SYSTEMATIC VARIABILITY IN PROTON AND COPPER(II) COMPLEXATION BY DISSOLVED ORGANIC MATTER FROM SURFACE FRESHWATERS

by

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Abstract

The reproducibility of potentiometric titrations using a copper (II) ion selective electrode increases directly as a function of dissolved organic matter (DOM) concentration, measured as dissolved organic carbon. Conditional stability parameters ($\mathrm{Log}(K_1)$, $\mathrm{Log}(K_2)$), calculated from titration curves using a 2-ligand Langmuir Isotherm, are also dependent on DOM concentration explaining 20% to 60% of the variation reported for these parameters in the literature. The effect of DOM concentration on Cu^{2+} complexation is greater for allochthonous DOM, which also exhibits higher charge density and higher affinity for Cu^{2+}, than autochthonous DOM ($p < 0.05$). The Cu^{2+} complexing properties of DOM collected from surface freshwaters depend primarily on allochthonous DOM and cumulative hydrologic residence time ($p < 0.05$) by approximating exposure to transformation and fractionation processes. Comparison to experimental transformation and fractionation processes in micro-reactors suggests that photochemical decomposition, microbial decomposition, and adsorptive fractionation could contribute to the observed pattern of Cu^{2+} complexation.

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List of Acronyms, Symbols and Units

ABS_{350}	Photometric absorbance at an optical wavelength of 350nm
CC_1, CC_2	Parameter values of complexing capacity empirically approximated from a 2-ligand Langmuir Isotherm that correspond to the density of organically complexed Cu^{2+} (mol C-g^{-1})
CD	Charge Density (mol C-g^{-1}) calculated as function of pH
CD_T	Total Charge Density (mol C-g^{-1})
CER	Cation Exchange Resin
C-g	Grams of carbon measured for DOM
CHRT	Cumulative Hydrologic Residence Time (yr)
Cu^{2+}	Copper (II)
CuL	Copper binding density (Cu-µmol C-g^{-1})
CuL_M	Molar concentration of Cu^{2+} binding sites (Cu-µmol L^{-1}) calculated from empirical titration curves at 1.0E-06 mol L^{-1} of free labile Cu^{2+} and ambient DOC concentrations
Cu-mol	Moles of Cu^{2+}
CV	Coefficient of Variation
d	Diameter
DO	Dissolved Oxygen (mg L^{-1})
DOC	Dissolved Organic Carbon (C-mg L^{-1})
DOM	Dissolved Organic Matter
EDTA	Ethylenediaminetetra-acetate
g	Grams
H^+	Hydrogen ion
HCl	Hydrochloric acid
HRT	Hydrologic Residence Time (yr)
ICP	Inductively Coupled Plasma
ISE	Ion Selective Electrode
K_f	Formation constant for the complexation of metal ions

KHP	Potassium Hydrogen phthalate
Kpa	Kilopascals
L	Liters
$\text{Log}(K_1)$, $\text{Log}(K_2)$	Parameter values empirically approximated from a 2-ligand Langmuir Isotherm that correspond to the binding affinity of organic ligands
M	Molar concentration
mg	Milligrams
mL	Milliliters
µmol	Micromoles
mn	Mean or average
mol	Moles
n	Sample size
nm	Nanometre
°C	Degrees Celsius
p	Probability that an observation is consistent with the null hypothesis
PAR	Photosynthetically Active Radiation
pH	The negative of the base-10 logarithm of hydrogen ion concentration
pK_a	Acid dissociation parameter – relates the concentration of an acid to the concentration of its ionization products
pK_w	Water formation constant
r	Correlation – measures the strength and direction of linear relationships
r^2	Square of the correlation – measures the fraction of the variation in the values of y that is explained by the least squares regression of y on x
RO	Reverse Osmosis
SAC_{350}	Specific Absorbance Coefficient calculated at a photometric ABS wavelength of 350nm

SD	Standard Deviation
UV	Ultraviolet
UVR	Ultraviolet Radiation

Preface

Research that was conducted for this thesis is part of a larger research initiative that focuses on systematic control over the concentration and composition of dissolved organic matter (DOM) in surface freshwaters. The following research was conducted but not included in this thesis: scavenging of DOM by iron hydroxide particles as a function of cumulative hydrologic residence time (CHRT); watershed control over DOM in alpine lakes; the relationship between optical properties of DOM and CHRT; and a comparative study of Cu^{2+} toxicity measured by rainbow trout bioassay, a copper (II) ion selective electrode and diffusion gradient thin film samplers.

1. Chapter 1. Introduction and Literature Review

1.1 Introduction

Dissolved organic matter (DOM) is approximately 40% carbon by weight (Perdue 1998), measured as dissolved organic carbon (DOC), and is transformed over time in the environment as a result of biological and chemical degradation processes (Buffle and De Vitre 1994; Brezonik 1994). Three of these processes are photochemical decomposition, microbial decomposition and adsorption (Wetzel 1983; Buffle and De Vitre 1994; Lindell *et al.* 2000). These types of processes will alter the chemical composition of DOM, which may influence its function as a metal complexing agent. DOM is composed of organic acids, which can complex metals and play an important role in regulating trace metal speciation dynamics in surface freshwaters (Sunda and Lewis 1978; Azenha *et al.* 1995; MacRae *et al.* 1999b). Therefore, a change in organic acidity may have a significant impact on aquatic ecosystems by an increase in metal toxicity. The objective of this research is to identify systematic variation in organic acidity and metal complexation as a function of DOM source and exposure to transformation and fractionation processes.

DOM is loaded to aquatic systems from two sources. The first is from the terrestrial environment (allochthonous) and the second is from within lakes (autochthonous) (Sposito 1981; Wetzel 1983; Fukushima *et al.* 1996). Allochthonous DOM is derived from the degradation of plant material, and is transported to lakes by various hydrologic pathways. Autochthonous DOM is derived from primary productivity within lakes (Sposito 1981; Wetzel 1983; Fukushima *et al.* 1996). These differences in derivation result in fundamental differences in chemical composition (Fukushima *et al.* 1996). Thus, the composition of

DOM loaded to surface freshwaters can be investigated using allochthonous and autochthonous sources of DOM for comparison purposes (Luider *et al.* in Press).

Photochemical decomposition, microbial decomposition and adsorption processes change the properties of DOM over time in the environment because these processes transform and fractionate DOM (Strome and Miller 1978; Geller 1986; Kieber *et al.* 1990; Gu *et al.* 1995). For example, DOM plays an integral role in metabolism because it is a fundamental food source for microbes (Wetzel 1983; Moran and Hodson 1990; Sun *et al.* 1997). Microorganisms, such as bacteria, may consume portions of dissolved organic molecules for energy. Excess carbon is transformed to microbial waste products, such as carbon dioxide, which are lost to surrounding waters (Wetzel 1983). Dissolved organic molecules are fractionated as a result of this decomposition process, and the overall chemical properties of DOM are changed (Kaplan and Newbold 1995; Volk *et al.* 1997; Moran *et al.* 2000).

Transformation and fractionation processes may also be coupled *in situ* to collectively change the properties of DOM over time. For example, photolysis may be coupled to microbial decomposition (Lindell *et al.* 1995; Miller and Moran 1997; Moran and Zepp 1997a). Natural ultraviolet radiation (UVR) can cleave simple organic molecules from DOM, which can stimulate microbial metabolism (Strome and Miller 1978; Wetzel *et al.* 1995; Moran and Zepp 1997a). Thus, photolysis and microbial decomposition have a combined influence on the concentration and composition of DOM in surface freshwaters.

Due to this combined influence, it is difficult to measure the individual effects of each process on the overall concentration and composition of DOM. The magnitude and rate of photochemical decomposition, microbial decomposition and adsorption processes is difficult to investigate in surface freshwaters because of the complex mixture of physical, biological and chemical activity occurring *in situ* (Wetzel 1983; Frimmel 1990; Buffle and De Vitre 1994). As a result, it is difficult to investigate the influence of specific transformation and fractionation processes on the metal complexing function of DOM.

Aspects of the complex nature of aquatic systems can be simplified by isolating transformation and fractionation processes in micro-reactors. The effects of each process on the concentration and composition of DOM can then be investigated directly, and compared to the transformation and fractionation of DOM in surface freshwaters (Buffle and De Vitre 1994; Gu *et al.* 1995). Furthermore, characteristic changes in DOM can be used to investigate the influence of each process on organic acidity and the metal complexing function of DOM.

Metal complexation is one of the most important functions of DOM, and it involves metal binding by organic ligands to form a single integral molecule (Hering and Morel 1988a; Buffle 1990; Christensen *et al.* 1999). It was determined in the 1970's that trace metal toxicity is a function of free ionic and not total metal concentrations (Brown *et al.* 1974; Pagenkopf *et al.* 1974; Mantoura *et al.* 1978; O'Shea and Mancy 1978). Thus, the ability of DOM to complex metals contributes to the regulation of trace metals in surface freshwaters (McKnight *et al.* 1983; Buffle 1990; Bruland 1992). For example, Cu^{2+} is readily complexed by DOM, which can reduce the toxicity of Cu^{2+} in surface freshwaters (Mantoura *et al.* 1978; McKnight and Morel 1979; Winner 1985; Hering and Morel 1988b; Meador 1991; Azenha *et al.* 1995; Erickson *et al.* 1996).

