number of kinetically distinguishable components, the initial concentration of each component, the concentration of each component at any specific time during the kinetic run and their rate constants. However, the results obtained by Graphite Furnace Atomic Absorption Spectrometry (GFAAS) using Chelex 100 as the competing ligand provide information that is chemically relevant.

**Chapter 5 Kinetic study of dissociation of Cu(II),
Pb(II), Cd(II), Ni(II), and Zn(II) complexes in
freshwater samples using the Competing
Ligand Exchange Method/Graphite
Furnace Atomic Absorption Spectrometry
(GFAAS).**

5.1 Introduction

Trace metals in the natural environment are essential nutrients and potential toxicants at elevated levels. The biological effects and biogeochemical cycling of trace metals are dependent on their chemical speciation [72,73]. Interactions of metal ions with humic substances control transport, bioavailability, and toxicity of metals in freshwaters. Since most of these trace metals in freshwaters are bound to humic substances, study of the metal-humate complexes is of primary importance in understanding the behaviour and fate of these trace metals in freshwaters.

Chapter 4 has dealt with the effects of the $[\text{metal}] / [\text{HA}]$ mole ratios on the dissociation rate of the metal-HA complexes in model systems. The above study is now extended to the dissociation of metal complexes in freshwaters. In freshwaters, trace metal ions, such as Ni(II), Cu(II), Cd(II) or Pb(II), exist as complexes with humic substances and other ligands [74]. Freshwater systems are usually non-equilibrium systems [3], and the importance of non-equilibrium processes in controlling metal ion speciation in the freshwater environment is now receiving increasing recognition [30,75]. Recently, several studies investigating the kinetics of metal-humic acid interactions have been reported [21,76,77].

Dissolved Organic Matter (DOM) in freshwaters is particularly important because of its high concentration; strong affinity for metals; and ubiquity in freshwaters, soils, and sediments. The most important component of DOM is Dissolved Organic Carbon (DOC). Humic substances (consisting of Humic Acid, Fulvic acid and Humin) constitute from

40% to 90% of DOC, and are the most numerous, naturally-occurring complexing agents of trace metals in freshwaters. The dominant tool for studying metal speciation has been the local equilibrium (or pseudoequilibrium) approximation [3]. The complexation reactions are assumed to be fast relative to other processes, and an equilibrium distribution among dissolved organic and inorganic species is assumed despite the fact that total metal concentration may vary as a result of some controlling biogeochemical process. However, natural systems are often not equilibrium systems. DOM, which is a heterogeneous, polyelectrolytic ligand system, can depart considerably in kinetic behaviour from simple ligands and may result in systems that are dynamic and far removed from equilibrium [78,79]; hence, equilibrium speciation methods may not apply [21]. In some instances, the rates of biogeochemical reactions may be influenced or even controlled by the rates of metal complexation reactions [79]. Furthermore, not much is known about the kinetics of metal complexation of trace metals by DOM and the relative reactivity of trace metals in freshwaters. Hence, new, kinetics-based chemical speciation methodologies are required to reflect more correctly the realities of freshwater systems, in which trace-metal release is often kinetically controlled. The objective of this study was to investigate the kinetic speciation of Cu(II), Pb(II), Cd (II), Ni(II), and Zn(II) within the context of natural waters as dynamic systems.

5.2 Experimental

5.2.1 Dissolved Organic Carbon (DOC)

The Dissolved Organic Carbon of the filtered water samples was determined using O I Analytical Model 1010 TOC Analyzer.

5.2.2 *Freshwater Samples*

Freshwater samples were collected in May 2001 and in June 2001 from several sampling sites in the Sudbury (Canada) area using pre-cleaned Teflon bottles. The sites were chosen upstream and downstream of the nickel-tailing ponds, and outflows from the treatment tanks receiving effluents from nickel ore processing and smelting operations. The samples were filtered through 0.45 μm membrane filters (CN-6 Metrice, 47 mm diameter, Gelman, Ann Arbor, MI) in our laboratory within 24 h of the sample collection to filter out the particulate matter. These filters, made of mixed cellulose esters, had the advantage of providing filtrates having low blanks for the trace metals. Each 0.45 μm filter was able to filter ~500 mL water sample without clogging. Immediately after the filtration, a subsample (250 mL) of the filtered sample was acidified with HNO_3 (Ultrex II) to pH 1.5 to preserve the sample integrity, i.e. the trace metals concentrations. The acidified samples were used to determine the concentrations of trace metals (Ni, Cu, Cd, Pb, Zn) using GFAAS. The remaining 250 mL of the filtered sample was stored in the dark at 4°C and was used for the kinetic measurement within 72 h of the filtration. All kinetic studies were done using the filtered water samples and following the experimental procedures described in chapter 4, and the Kinetic Model described therein. The kinetic study was done using a fixed pH, ionic strength, and temperature (23°C) for each kinetic run.