The contribution of organic complexation as a regulating process of free ionic metals, such as Cu^{2+}, has been investigated by many different researchers (Playle and Dixon 1993;

Marr *et al.* 1999; MacRae *et al.* 1999a). DOM has been observed to significantly influence free ionic Cu^{2+} concentrations in lake systems (Pagenkopf *et al.* 1974; Sunda and Lewis 1978). However, the protective effects of DOM are unclear at environmentally significant Cu^{2+} concentrations, which can be as low as 2.0×10^{-10} M (Anderson and Morel 1978). Therefore, organic complexation may not completely reduce the toxicity of Cu^{2+}, and only thermodynamically or kinetically stable Cu^{2+} complexes may be important from an eco-toxicological perspective.

The function of DOM as a Cu^{2+} complexing agent may also change over time as DOM is transformed and fractionated in surface freshwaters. Transformation and fractionation processes alter the chemical composition of DOM, which may inhibit the formation of stable metal complexes. Subsequently, the susceptibility of surface freshwaters to Cu^{2+} toxicity may vary as a function of exposure to transformation and fractionation processes. Therefore, the influence of transformation and fractionation processes on the Cu^{2+} complexing function of DOM needs to be investigated.

1.2 Hypothesis

Organic acidity and Cu^{2+} complexation by DOM vary systematically according to DOM source and exposure to environmental transformation and fractional processes.

1.3 Significance

This research addresses one of the most important topics in environmental toxicity. Freshwater metal contamination is quickly becoming a global problem because of the atmospheric transport of metal contaminants (Nriagru and Pacyna 1988). Dissolved organic matter (DOM) is one of the most significant substances in freshwater that is available in sufficient concentrations to mediate metal toxicity (Perdue 1998; Rozan and Benoit 1999).

As global populations continue to rise, metal contamination will undoubtedly be a problem of the future as well as the present (Runnels *et al.* 1992; Myers 1993; Cohen 1995). In order to sustain the biodiversity of aquatic flora and fauna, resource management guidelines must better address metal contamination (Savage 1995; Naeem and Li 1997). A better understanding of the role of DOM in metal complexation processes will aid in the development of these resource management guidelines.

The sensitivity of lakes to metal contamination will be a function of DOM concentration and composition. However, these properties of DOM vary among systems and may change as a function of exposure to transformation and fractionation processes. Therefore, it is crucial to determine how changes in DOM influence metal complexing processes, especially since changes in global climate may increase transformation and fractionation processes, such as photolysis (Kerr and McElroy 1993; Schindler *et al.* 1997).

Defining the effects of DOM transformation and fractionation on metal complexation is critical to the development of site specific metal regulations. If metal sensitive lakes can be identified on the basis of DOM concentration and composition, then more specific management guidelines can be developed and implemented. It is not until the role of organic metal complexation is better understood that the problem of metal contamination can be better regulated.

1.4 Literature Review

1.4.1 Systematic variability of DOM

The concentration and composition of dissolved organic matter (DOM) in aquatic systems is dependent on loading from allochthonous and autochthonous sources and exposure to transformation and fractionation processes. Exposure to transformation and fractionation processes is approximated by hydrologic residence time (HRT; Eq 1.1)

$$\mathrm{HRT}(yr) = \frac{\text{Total Lake Volume}\left(m^3\right)}{\text{Annual Hydrologic Outflows}\left(m^3\, yr^{-1}\right)} \qquad \text{(Eq. 1.1)}$$

For example, changes in colour and dissolved organic carbon (DOC) concentration are inversely dependent on HRT for enclosure experiments (Curtis and Schindler 1997). Similarly, HRT was used to approximate losses of DOM due to mineralization and sedimentation in lakes (Engstrom 1987). Therefore, HRT can be used as an independent variable to investigate the effects of transformation and fractionation processes on organic acidity and organic Cu^{2+} complexation.

Increasing HRT will be associated with a transition from reactive (labile) to less reactive (refractory) DOM (Luider *et al.* in Press). For example, systems that exhibit a long HRT should exhibit an accumulation of photo-refractory DOM and a decrease in photochemical activity (Lindell *et al.* 2000). As DOM is exposed to more ultra violet radiation (UVR), photochemically labile components of DOM will be preferentially consumed in photochemical reactions (Brezonik 1994). The refractory components of DOM will resist photochemical degradation processes and will accumulate over time (Lindell *et al.*

2000). Therefore, the rate of photochemical decomposition processes will decrease over time as refractory DOM accumulates, (Clair and Soyer 1997; Lindell *et al.* 2000).

The same reasoning can be applied to other transformation and fractionation processes. For example, as DOM is decomposed by microbial assemblages, biologically reactive components of DOM will be preferentially consumed and biologically unreactive components will accumulate (Brady et al. 2000). As the labile components of DOM are consumed over time, the bioavailability of DOM is reduced (Leff and Meyer 1991; Brady et al. 2000). There have been similar findings for adsorption of DOM to $Fe(OH)_{3(s)}$ (Luider *et al.* in Press).

This transition from labile to refractory DOM with increasing HRT will vary among aquatic systems because some systems may receive DOM that has been pre-exposed to transformation and fractionation processes. As a result, DOM entering a system may exhibit a high proportion of refractory DOM. Therefore, lakes in series may result in a cumulative effect of HRT. As DOM passes from one lake to the next, the accumulation of refractory DOM would successively increase. This effect can be approximated by cumulative hydrologic residence time (CHRT)(Luider *et al.* in Press).

1.4.2 Allochthonous and Autochthonous DOM

Dissolved organic matter (DOM) is loaded to aquatic systems from allochthonous and autochthonous sources. Sources of allochthonous DOM from the terrestrial environment primarily depend on the chemical and biological breakdown of plant material (Wetzel 1983; Fukushima *et al.* 1996; McKnight and Aiken 1998). Leaching processes in soils will mobilize soluble organics, which will result in influxes of dissolved organic acids to creeks and streams (Singer and Munns 1987). The bulk of these leachates are derived from animal and microbial digestion of plant material (Ertel *et al.* 1984; Singer and Munns 1987; Carter 1996; Fukushima *et al.* 1996; Hessen *et al.* 1997; McKnight and Aiken 1998).

The concentration and composition of allochthonous DOM produced from soils will generally be a function of carbon supply from terrestrial plant material and regional climate (Carter 1996; Hessen *et al.* 1997; Curtis 1998). The type of terrestrial plant material will affect the composition of DOM produced (Ertel *et al.* 1984; Singer and Munns 1987; Carter 1996; McKnight and Aiken 1998). The concentration of DOM produced will depend on the supply of plant material, temperature and soil moisture (Singer and Munns 1987; Carter 1996; Curtis 1998). Subsequently, the mobilization of organic matter will depend on hydrologic flows through the watershed, which will largely be a function of precipitation and basin morphology (Wetzel 1983; Schindler *et al.* 1997; Curtis 1998).

DOM produced from the terrestrial environment consistently has a few characteristic properties (McKnight and Aiken 1998). Allochthonous DOM is usually richly coloured as a result of high aromaticity and is thought to be chemically polyfunctional, which means that it exhibits a variety of chemical functional groups (Meili 1992; Brezonik 1994; Coveney and Wetzel 1995). Although, carboxyl and hydroxyl groups are thought to constitute the bulk of the chemical functional groups for allochthonous DOM (Buffle *et al.* 1990; Manahan 1994).

In contrast to allochthonous DOM, autochthonous DOM is derived internally within a lake system (Wetzel 1983; Fukushima *et al.* 1996). The production of autochthonous DOM is primarily derived from the by-products of primary production within lakes, which is generally dominated by phytoplankton (Wetzel 1983). Factors controlling primary productivity with lakes include nutrient availability, temperature, dissolved oxygen (DO) concentrations and photosynthetically active radiation (PAR) (Wetzel 1983; Roberts *et al.* 1992; Zhang and Prepas 1996). Each of these parameters vary temporally as a result of physical, biological and chemical lake processes and seasonal climate changes (Wetzel 1983). Therefore, the greatest influx of autochthonous DOM will occur when the optimal combination of conditions is achieved, which will generally occur in the spring.

Although the exact composition is difficult to determine, autochthonous DOM is thought to be composed of amino acids, proteins and polysaccharides (Buffle and De Vitre 1994; Brady et al. 2000). These types of molecules exhibit less aromatic character and less colour, as compared to allochthonous DOM (Meili 1992; Buffle and De Vitre 1994; Curtis 1998). These types of molecules are also generally smaller and less complex (Buffle and De Vitre 1994) (Buffle 1990). Carboxyl and hydroxyl functional groups will be present, but in lesser abundance than allochthonous DOM (Zumstein and Buffle 1989; Buffle and De Vitre 1994).

Differences in chemical composition cause allochthonous and autochthonous DOM to function differently. As in any chemical process, the composition of the reactants will determine the outcome of the reaction (Kotz et al. 1994; Radel and Navidi 1994). For example, many components of allochthonous DOM are chemically inactive or refractory and resist transformation and fractionation processes (Filip 1985; Manahan 1994). In contrast, organic compounds released during autochthonous DOM production may be chemically reactive or labile (McKnight and Morel 1979; Wilhelm and Trick 1994; Meador *et al.* 1998). It is useful to consider allochthonous and autochthonous sources of DOM separately because the bulk properties of DOM in aquatic systems can be simplified, even though the bulk of DOM is derived from allochthonous sources (Curtis 1998). The characteristic properties of each source of DOM can be compared as end-members to the function of DOM in surface freshwaters (Luider *et al.* in Press).