5.3 **QA / QC**

Quality control for the trace metal analysis included repeated injections and periodic (two in every two-hour operation of the equipment) analysis of Certified Reference Standard, NIST 1643d. A prior set of samples was re-run if the analyzed value differed

from the Reference Standard's certified value by $> 20\%$. The relative standard deviation among replicate determinations was typically $\leq 5\%$.

5.4 Results and Discussion

Table 8 presents the pH and conductivity of the water samples as received. The results of kinetic study are tabulated in Tables 9, 10, 11, and 12, and are plotted in figures 21, 22, 23, and 24, respectively. From these Tables and Figures the following generalizations can be made. The mole ratios of $[\text{metal}]/[\text{DOC}]$ generally determines the percentage of the metal that is labile. However, since humic substances (HS) dominate DOC, the metal-DOC complex formation can be considered in a simplified form in terms of metal-HS complex formation. Humic substances have two types of binding sites. 1-10% of their total binding sites are strong sites and 90-99% are weak sites. Based on the results obtained for Ni(II) and Cu(II) from various water samples, it can be seen that a large percentage of these trace metals are labile when the $[\text{metal}]/[\text{DOC}]$ mole ratio is as high as $\sim 10^{-3}$. However, as shown in Table 9, a higher proportion of labile Zn(II) species occurs at a $[\text{metal}]/[\text{DOC}]$ mole ratio of about 10^{-5} . In general, the Zn(II) complexes in the three water samples from Sudbury, all of which have $[\text{metal}]/[\text{DOC}]$ ratios of about 10^{-5} , appear to be more labile than Ni(II) and Cu(II) complexes from other samples with similar $[\text{metal}]/[\text{DOC}]$ ratios. This suggests that Zn(II) tends to form weaker complexes with DOC than Ni(II) and Cu(II) at similar metal loading levels. Of the three metals investigated, Cu(II) appears to form the strongest metal complexes. This is to be expected, as Cu(II) forms d^9 octahedral complexes, which characteristically undergo a type of stabilizing tetragonal distortion known as the Jahn-Teller effect. The ground electronic configuration of an octahedral Cu(II) d^9 complex is orbitally degenerate, thus

the complex distorts via elongation of the two axial bonds and shortening of the four equatorial bonds so as to remove the degeneracy and achieve a lower energy. Although the Jahn-Teller effect is most often observed in Cu(II) complexes, this phenomenon is not restricted to copper.

Ionic strength influences the binding of metals to HS; however, it is not an increase in affinity to the sites that is observed, but a decrease. This is because at high ionic strength, natural waters typically contain high concentrations of spectator ions (e.g. Na^+ , K^+), and major cations (e.g. Ca^{2+} and Mg^{2+}). In effect, these cations will screen the negative charges of the HS, making it more difficult for the incoming trace metal ion to “see” the sites. The outer surface of the HS are covered with negative charges, but since the medium is water, all these charges will be coordinated to polar water molecules, with the hydrogen atoms (positive dipole) pointing towards the negative charges and the oxygen atoms (negative dipole) pointing outwards towards the bulk solution. This creates the so-called electrical double layer. Major cations which are much more abundant than trace transition metal ions create electrostatic bonds (ion-pairs) which is a long range bond. This screening effect, and also the presence of some competition with Ca^{2+} and Mg^{2+} for the sites makes it more difficult for transition and other divalent metal ions to bind with HS, and therefore more of the metal will be available for uptake into the biota.

The non-linearity of the equations results in a proportionally much greater effect of the di- and trivalent ions on the surface potential. This is again important in waters with high ionic strength due to major cations Mg^{2+} and Ca^{2+} . These two ions will dominate the coulombic attractions of medium-sized polyacids and diminish the tendency of HS to

complex with trace metals both by competition for specific sites on the HS, and by decreasing the coulombic attraction through shielding of the neighbouring ionized groups

Although the results for model solutions with Humic acid in Chapter 4 reveal an obvious trend of increasing lability of metal complexes with increasing [metal]/[DOC] ratio, this trend was not perfectly followed in results obtained for the real samples. Table 9, and Figure 21 display the kinetic speciation results for Zn(II) complexes in three soil solution samples from the Sudbury area. Based on the [metal]/[DOC] ratios, the lability of metal complexes should increase from Site 2, through to Site 1, to Site 3. However, Zn(II) complexes for Site 2 appear to be more labile than those from Site 1, despite the lower [metal]/[DOC] ratio in that sample. A similar discrepancy is noted in the trends for Ni(II) (Table 10, Figure 22) and Cu(II) complexes (Tables 11, 12, Figures 23, 24), where lability results for certain samples appear to be out of the expected order based on their [metal]/[DOC] ratios. This is likely a result of influence of other factors, such as the presence of large concentrations of Ca^{2+} and Mg^{2+} , as well as differences in pH, ionic strength, and the nature of the DOC between different samples.