1.4.2.1 Isolating allochthonous and autochthonous DOM

Allochthonous DOM can most easily be isolated within creeks and streams because the DOM in these type of systems is usually primarily from allochthonous sources (Cummins *et al.* 1982; Wetzel 1983; Bilby and Bisson 1992). Phytoplankton and attached algae can produce small amounts of autochthonous DOM, but this source of DOM is minimized in smaller streams because photosynthetically active radiation (PAR) is limited by riparian canopy (Wetzel 1983; Bilby and Bisson 1992). Therefore, smaller streams should provide a sufficiently isolated source of allochthonous DOM.

Autochthonous DOM is more difficult to isolate because most systems contain a mixture of DOM from allochthonous and autochthonous sources. The majority of DOM is loaded from allochthonous sources and allochthonous DOM is generally ubiquitous in freshwaters as a result (Wetzel 1983; Zumstein and Buffle 1989; Curtis 1998). One approach to alleviating this problem is to produce autochthonous DOM within an enclosure (Fukushima *et al.* 1996). Primary producers can be isolated in a mesocosm so that the growth environment can be controlled to maximize productivity and allochthonous sources of DOM can be excluded.

1.4.3 Transformation and Fractionation of DOM

1.4.3.1 Photochemical Decomposition

Photochemical decomposition will contribute to changes in the concentration and composition of dissolved organic matter (DOM) over time and will occur directly as a function of UVR (Brezonik 1994; Wetzel *et al.* 1995; Morris *et al.* 1995). UVR is composed of photons of energy according to Planck's Law (Radel and Navidi 1994; Kotz et al. 1994; Brezonik 1994), and DOM contains chromophores that absorb photons of energy, giving rise to photochemical reactions (Brezonik 1994; Clair and Soyer 1997). Photochemical reactions can result in chemical bond rearrangements and bond dissociation, which can result in the transformation and fractionation of DOM in surface freshwaters (Kieber *et al.* 1990; Skoog and Leary 1992; Brezonik 1994).

These types of photochemical processes will alter the chemical and optical characteristics of DOM. Photolysis can result in net losses of carbon, decreases in colour and changes in the chemical composition of DOM, such as a changes in carboxyl character (Kieber *et al.* 1990; Kieber *et al.* 1999; Lindell *et al.* 2000). The degree of these alterations will depend on the initial concentration and composition of DOM in surface freshwaters, the duration of exposure and the intensity of UVR (Mopper *et al.* 1991; Brezonik 1994; Wetzel *et al.* 1995).

Latitudes north and south of the equator will experience seasonal variation in UVR. In northern latitudes, UVR will decrease over winter and increase over summer (Ahrens 1998). Thus, photochemical activity will potentially be maximized throughout the spring and summer. However, transmittance can also regulate availability and intensity. For example, ice and snow cover can reduce the amount of UVR that enters the water column (Wetzel

1983; Ahrens 1998). Similarly, cloud cover and atmospheric particles can result in decreases in UVR (Ahrens 1998). The seasonal variation of these parameters will generally be a function of solar elevation and climate (Ahrens 1998).

Photochemical decomposition of DOM is also coupled to other processes *in situ*, such as photosynthesis and metabolic activity. The UVR and light attenuating properties of DOM can regulate photosynthesis and metabolic activity by regulating the euphotic zone. Highly concentrated and coloured DOM will effectively protect microorganisms from harmful UVR, but will also limit photosynthetic activity (Morris *et al.* 1995; Sommaruga *et al.* 1999). In contrast, low concentrations of weakly coloured DOM may result in a high transmittance of light, which may promote photosynthetic activity, but subsequently inhibit metabolism by increased exposure to harmful UVR (Lind and Hongue 1994).

There is also the possibility of a positive feedback mechanism. Biodegradation may increase the susceptibility of DOM to photochemical reactions (Miller and Moran 1997). Similarly, photo-transformation and fractionation of DOM can produce simple organic molecules that can stimulate microbial activity (Wetzel *et al.* 1995; Moran and Zepp 1997b; Lindell *et al.* 2000). Therefore, photochemical decomposition and microbial decomposition processes may function interdependently to degrade DOM in surface freshwaters.

1.4.3.2 Microbial Decomposition

Microbial decomposition of dissolved organic matter (DOM) will contribute to changes in the concentration and composition of DOM over time, and will occur directly as a function of metabolism. DOM is readily utilized by aquatic microbes, such as bacteria, as a fundamental energy source by processes of assimilation and respiration (Wetzel 1983; Brady et al. 2000). The result of these processes is the complete or partial breakdown of DOM.

Complete breakdown results in the total transformation of an organic molecule and typically occurs as a result of carbon assimilation into microbial cell structures (Moran and Hodson 1990). Assimilated carbon is digested by the mechanism of respiration and therefore, some carbon is lost to carbon dioxide. Assimilated carbon can also be released as microbial exudates and cellular debris, which can result in influxes of simple sugars, proteins and simple fatty acids into the water column (Brady et al. 2000). Influxes of these compounds into the water column (Park *et al.* 1997) (Buffle and De Vitre 1994)may result in changes to the chemical and optical characteristics of DOM (Fukushima *et al.* 1996).

Partial breakdown of DOM occurs by the same process as complete breakdown except only a fraction of an organic molecule is assimilated. The usable fractions of DOM will be preferentially consumed over time (Geller 1986; Sun *et al.* 1997). As portions of an organic molecule are removed and transformed, the original organic molecule is fractionated. Therefore, fractionation may result in a selective decrease in the molecular weight of organic molecules over time, which may influence the characteristic properties of DOM (Leff and Meyer 1991; Sun *et al.* 1997). The chemical and optical characteristics of DOM will likely change over time as a result (Sun *et al.* 1997).

The degree DOM is decomposed will vary as a function of microbial activity. Microbial activity will depend on nutrient availability, the concentration and composition of DOM, temperature, dissolved oxygen (DO) and photosynthetically active radiation (PAR) (Wetzel 1983; Brady et al. 2000). Each of these variables will vary seasonally, which results in temporal variation of microbial decomposition processes. Conditions that will maximize microbial activity within a lake will generally occur after the spring turnover and the spring melt when nutrient availability, DOM and DO concentrations will be maximized (Wetzel 1983). As a result, microbial assemblages will usually exhibit maximum productivity for approximately two months in the spring (Wetzel 1983).

1.4.3.3 Adsorption to Iron Oxyhydroxides and Hydroxides

Adsorption to iron (Fe) oxides will contribute to changes in the concentration and composition of dissolved organic matter (DOM) over time, and will occur directly as function of surface complexation processes (Buffle and De Vitre 1994). Functional groups of DOM can react with Fe oxyhydroxide and hydroxide particle surfaces (Schlautman and Morgan 1994; Buffle and De Vitre 1994; Gu *et al.* 1995). This reaction is known as surface complexation and can result in complete or partial fractionation of DOM, resulting in net losses of DOM from surface freshwaters (Buffle and De Vitre 1994; Lambert and Graham 1995; Luider *et al.* in Press).

The primary source of Fe in lakes arises from the terrestrial environment (Buffle and De Vitre 1994). Thus, the spring melt should result in an influx of Fe to aquatic systems. The majority of ferrous species (Fe^{2+}) are oxidized to ferric species (Fe^{+3}) in surface freshwaters, typically occurring as iron oxyhydroxide and hydroxide ($Fe(OH)_{3(s)}$) precipitates, which can then fractionate DOM (Wetzel 1983; Buffle and De Vitre 1994; Stumm and Morgen 1995).

As Fe particles enter a lake system they will pass through surface freshwaters and settle to the sediment-water interface (Buffle and De Vitre 1994). During settling, chemical adsorption and desorption processes can result in the redistribution of DOM (Buffle and De

Vitre 1994; Gu *et al.* 1994). For example, under high redox conditions Fe^{+3} can complex organic ligands, nutrients and trace metals, resulting in Fe-hydroxide and Fe-phosphate species (Buffle and De Vitre 1994). As the Fe particulates settle through the water column, the ambient redox conditions can fall (Wetzel 1983; Buffle and De Vitre 1994). The subsequent reduction of oxidized iron complexes can result in the release of complexed surface groups (Manahan 1994; Stumm and Morgen 1995). The result is a net loss of DOM from surface freshwaters, and the loss may be specific to organic functional groups that are susceptible to surface complexation.

1.4.4 Copper in Aquatic Systems

1.4.4.1 Sources and effects

More research focus has been given to the topic of Cu^{2+} contamination in the last 20 to 30 years because it is a growing problem and because it can have serious toxicological effects in aquatic environments. Contamination of Cu^{2+} has been steadily increasing over the last 400 years (Sposito 1981; Nriagru 1996). Most aquatic systems have been estimated to exhibit up to a seven fold increase in Cu^{2+} concentrations as compared to background concentrations, which on a global scale has been approximated as 3.9×10^{-8} mol L^{-1} (Nriagru and Pacyna 1988). Furthermore, Cu^{2+} contamination is no longer considered a localized problem. Although global estimates are vague, metal contamination is quickly becoming of global significance (Nriagru and Pacyna 1988).

Increases in Cu^{2+} concentrations within aquatic systems are not directly related to increases in toxicity because it was determined in the 1970's that Cu^{2+} toxicity is strongly correlated to free ionic or labile Cu^{2+} rather than total concentrations. It was determined that free ionic Cu^{2+} within aquatic systems can be regulated by inorganic and organic complexation processes (Brown *et al.* 1974; Pagenkopf *et al.* 1974; Mantoura *et al.* 1978; O'Shea and Mancy 1978). Similar studies followed throughout the 1970's and the early 1980's (Sunda and Guillard 1976; Andrew *et al.* 1978; Sunda and Lewis 1978; Wood 1983; McKnight *et al.* 1983; Borgmann and Ralph 1984). The more recent investigations address site specific parameters, such as pH, water hardness and alkalinity, which can influence Cu^{2+} complexation processes and the subsequent availability of Cu^{2+} to aquatic organisms (Cusimano *et al.* 1986; Lauren and McDonald 1986; Welsh *et al.* 1993; Welsh *et al.* 1996).

Depending on the organism, free labile Cu^{2+} can be toxic in very low concentrations. For example, at concentrations of approximately 2.0×10^{-10} mol L^{-1} dinoflagellates were observed to be 100% non-motile (Anderson and Morel 1978). At higher concentrations, such as 3.2×10^{-5} mol L^{-1}, whole microbial ecosystems can be killed (Meador *et al.* 1998). In contrast, the freshwater algal species *Oocystis pusilla* was found to be uninhibited by free labile Cu^{2+} concentrations ranging from 8×10^{-6} to 20×10^{-6} mol L^{-1} (Meador *et al.* 1998).

It should also be noted that Cu^{2+} is a micronutrient (Wood 1983; Wetzel 1983; Buffle and De Vitre 1994; Azenha *et al.* 1995). Therefore, a sensitive balance exists between deficiency and excess of available Cu^{2+} concentrations. The uptake and resulting toxicological effect of Cu^{2+} also varies among organisms (Ariza et al. 1999). In general however, the environmentally relevant range, from a toxicology perspective, seems to be from Cu^{2+} concentrations of approximately 1.0×10^{-6} mol L^{-1} down to the detection limits of most analytical techniques.

1.4.4.2 *Complexation of Cu^{2+} by DOM*

The availability of complexed Cu^{2+} varies according to the type of complex that is formed (Buffle 1990). Under typical pH and redox conditions the aqua Cu^{2+} complex ($Cu(H_2O)_4^{+2}$) will dominate, which is considered as free ionic or labile Cu^{2+}(Meador 1991; Kotz et al. 1994; Bricker and Jones 1995). However, Cu^{2+} can also form complexes that are unavailable to aquatic organisms (Wetzel 1983; Buffle 1990; Manahan 1994; Milne *et al.* 1995) because Cu^{2+} can be complexed by a variety of different ligands and bonding arrangements (Kettle 1969; Wulfsberg 1987; Radel and Navidi 1994). For example, if a ligand binds to more than one site on a metal cation, then it is referred to as a chelating agent (Kettle 1969; Buffle 1990; Martell and Hancock 1994). These different chemical and structural combinations of ligands will determine the availability of complexed Cu^{2+} to undergo other reactions, which is usually discussed in terms of thermodynamic and kinetic stability (Buffle 1990; Martell and Hancock 1994). Cu^{2+} will preferentially reside in the most stable form possible, which is why weakly complexed Cu^{2+}, such as $Cu(H_2O)_4^{+2}$, can be bioavailable (Buffle 1990; Buffle and De Vitre 1994; Martell and Hancock 1994).

Thermodynamic stability will depend inversely on the free energy associated with a metal complex under equilibrium conditions (Radel and Navidi 1994; Kotz et al. 1994). Complexation reactions that are associated with low free energy will be more stable, and thus more thermodynamically preferential (Kettle 1969; Buffle 1990; Kotz et al. 1994). Kinetic stability involves the mechanism that facilitates the complexation reaction (Radel and Navidi 1994; Kotz et al. 1994). A reaction may be thermodynamically favoured, but is limited by a viable mechanism that will produce thermodynamically stable end products (Kettle 1969; Kotz et al. 1994).

Thermodynamic stability depends on two types of interactions between the metal and the ligand. The first is the electrostatic and covalent interaction between the metal and ligand (Buffle 1990; Kotz et al. 1994). The second is the coordination geometry between the metal and ligand, which involves a discussion of chelation (Wulfsberg 1987; Radel and Navidi 1994; Kotz et al. 1994).

One way of approximating the electrostatic and covalent interaction between a metal and ligand is by hard and soft character (Kettle 1969; Cotton and Wilkinson 1976; Wulfsberg 1987; Buffle and De Vitre 1994). Hard cations preferentially participate in electrostatic interactions and will bind with hard donor atoms. Soft cations preferentially participate in covalent interactions and bind with soft donor atoms (Cotton and Wilkinson 1976; Wulfsberg 1987; Buffle and De Vitre 1994).

The hardness of cations is determined on the basis of charge and radius (Cotton and Wilkinson 1976; Wulfsberg 1987; Buffle and De Vitre 1994). Small, highly charged cations, such as hydrogen, will be compact and resist electrostatic polarization (Cotton and Wilkinson 1976; Radel and Navidi 1994; Buffle and De Vitre 1994). These metals include the group I and II metals. In contrast, soft cations include the heavier transition metals, such as mercury and lead, which are usually larger, exhibit more complex valence configurations and tend to be more polarizable (Cotton and Wilkinson 1976; Radel and Navidi 1994; Buffle and De Vitre 1994). Cu^{2+} has both hard and soft characteristics and will function as both a hard and soft metal cation (Cotton and Wilkinson 1976; Wulfsberg 1987; Buffle and De Vitre 1994).

Hard and soft ligand donors can be separated into two groups; inorganic and organic. Hard inorganic ligands in natural waters will generally include simple molecules, such as carbonates, sulfates and phosphates, which are rich in oxygen (Buffle and De Vitre 1994).

Hard organic ligands include carboxyl, hydroxyl and phenolic groups, which are also rich in oxygen (Buffle and De Vitre 1994). In contrast, soft inorganic and organic ligands will include nitrogen and sulfur species, such as sulfhydryl (-SH) groups (Wetzel 1983; Wulfsberg 1987; Buffle and De Vitre 1994).

The interaction of hard metals and ligands does not usually produce stable complexes, probably because of thermodynamic or kinetic instability. In contrast, soft metals can form very stable complexes with soft ligands (Cotton and Wilkinson 1976; Wulfsberg 1987; Buffle and De Vitre 1994). The main difference between hard and soft metal interactions is the covalent nature of the bonds involved. Soft metals are larger, more easily polarized, and react with soft ligands that readily exhibit a lone pair of electrons (Kotz et al. 1994; Radel and Navidi 1994; Buffle and De Vitre 1994). These conditions promote strong covalent interactions, which may increase the stability of metal complexes (Kotz et al. 1994; Radel and Navidi 1994). Therefore, the covalent nature of soft metal interactions likely contributes to stability, and may be a useful consideration when investigating Cu^{2+} complexation processes.

Chelation reactions can also increase thermodynamic stability because a ligand binds to a central metal ion in two or more places (Martell and Hancock 1994). Stability is increased when donor groups of the ligand are approximately positioned to coordinate the central metal ion (Kotz et al. 1994; Martell and Hancock 1994). The free energy of the complex is lowered "because of a built in rigidity [that] results more or less in the donor groups being frozen into a position favorable for complex formation" (Martell and Hancock 1994). Thus, chelating agents are usually large molecules with a functional group orientation that may favour metal complexation.

A good example of a chelating agent is ethylenediaminetetraacetate (EDTA). EDTA contains six potential binding sites that can interact with a metal ion (Manahan 1994; Radel and Navidi 1994). Four of these sites are carboxylic groups, which would be considered hard donors that would not preferentially react with soft metals (Buffle and De Vitre 1994; Radel and Navidi 1994). However, EDTA is one of the strongest known complexing agents of heavy metal species, such as Cu^{2+}, Pb^{2+} and Hg^{2+} (Morel and Hering 1993; Manahan 1994).

Therefore, the stability of Cu^{2+} complexes will depend on a combination of variables. The electrostatic and covalent interactions between Cu^{2+} and ligands of DOM may influence complex stability. However, large molecules with a plethora of potential donor sites, such as organic acids of DOM, may chelate Cu^{2+}, which can increase stability. Therefore, chelation processes may also be important to consider when investigating Cu^{2+} complexation processes.

The stability of metal complexes is conventionally quantified by the formation constant (K_f) of the complexation reaction, which is essentially equal to the concentration of the reaction products [Cu-Ligand$_i$] divided by the concentration of the reactants [Cu^{+2}; Ligand] under equilibrium conditions (Radel and Navidi, 1994; Kotz et al, 1994)(Equation 1.2).

$$K_f = \frac{[Cu - ligand_i]}{[Cu^{+2}][Ligand]^i} \qquad \text{(Eq 1.2)}$$

Metals will preferentially reside in the most stable form possible. Therefore, a strong shift in complexation reactions to the metal complex side of the equilibrium indicates a preferentially stable state, which is quantified by a higher K_f. A good example is the reaction between free ionic Cu^{2+} and the chelating agent EDTA. The formation constant for EDTA and Cu^{2+} is approximately 3.16×10^{20}, which means that under equilibrium conditions essentially all free ionic Cu^{2+} is complexed by EDTA (Morel and Hering 1993).