Table 8 pH and conductivity of water samples from the Sudbury Mines area and the Rouyn Noranda area, collected in May 2001.

Sample name	pH of the sample as received	Conductivity, mS/m
Site 1B Sudbury Organic lysimeter	4.9	5.31
Site 2B Sudbury Organic lysimeter	4.0	4.55
Abitibi Site 3 Aiguebelle lys.0cm	3.3	15.97
Abitibi Site 3 Aiguebelle lys.30cm	3.9	3.61
Abitibi Site 2 Clericy lysimeter 0 cm	3.0	7.12
Sudbury Site 1	5.3	3.7
Sudbury Site 2	5.6	2.9
Sudbury Site 3	5.2	9.3
Sudbury Openfield Site 1	4.5	2.10
Sudbury Throughfall Site #1	4.8	3.57

Table 9 Kinetic parameters of the Zn(II)-DOC complexes in the Sudbury area water samples collected in June 2001

Sample No.	[Zn(II)]/[DOC] mol/g	Kinetically distinguishable components		Dissociation rate coefficients of	
				C ₁	C ₂
		C ₁ (%)	C ₂ (%)	k ₁ × 10 ³ (s ⁻¹)	k ₂ × 10 ⁵ (s ⁻¹)
Sudbury Site 1	8.56 × 10 ⁻⁵	86 ± 4	9.6 ± 3.8	0.43 ± 0.1	<10 ⁻⁶ (inert)
Sudbury Site 2	1.51 × 10 ⁻⁶	68.4 ± 6.8	31.3 ± 5.9	3.8 ± 0.8	16.2 ± 5.8
Sudbury Site 3	4.13 × 10 ⁻⁵	84.6 ± 3.7	18.2 ± 2.9	4.8 ± 0.4	16 ± 5

The uncertainty represents 95.5 % confidence limit from the non-linear regression analysis.

Table 10 Kinetic parameters of the Ni(II)-DOC complexes in the Sudbury area water samples collected in June 2001.

Sample No.	[Ni(II)]/[DOC] mol/g	Kinetically distinguishable components		Dissociation rate coefficients of	
				C ₁	C ₂
		C ₁ (%)	C ₂ (%)	k ₁ × 10 ³ (s ⁻¹)	k ₂ × 10 ⁵ (s ⁻¹)
Site 1B					
Sudbury Organic lysimeter	1.2 × 10 ⁻⁴	23.6 ± 1.6	76 ± 1	12.0 ± 1.8	17.2 ± 0.5
Site 2B					
Sudbury Organic lysimeter	2.5 × 10 ⁻³	63.6 ± 7.9	36.4 ± 7.4	3.8 ± 0.9	19 ± 7
Abitibi Site 3					
Aiguebelle lys. 0 cm	6.4 × 10 ⁻⁴	39 ± 8	62.2 ± 8.3	1.5 ± 0.7	5.8 ± 2.7

The uncertainty represents 95.5 % confidence limit from the non-linear regression analysis.

Table 11 Kinetic parameters of the Cu(II)-DOC complexes in the Sudbury and Rouyn Noranda area water samples collected in June 2001.

Sample No.	[Cu(II)]/[DOC] mol/g	Kinetically distinguishable components		Dissociation rate coefficients of	
				C ₁	C ₂
		C ₁ (%)	C ₂ (%)	k ₁ × 10 ³ (s ⁻¹)	k ₂ × 10 ⁵ (s ⁻¹)
Site 1B					
Sudbury Organic lysimeter	1.44 × 10 ⁻⁴	16.0 ± 1.5	84.0 ± 0.7	39.2 ± 16.9	11.2 ± 0.3
Site 2B					
Sudbury Organic lysimeter	8.2 × 10 ⁻³	35.0 ± 2.1	65.0 ± 1.5	10.7 ± 1.4	28.4 ± 1.0
Abitibi Site 3					
Aiguebelle lys. 0 cm	7.2 × 10 ⁻³	34.7 ± 3.6	65.3 ± 3.8	1.1 ± 0.2	6.9 ± 1.1
Abitibi Site 2					
Clericy lys. 0 cm	2.97 × 10 ⁻⁵	11.4 ± 1.7	88.6 ± 1.3	3.3 ± 1.0	6.4 ± 0.3

The uncertainty represents 95.5 % confidence limit from the non-linear regression analysis.