Since K_f values also provide a measure of stability, they approximate the susceptibility of organically complexed Cu^{2+} to competing ligands, such as biological surfaces (Buffle and De Vitre 1994). For example, EDTA may complex Cu^{2+} bound by DOM because the K_f value associated with EDTA-Cu complexes is high. Similarly, if biological surfaces provide higher stability, then Cu^{2+} will preferentially bind to the biological surfaces (Buffle and De Vitre 1994).

These types of substitution reactions will be governed by the dissociation and formation kinetics between each stepwise reaction in the overall process (Kettle 1969; Kotz et al. 1994; Radel and Navidi 1994). However, not all reactions are reversible. Thus, more thermodynamically stable situations may exist, but a mechanism to facilitate the reaction may not. Stable complexes will be unlikely to undergo dissociation because the activation energy required to facilitate the reaction is high (Kotz et al. 1994; Radel and Navidi 1994). In contrast, unstable complexes, such as many simple inorganic complexes, will readily undergo dissociation reactions and more thermodynamically stable organic metal complexes may result (Morel and Hering 1993; Brezonik 1994).

The overall interaction between metal cations and ligands will depend on the ambient chemical conditions. Dissociation and formation reactions will be influenced by pH, group I and II metal concentrations and redox potential (Wetzel 1983; Buffle and De Vitre 1994; Brezonik 1994). Hydrogen (H^+) behaves like a hard metal and will not necessarily form strong complexes; however, at low pH values the concentration of H^+ will drastically exceed trace metal concentrations, which will result in strong competition for ligands (O'Shea and Mancy 1978; Morel and Hering 1993). Even if only a small proportion of H^+ are active, the majority of ligands will become protonated.

High pH values can influence the interaction between metals and ligands through the formation of inorganic metal hydroxide complexes. Cations in solution act as Lewis acids, which results in aqua metal complexes (Kotz et al. 1994; Radel and Navidi 1994). The hydroxide ion is an excellent Lewis base (Kotz et al. 1994; Radel and Navidi 1994). As hydroxide concentrations increase with increasing pH, metal hydroxides will be preferentially formed. Depending on the Lewis character of the transition metal, hydroxide precipitates will form with increasing pH (Kotz et al. 1994; Radel and Navidi 1994).

Group I and II metal cations can also result in competitive effects. Group I and II metals, such as sodium, magnesium and calcium, can be present in moderately high concentrations (Wetzel 1983). These metals can influence trace metal complexation reactions in two ways. Firstly, group I and II metals can compete for complexing sites, either on DOM or biological surfaces (Playle and Dixon 1993; Richards and Playle 1998). The resulting complexes are usually weak and probably do not significantly affect Cu^{2+} toxicity (Lauren and McDonald 1986; Buffle and De Vitre 1994; Brezonik 1994; Erickson *et al.* 1996). Secondly and most importantly, high concentrations of hard metals can influence the polyelectric field at the surface of organic molecules (Tipping 1993; De Wit *et al.* 1993a; Buffle and De Vitre 1994; Milne *et al.* 1995). The net negative character and chemical function of DOM as a metal complexing agent may be reduced as a result.

Finally, redox conditions can influence complexation processes by affecting the oxidation states of metals and ligands (Buffle 1990; Stumm and Morgen 1995). For example, interactions between Cu^{2+} and Fe hydroxide surfaces may change according to redox conditions. Under sufficiently negative redox conditions iron hydroxide can be reduced from ferric to ferrous iron species, which may subsequently react to form reduced species, such as iron sulphides (Wetzel 1983; Buffle and De Vitre 1994). Cu^{2+} will likely be released when these reduced iron species form and similar reactions may occur with ligands of DOM.

1.4.4.3 Measuring Organic Acidity and Cu^{2+} Complexation

Copper (Cu^{2+}) complexation by DOM can be quantified by a number of different approaches. One approach is organic acidity, which accounts for the concentration and composition of organic acids that comprise DOM (Lydersen 1998). Another approach is to directly quantify organic Cu^{2+} complexation (Buffle *et al.* 1977; Saar and Weber 1980; Hales *et al.* 1999). Potentiometric titration analyses can be used in each approach (Midgely and Torrance 1991), thus providing information on organic pH buffering and Cu^{2+} complexation. Furthermore, only a fraction of organic acids will realistically contribute to Cu^{2+} complexation. This active fraction of organic acids can be quantified by comparing measures of organic acidity to measures of Cu^{2+} complexation.

Organic acidity is measured by titrating samples of acidified DOM with sodium hydroxide (NaOH) and recording the subsequent changes in pH with a pH electrode. Concentrations of organically complexed protons (H^+) are then calculated by difference of added hydroxide (OH^-) ion concentrations and measured changes in pH according to the water formation constant (pK_w) to produce a charge density measure (CD). The resulting titration data is modeled to produce total charge density (CD_T) and acid dissociation (pK_a)

parameters (Milne *et al.* 1995; Lydersen 1998), which provide an approximation of total ionizable binding site density and the pH of binding site ionization respectively.

The determination of CD_T and pK_a parameters is limited by sensitivity to low concentrations of organically bound H^+ because the relevant pH range of organic acid dissociation is generally from 2.5 to 4.5 (Leuenberger and Schindler 1986; Tipping and Hurley 1992; De Wit *et al.* 1993b; Driscoll and Lehtinen 1994; Lydersen 1998), and because pH measurements are typically only accurate to ±0.1 pH units (Midgely and Torrance 1991). These accuracy limitations correspond to H^+ ion concentrations that range from 1.5×10^{-3} to 2.0×10^{-4} M respectively from pH 2.5 to 3.5. Concentrations of organically complexed H^+ must sufficiently exceed these concentrations to measure organic acidity with confidence.

Organic Cu^{2+} complexation is measured similarly to organic acidity. Samples of DOM are titrated with copper sulfate ($CuSO_4$) and the subsequent increase in the concentration of Cu^{2+} ions is measured with a cupric ion selective electrode (ISE). Concentrations of organically complexed Cu^{2+} are then determined by difference of added and measured Cu^{2+} according to Nernstian response of the ISE (Midgely and Torrance 1991). The resulting titration data is typically modeled to produce complexing capacity (CC) and conditional stability (Log(K)) parameters (Bresnahan *et al.* 1978; McKnight *et al.* 1983; Xue and Sunda 1997; MacRae *et al.* 1999b), which empirically correspond to the density of complexed Cu^{2+} (CuL) for a given concentration of free labile Cu^{2+} (Buffle 1990).

Potentiometric analyses of organic Cu^{2+} complexation are sensitive to experimental conditions, such as temperature and most importantly, the ionic composition of the sample matrix (Avdeef *et al.* 1983; Belli and Zirino 1993), which is why addition of an ionic strength adjuster is typically recommended (Midgely and Torrance 1991). The ionic composition of the sample matrix will influence processes of Cu^{2+} complexing by a change in Cu^{2+} ion activity. Similarly, cationic species in solution, especially other transition metals, can compete with Cu^{2+} for binding sites (Lauren and McDonald 1986; Meador 1991; Tipping 1993), and interfere with the Nernstian response of the ISE by changing the activity coefficient for Cu^{2+} (Midgely and Torrance 1991). Therefore, variation in the ionic composition of the sample matrix among samples can increase instrumental variation, which is a particularly important consideration when low concentrations of free ionic Cu^{2+} are being investigated (Avdeef *et al.* 1983).

It is important to consider low concentrations of free ionic Cu^{2+} because environmentally significant levels of Cu^{2+} complexation by DOM should be addressed. It is ligands that have a high affinity for Cu^{2+} that will effectively reduce the availability of Cu^{2+} in surface freshwaters by forming stable complexes (Vasconcelos *et al.* 1997; Marr *et al.* 1999). Furthermore, Cu^{2+} toxicity ranges widely from approximately 1.0×10^{-10} to 1.0×10^{-6} M among aquatic biota. Concentrations of high affinity ligands that buffer Cu^{2+} to within this range may be below the detection limits of potentiometric titration analyses in some surface freshwaters (Buffle *et al.* 1990). Therefore, potentiometric titration analyses may not provide an accurate approximation of organic Cu^{2+} complexation *in situ* for some surface freshwaters because of sensitivity limitations to high affinity Cu^{2+} binding sites.

1.4.4.4 Pretreatment requirements

The sensitivity of potentiometric titration analyses to organically complexed H^+ concentrations and high affinity Cu^{2+} sites can be maximized by increasing the total concentration of organic acids and exposing samples to a cation exchange resin (CER). The total concentration of organic acids can be increased by sample concentration in a reverse osmosis (RO) concentrator (Serkiz and Perdue 1990; Clair *et al.* 1991), and will increase the signal to noise ratio for potentiometric analyses. Exposure of samples to the CER replaces inorganic cations in solution, such as Mg^{+2} and Ca^{+2}, with hydrogen ions, which will minimize differences in the ionic composition of sample matrices, thus reducing instrumental variation.

Each of the pretreatment processes may increase the sensitivity of potentiometric titration analyses, but may also impose certain limitations that are relevant to an investigation of Cu^{2+} complexation. Sample concentration by RO will increase ionic strength, which may change the spatial orientation of potential Cu^{2+} binding ligands to account for the increased concentration of repulsive and attractive forces. Similarly, exposure of samples to the CER may alter Cu^{2+} complexation chemistry by reducing competition with other cations. Therefore, results of potentiometric analyses are subject to these experimental conditions.

1.5 Conclusions of Introduction and Literature Review

Dissolved organic matter (DOM) is composed of organic acids and is an important regulator of trace metals, such as Cu^{2+}, in surface freshwaters. Ligands of DOM can complex Cu^{2+} and these complexation processes will vary according to the concentration and composition of organic acids in aquatic systems. The overall objective of this thesis is to identify systematic variation in organic acidity and Cu^{2+} complexation processes as a function of DOM loading and increasing hydrologic residence time (HRT). DOM loading from allochthonous and autochthonous sources will influence the initial concentration and composition of DOM and HRT provides a good approximation of DOM exposure to transformation and fractionation processes. Three of these processes include photochemical decomposition, microbial decomposition and adsorption, all of which can change the concentration and composition of DOM over time.

The first step in addressing the overall objective of this thesis is to determine how to measure organic acidity and Cu^{2+} complexation by DOM. These issues are considered in chapter 2 of this thesis, where the reproducibility H^+ and Cu^{2+} potentiometric titration analyses is measured, and the effects of increasing dissolved organic carbon (DOC) concentration on potentiometric titration analyses is investigated.

The second step in addressing the overall objective of this thesis is to test the hypothesis that organic acidity and Cu^{2+} complexation vary systematically as a function of DOM loading and HRT. This hypothesis is tested in chapter 3 of this thesis, where the Cu^{2+} complexing properties of DOM are compared among samples of allochthonous and autochthonous DOM and among samples of DOM from surface freshwaters spanning a gradient in cumulative hydrologic residence time (CHRT). In addition, DOM is treated in micro-reactors that maximize rates of photochemical decomposition, microbial decomposition and adsorption processes to better determine how each process contributes to changes in organic Cu^{2+} complexation in surface freshwaters.

2. Chapter 2. Allochthonous and autochthonous dissolved organic carbon (DOC) concentration dependence of proton and copper(II) potentiometric titration analyses

2.1 Abstract

The dependence of proton (H^+) and copper (Cu^{2+}) potentiometric titration analyses on dissolved organic carbon (DOC) concentration was investigated for allochthonous and autochthonous sources of dissolved organic matter (DOM) using a Langmuir Isotherm model. Parameter values and their reproducibility from the model were calculated for a gradient of DOC concentrations ranging from approximately 5 to 435 C-mg L^{-1}. Organic acid composition was controlled by concentrating samples in a reverse osmosis (RO) concentrator and then serially diluting samples to produce the DOC concentration gradient. Results indicate that the reproducibility of charge density (CD) and acid dissociation (pK_a) parameters is limited by the accuracy of a pH electrode at ambient DOC concentrations in the majority of aquatic systems. The reproducibility of Cu^{2+} complexing capacity (CC_1, CC_2) and conditional stability ($Log(K_1)$, $Log(K_2)$) parameters is mainly limited by a low density of strong Cu^{2+} binding sites, particularly in the case of autochthonous DOM. In addition, condition stability parameters ($Log(K_1)$, $Log(K_2)$) are inversely dependent on titration end-point concentrations of free labile Cu^{2+}. The result is higher $Log(K_1)$ and $Log(K_2)$ values with lower titration end point concentrations. This effect is similar to the dependence of parameter values on DOC concentration with respect to Cu^{2+} complexation, which is consistent with a combination of two processes; multidentate complexation of Cu^{2+} and occlusion of Cu^{2+} binding ligands. Regardless of the mechanism, DOC concentration could explain 20% to 60% of the reported variation in parameter values of the Langmuir Isotherm model. Therefore, DOC concentration should be considered in the comparison of organic H^+

and Cu^{2+} complexation processes among different systems or sources of DOM. This research indicates that allochthonous DOM exhibits a higher total charge density (CD_T) and a higher density of higher affinity Cu^{2+} complexing sites, as compared to autochthonous DOM.

2.2 Introduction

Dissolved organic matter (DOM), typically measured analytically as dissolved organic carbon (DOC), is partly composed of organic acids that buffer pH and contribute to the complexation of trace metals in surface freshwaters (Perdue and Lytle 1983b; De Wit *et al.* 1993b; Lydersen 1998). The ability of DOM to complex metals has been studied for some time and is one of the most important factors affecting trace metal complexation in aquatic systems (McKnight *et al.* 1983; Buffle 1990; Buffle and De Vitre 1994). Organic acidity and metal complexation processes are of particular concern in surface waters of freshwater ecosystems because of acidic rain and anthropogenic metal contamination (Nriagru and Pacyna 1988; Urban *et al.* 1990; Lydersen 1998).

The ability of DOM to control pH and to complex metals, such as copper (Cu^{2+}), will vary according to the concentration and composition of weak organic acids (Morel and Hering 1993; Buffle and De Vitre 1994; Buffle and De Vitre 1994). These properties of DOM are typically investigated directly by potentiometric titration analyses because free labile (or non-complexed) H^+ and Cu^{2+} ion activity can be measured under the appropriate experimental conditions with a pH and cupric ion selective electrode (ISE), respectively (Sjöberg and Lövgren 1993; Lydersen 1998). Experimental conditions, such as pH, temperature and ionic strength, must be controlled, and measurements should be made within the appropriate sensitivity window for potentiometric titrations to be as reproducible and accurate as possible (Midgely and Torrance 1991; Hales *et al.* 1999).

Measurements of proton (H^+) activity in water are routinely conducted between pH 3 and 10 (Leuenberger and Schindler 1986; Ephraim *et al.* 1989; Midgely and Torrance 1991), but the literature is less conclusive for measurements of free labile Cu^{2+}. A cupric ISE can be calibrated to produce a linear Nernstian response between pCu 3 (1.0×10^{-3} mol L^{-1}) and 19 (1.0×10^{-19} mol L^{-1}) in buffered solutions because excess ligands counter the dissolution of Cu^{2+} from the electrode surface that cause drift in response (Avdeef *et al.* 1983; Hales *et al.* 1999). Manufacturers however, recommend that the lower end of the linear calibration range is approximately between 1.0×10^{-7} and 1.0×10^{-8} mol L^{-1} in non-buffered solutions. The latter corresponds to a range that is more typically used for collecting potentiometric titration data because of the relative ease in the calibration procedure and the relevance to natural systems (Cabaniss and Shuman 1988; Xue and Sunda 1997; Hales *et al.* 1999; MacRae *et al.* 1999b).

Potentiometric data for titration curves of DOM reflect a continuous distribution of organic ligand types that can be discretely modeled to empirically investigate the average bulk properties of organic ligands. Dissociation of organically bound H^+ from organic acids is pH dependant and is measured as charge density (CD), which can be modeled with a Langmuir Isotherm model to approximate an acid dissociation (pK_a) parameter (Buffle 1990). Similarly, Cu^{2+} complexation and free labile Cu^{2+} activity can be approximated for the determination of complexing capacity (CC_1, CC_2) and conditional stability ($\log(K_1)$, $\text{Log}(K_2)$) parameters. A minimal number of free parameters are included in the model according to tests of significance using least-squares regression analyses, and therefore, these parameters do not reflect specific ligand classes, but rather an empirical means of comparison (Perdue and Lytle 1983b; Town and Filella 2000).

The reproducibility of parameter values from the Langmuir Isotherm model depends on the sensitivity of potentiometric titration analyses to organically complexed H^+ and Cu^{2+} ions. This issue is particularly relevant to titrations of H^+ complexation because the bulk of organic acid dissociation typically occurs below pH 3 (Lydersen 1998). Therefore, most of the acid dissociation occurs throughout a low pH range where small changes in proton activity are difficult to discern from the high background signal of H^+ ions. Cu^{2+} titrations may have similar sensitivity limitations in weakly buffered samples with low total concentrations of Cu^{2+} binding sites (Avdeef *et al.* 1983), as indicated by a low DOC concentration and a low density of Cu^{2+} binding ligands (Midgely and Torrance 1991).

The upper and lower extreme of organic Cu^{2+} complexation is generally observed in DOM that is derived from the catchment (allochthonous), and DOM that is derived from primary production (autochthonous), respectively (McKnight and Aiken 1998; Richards *et al.* 2001). The bulk of DOM in aquatic systems originates from allochthonous sources (Meili 1992) and is characteristic of a higher complexing capacity (CC_1,CC_2) of higher affinity binding sites (higher $Log(K_1)$ and $Log(K_2)$), as compared to autochthonous DOM (Town and Filella 2000). Consequently, there is greater precision for measurements of Cu^{2+} complexation in allochthonous DOM. However, current research suggests that Cu^{2+} binding varies among systems (Hirose 1994; Rozan and Benoit 1999), and ambient concentrations of DOC vary significantly among surface water samples, ranging from less than 1 to over 100 C-mg L^{-1} in extreme cases (Curtis and Adams 1995; Curtis and Schindler 1997; Curtis 1998). Titration analyses conducted on samples with low Cu^{2+} binding density and low DOC concentrations may not accurately reflect complexation reactions that are relevant to real world concentrations of Cu^{2+}, which have been approximated at 3.97×10^{-8} (SD = 4.35E-08) mol L^{-1} (Wetzel 1983; Borg 1995).