Table 12 Kinetic parameters of Cu (II)-DOC complexes in Sudbury area water samples collected in June 2001.

Sample No.	[Cu(II)]/[DOC] mol/g	Kinetically distinguishable components		Dissociation rate coefficients of	
				C ₁	C ₂
		C ₁ (%)	C ₂ (%)	k ₁ × 10 ³ (s ⁻¹)	k ₂ × 10 ⁵ (s ⁻¹)
Sudbury Openfield	5.6 × 10 ⁻³	53.0 ± 4.4	48.8 ± 4.2	3.0 ± 0.5	19.2 ± 2.5
Site 1					
Sudbury Throughfall #1	2.24 × 10 ⁻⁵	9.7 ± 3.3	91.7 ± 2.8	3.6 ± 2.6	15.0 ± 0.9
Abitibi Site 3					
Aiguebelle lys. 30 cm	6.5 × 10 ⁻⁵	—	98.0 ± 0.5	—	3.7 ± 0.2

The uncertainty represents 95.5 % confidence limit from the non-linear regression analysis.

Figure 21 Effect of Zn / DOC (mol/g) ratio on the kinetic lability of the Zn(II)-DOC complexes in the Sudbury area water samples collected in July 2001. The error bars represent 95.5% confidence limit.

(○): Sudbury Site 1 $[\text{Zn(II)}]/[\text{DOC}] = 8.56 \times 10^{-5} \text{ mol/g}$;

(◇): Sudbury Site 2 $[\text{Zn(II)}]/[\text{DOC}] = 1.5 \times 10^{-6} \text{ mol/g}$;

(△): Sudbury Site 3 $[\text{Zn(II)}]/[\text{DOC}] = 4.1 \times 10^{-5} \text{ mol/g}$

Figure 22 Effect of Ni/ DOC (mol/g) ratio on the kinetic lability of the Ni (II)-DOC complexes in the Sudbury area and the Rouyn Noranda water samples collected in May 2001. The error bars represent 95.5% confidence limit.

(O):Site 1B Sudbury Organic lysimeter $[\text{Ni(II)}]/[\text{DOC}] = 1.2 \times 10^{-4} \text{ mol/g}$;

(□):Site 2B Sudbury Organic lysimeter $[\text{Ni(II)}]/[\text{DOC}] = 2.5 \times 10^{-3} \text{ mol/g}$;

(Δ):Abitibi site 3 Aiguebelle lys.0 cm $[\text{Ni(II)}]/[\text{DOC}] = 6.4 \times 10^{-4} \text{ mol/g}$

Figure 23 Effect of Cu / DOC (mol/g) ratio on the kinetic lability of the Cu(II)-DOC complexes in the Sudbury area and the Rouyn Noranda water samples collected in May 2001. The error bars represent 95.5% confidence limit.

(○): Site 1B Sudbury Organic lysimeter $[\text{Cu(II)}]/[\text{DOC}] = 1.4 \times 10^{-4}$ mol/g;

(□): Site 2B Sudbury Organic lysimeter $[\text{Cu(II)}]/[\text{DOC}] = 8.2 \times 10^{-3}$ mol/g;

(△): Abitibi Site 2 clericy lys.0 cm $[\text{Cu(II)}]/[\text{DOC}] = 2.97 \times 10^{-5}$ mol/g;

(▽): Abitibi Site 3 Aiguebelle lys.0 cm $[\text{Cu(II)}]/[\text{DOC}] = 7.2 \times 10^{-3}$ mol/g

Figure 24 Effect of Cu / DOC (mol/g) ratio on the kinetic lability of the Cu(II)-DOC complexes in the Sudbury area and the Rouyn Noranda water samples collected in November 2001. The error bars represent 95.5% confidence limit.

(○): Openfield #1 $[\text{Cu(II)}]/[\text{DOC}] = 5.6 \times 10^{-3} \text{ mol/g}$;

(□): Throughfall #1 $[\text{Cu(II)}]/[\text{DOC}] = 2.24 \times 10^{-5} \text{ mol/g}$;

(△): Abitibi site #3 Aiguebelle lys.30 cm $[\text{Cu(II)}]/[\text{DOC}] = 6.5 \times 10^{-5} \text{ mol/g}$;

5.5 Conclusions

The ability of the kinetic model to correctly identify the relative rates of metal complex dissociation in both model solutions and freshwaters supports the conclusion that the speciation model has chemical significance. The practical application of this work is that it is possible to extrapolate results from model solutions to freshwater systems because the relative rates are correctly diagnosed. The results from this work have a direct implication on predicting free metal ion availability of the above metals and, hence, their bioavailability [80,81] in the freshwater environment.

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