Sample concentration by reverse osmosis (RO) and exposure to a cation exchange resin (CER) increases the sensitivity of potentiometric titration analyses to organically bound

H^+ and Cu^{2+} ions by increasing the total concentration of organic acids, and particularly high affinity Cu^{2+} complexing sites (Serkiz and Perdue 1990; Clair *et al.* 1991). However, sample concentration imposes potential limitations to the interpretation of Cu^{2+} titration data because a 1:1 ligand-metal ratio is typically assumed (Buffle 1990; Morel and Hering 1993). This ratio may increase as a function of DOC concentration because of physicochemical changes to organic ligands of DOM. For example, Cu^{2+} may undergo processes such as multidentate complexation or binding site occlusion with increasing DOC concentration. Multidentate complexation would be consistent with an increased affinity for Cu^{2+} complexation (higher $Log(K_1)$ and $Log(K_2)$) and both processes would be consistent with a decrease in complexing capacity (lower CC_1 or CC_2) (Manahan 1994; Stumm and Morgen 1995).

There were a number of objectives to this research. The first objective was to assess the reproducibility of H^+ and Cu^{2+} potentiometric titration analyses, with particular reference to organic acid dissociation below 3 and high affinity Cu^{2+} complexing sites. The second objective was to determine the sensitivity of the Langmuir Isotherm model to titration end-points of free labile Cu^{2+} concentration. Titration data were modeled with the Langmuir Isotherm at each successive titration point for samples of DOM. The third objective was to test for effects of increasing DOC concentration on organic Cu^{2+} complexation, as indicated by parameter values of the Langmuir Isotherm model. We controlled for organic acid composition by concentrating and then serially diluting samples of DOM to provide a DOC concentration gradient. All analyses were compared among split samples of allochthonous and autochthonous DOM to determine the influence of organic acid composition on each research objective.

2.3 Methods

2.3.1 Sample Collection and Preparation

Allochthonous dissolved organic matter (DOM) was collected from a first order stream in the Okanagan Basin of British Columbia Canada (50° 47' N, 15° 40' W). Autochthonous DOM was produced in a clear acrylic mesocosm (1 m^3) with almost 100% light transmittance throughout the visible range. The mesocosm was filled with dissolved organic carbon (DOC)-free deionized water, amended with salts and nutrients, and inoculated with plankton collected onto a glass fiber filter (Whatman GF/C) from a eutrophic lake. Reagent grade salts were added to simulate 1/10th of the concentrations of global average river water (Wetzel 1983). Concentrations of phosphorus were amended to 0.1 mg L^{-1}, but no nitrogen was added to favor the growth of cyanobacteria, with the dominant taxon being *Anabaena sp.* The mesocosm was stored outdoors in a locked compound for four months (from May to August), where it was exposed to ambient lighting conditions. *In vivo* fluorescence, measured at a wavelength of 660nm with an excitation wavelength of 436nm on a Shimadzu RF 1501 spectrofluorophotometer, and DOC concentration were measured weekly to monitor the growth of the plankton community and production of autochthonous DOM. Autochthonous DOM was collected at the beginning of the stationary phase of plankton growth.

Approximately 200 L of water from allochthonous and autochthonous sources were collected and filtered through 142mm-glass fiber filters to remove seston, however colloidal and bacterial size fractions are generally not removed by this process. The samples were then concentrated using a stainless steel reverse osmosis (RO) concentrating unit (Limnological Research Corp., Kelowna, B.C.). The RO unit consisted of a stainless steel

Delcon centrifugal pump, a stainless steel holding tank and a Filmtec FT30 US Filters thin composite RO membrane housed in a stainless steel cartridge. Operating pressure was between 700 and 1200 kPa. The permeate was discarded and the retentate was recycled to a final volume of 5 to 10 L. Concentrated sample was drained into acid washed glass bottles and stored at 4°C.

Concentrated samples were exposed to an Amberlite IR-118H cation exchange resin (CER) to replace potentially interfering cations with H^+ and to fully protonate organic acids. The resin was replenished with 4 mol L^{-1} HCl and washed ten times with ultra pure water between each sample treatment. Leaching of organics and inorganics from the resin was negligible after the tenth wash with ultra pure water. The resin was added to samples until further additions failed to lower pH (generally about pH 2).

Concentrated samples that were exposed to the cation exchange resin were then serially diluted to produce a concentration gradient of DOC for allochthonous and autochthonous DOM. DOC concentrations ranged from 3.7 to 437.6 C-mg L^{-1} for allochthonous DOM and from 3.5 to 435.1 C-mg L^{-1} for autochthonous DOM. Diluted samples were then split into triplicate samples for each analysis.

DOC concentration was measured for all samples with a Shimadzu TOC-5000A Total Organic Carbon Analyzer. Dissolved inorganic carbon was removed by acidifying samples ($pH < 2$) with select grade hydrochloric acid (2N HCl) and purging with oxygen for seven minutes before analysis. The coefficient of variation for instrumental response was consistently less than 2% and the detection limit of the instrument was 0.05 C-mg L^{-1} at an injection volume of 750 μL.

2.3.2 Organic Acidity

Organic acidity was measured by titrating 20 mL of each diluted sample with 0.05 mol L^{-1} NaOH (standardized to 0.05 mol L^{-1} potassium hydrogen phthalate (KHP)) from pH 2.0 to 6.0 using a QC Mantech Autotitrator and a glass double junction Ag-Ag/Cl pH electrode. The pH electrode was calibrated using the autotitrator with stabilization criteria of ± 0.05 pH/min according to a 3-point calibration curve (pH 2, 4 and 7; $p < 0.01$, $r^2 = 0.99$)(Ephraim *et al.* 1989). Experimental conditions used for calibration were the same as for sample analyses, including ambient temperature (20.1°C, SD = 0.8°C, n = 2848) and constant lighting conditions with a final amended ionic strength of 0.1 mol L^{-1} $NaNO_3$ (Midgely and Torrance 1991; Sjöberg and Lövgren 1993).

Charge density (CD) and acid dissociation (pK_a) parameters were calculated for each sample from corrected titration data between pH 2.0 and 6.0 (Equations 2.1, 2.2 and 2.3). Titration data above pH 6 were not included in the model because there was a negligible increase in CD. Corrections to titration data included background inorganic OH^- consumption from the formation of H_2O and the dissociation of HSO_4^-. Sulfate (SO_4^{2-}) concentrations required for HSO_4^- corrections were measured using a Waters 501 HPLC with a Waters 431 Continuity detector and a Waters IC PAC 4.6 x 50mm anion column.

$$CD = \left[\frac{(OH_A)-(OH_{BC})-(OH_m)}{DOC}\right] \qquad \text{Eq 2.1}$$

where, OH_A is equal to OH^- added (mol L^{-1}), OH_{BC} is equal to the adjustment for inorganic OH^- consumption (mol L^{-1}), OH_m is equal to OH^- measured (mol L^{-1}), and DOC is in units of C-g L^{-1}. Total charge density (CD_T) was calculated operationally as CD from pH 2 to 6 and is expressed as mol C-g^{-1}.

The acid formation parameter (K_b) was calculated using Systat 8.0 non-linear regression analyses according to equation 2.2.

$$CD = CC\frac{K_b[M]}{1+K_b[M]} \qquad \text{Eq 2.2}$$

where CD is equal to charge density (mol C-g^{-1}), CC is complexing capacity (mol C-g^{-1}), which is equivalent to CD_T, and M is equal to OH_m (mol L^{-1}). All non-linear regression analyses were significant ($p < 0.01$) with only two free parameters in the model. Subsequently, the acid dissociation parameter (pK_a) is calculated according to equation 2.3 and was found to be consistent with empirical titration curves in each case.

$$pK_a = 14 - \log(K_b) \qquad \text{Eq 2.3}$$

2.3.3 Copper Titrations

Copper (Cu^{2+}) complexation was investigated by titrating 20 mL of each sample with 1.0E-05 mol L^{-1} $CuSO_4$ (standardized to 1.0E-05 mol L^{-1} ethylenediamine tetraacetate (EDTA)) using a QC Mantech Autotitrator with an Orion solid state ion selective electrode (ISE) and a Ag-Ag/Cl sure flow double junction reference half electrode. Free labile Cu^{2+} concentrations were determined from electrode response by calibrating the electrodes from 5.0×10^{-8} to 1.0×10^{-6} mol L^{-1} Cu^{2+} using the autotitrator with stabilization criteria of ± 0.1

mV/min. The electrodes were conditioned with EDTA, rinsed with ultra pure water, and the Cu^{2+} ISE was polished between each titration to minimize electrode fouling and carry over of organic or inorganic contaminants. Nernstian response was consistently observed (29.5 ± 0.4 mV pCu^{-1}, n = 5) under the same operational experimental conditions used for sample titrations.

Samples were prepared for titration by adjusting ionic strength to 0.1 mol L^{-1} with $NaNO_3$ to overwhelm small differences in ionic strength. In addition, pH was adjusted to 6 (± 0.1 pH units) for comparison with previous research, to avoid interference's due to inorganic speciation (Midgely and Torrance 1991), and because organic acid dissociation was negligible at higher pH values. pH was adjusted under equilibrium conditions with atmospheric CO_2, let stand for 2-3 hours and re-adjusted prior to running titrations with stabilization criteria of ± 0.05 pH/min. In addition, samples were titrated at room temperature (20.1°C, SD = 0.8°C, n = 2848) and under constant lighting conditions.

The titration end-point was operationally set to 1.0 μmol L^{-1} free labile Cu^{2+}, as determined by the response of the ISE, because higher free labile Cu^{2+} concentrations are not relevant to typical freshwaters (Rozan and Benoit 1999). Free labile Cu^{2+} concentrations below the lower end of the linear calibration curve were approximated assuming Nernstian response from the ISE because Cu^{2+} was buffered by excess ligands of DOM (Avdeef *et al.* 1983; Hales *et al.* 1999). This assumption is conservative with respect to the Cu^{2+} complexing properties of DOM because dissolution of Cu^{2+} from the surface of the ISE results in an underestimate of complexed Cu^{2+}.

Complexing capacity (CC_1, CC_2) and conditional stability ($\log(K_1)$, $\log(K_2)$) parameters were calculated from titration data by correcting for initial residual Cu^{2+} that was not exchanged in the CER process, and then modeling titration data using a 2-ligand Langmuir Isotherm discrete binding model (Buffle 1990)(Equation 2.4).

$$v = CC_1 \frac{K_1[M]}{1+K_1[M]} + CC_2 \frac{K_2[M]}{1+K_2[M]} \qquad \text{Eq 2.4}$$

where, v is equal to the moles of bound copper (Cu^{2+}) per gram of carbon (Cu-mol C-g^{-1}), CC is equal to complexation capacity (mol C-g^{-1}), M is equal to free labile Cu^{2+} concentration (mol L^{-1}), and K_1 *and* K_2 are equal to the average conditional stability parameters for high and low affinity Cu^{2+} binding sites respectively. It was assumed that the initial residual Cu^{2+} in samples was complexed, thereby adding to the total complexing capacity (Town and Filella 2000). Initial residual Cu^{2+} was determined by inductively coupled plasma (ICP) analyses on a Leeman Labs PC1000.

A minimum number of free parameters were included in the model according to tests of significance using Systat 8.0. A 1-ligand model was not significant ($p > 0.05$) so a 2-ligand model was used. All non linear regression analyses using the 2-ligand model were significant ($p < 0.05$), and parameter values were consistent with empirical titration data. Furthermore, CC_1 and CC_2 parameter values were compared to Cu-binding density (CuL, Cu-mol C-g^{-1}) among samples, which was operationally calculated at 1.0×10^{-6} mol L^{-1} of free labile Cu^{2+} from each of the titration curves.

2.4 Results

2.4.1 Organic Acidity

The lower detection limit of charge density (CD) with respect to dissolved organic carbon (DOC) concentration was calculated to be approximately 435 C-mg L^{-1} ($p > 0.05$) by comparison to titrations of DOC free water. Error associated with the CD model was approximately 0.01 pH units (SD = 0.009, n = 102), and 0.002 mol L^{-1} (SD = 8.5E-04, n = 3) of H^+ complexation was measured in DOC free water. This background H^+ complexation was within the reported accuracy limitations (± 0.1 pH units) of the pH electrode ($p > 0.1$) throughout the lower end of the pH titration range (approximately 2.0 to 2.5). Given these limitations to measuring pH and subsequently calculating CD, base titrations conducted on samples with a DOC concentration below the detection limit were not included in comparisons of allochthonous and autochthonous DOC.

Total charge density (CD_T) ranged from 0.024 to 0.006 H-mol C-g^{-1} independent of DOC concentration for allochthonous and autochthonous DOM. Similarly, pK_a values ranged from 2.4 to 3.1 (Figure 2.1). The reproducibility (95% confidence interval) of total charge density (CD_T) and acid dissociation parameters (pK_a) was better for allochthonous dissolved organic matter (DOM) by 15% to 30%, compared to autochthonous DOM, and generally decreased with increasing DOC concentration (Figure 2.1).

Differences in CDT and pKa measures for allochthonous and autochthonous DOM were assessed using a 1-factor ANOVA. The CDT of allochthonous DOM was significantly higher than CDT for autochthonous DOM by a factor of approximately 2.3 ($p < 0.05$, n = 3; Figure 2.1), with mean values of 0.018 and 0.008 H-mol C-g-1 respectively. In contrast to CDT measures, the pKa of allochthonous and autochthonous DOM was statistically similar

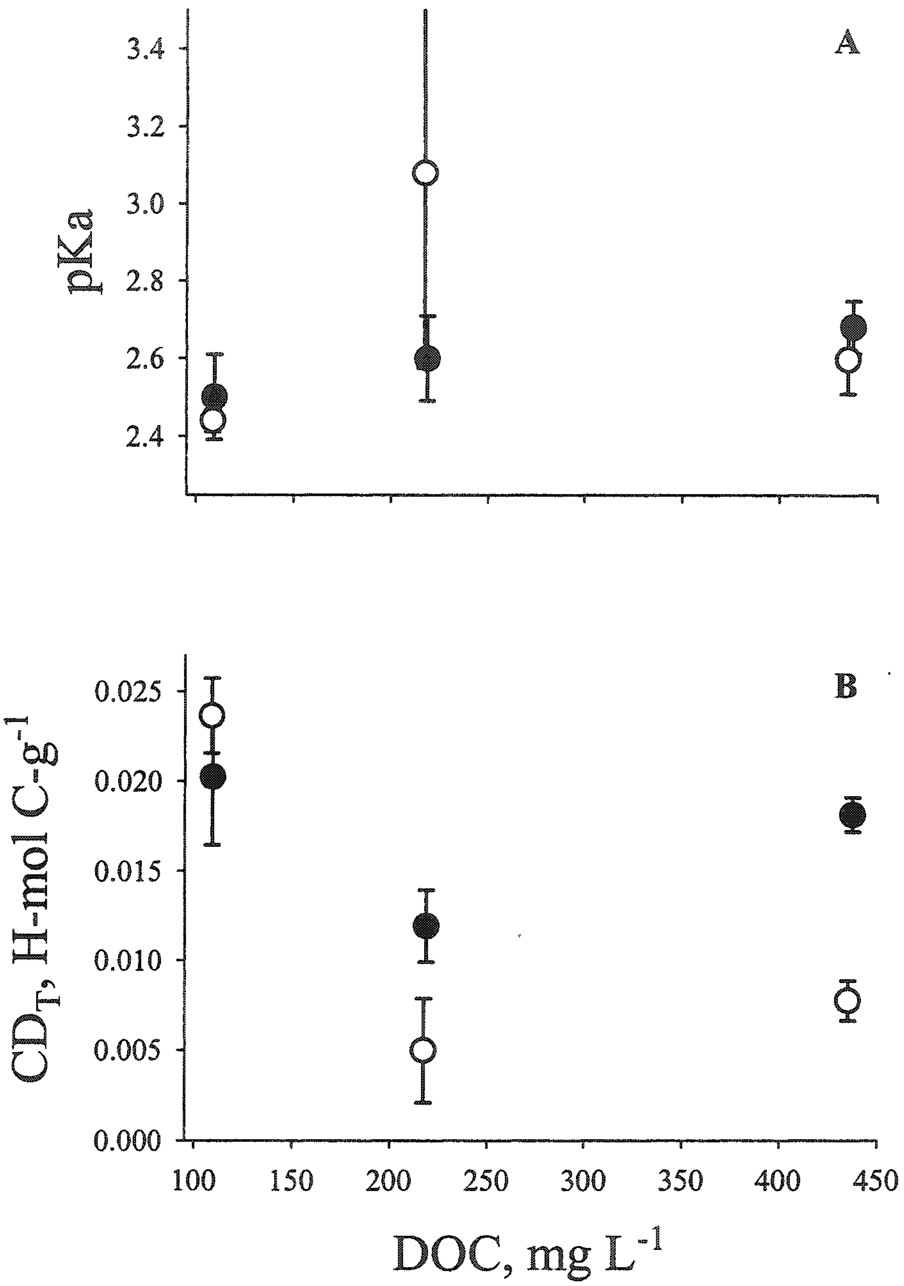

Figure 2.1. Parameter values for organic acidity plotted against dissolved organic carbon (DOC) concentration for allochthonous (closed circle) and autochthonous (open circle) sources of DOM. The 95% confidence interval is shown for all data points (n = 3). A. Acid dissociation constants (pK$_a$). B. Total charge density (CD$_T$).

at about 2.6 ($p > 0.05$, $n = 3$). Both measures for allochthonous and autochthonous DOM are within the reported range of variation and approximately correspond to the upper and lower extreme respectively for CD_T (Table 2.1). CD_T typically ranges from 0.0033 to 0.022 mol C-g^{-1} (mn = 0.011, SD = 0.0040, n = 22) and pK_a values typically range from 2.1 to 4.8 (mn = 3.38, SD = 0.58, n = 48) for comparable titration ranges (Leuenberger and Schindler 1986; Buffle 1990; Tipping *et al.* 1990; Tipping and Hurley 1992; De Wit *et al.* 1993b; Driscoll and Lehtinen 1994; Manuza *et al.* 1995; Milne *et al.* 1995; Kinniberg *et al.* 1996; Lydersen 1998).

2.4.2 Copper Complexation

Initial residual Cu^{2+} concentrations in each sample prior to Cu^{2+} titrations were all less than 0.32 μmol L^{-1} and were below the detection limit of the ICP analysis at DOC concentrations below approximately 109 C-mg L^{-1} ($p < 0.01$). Measurements of free labile Cu^{2+} suggest that more than 99.9% of this initial residual Cu^{2+} is complexed, even in autochthonous samples. Titration data were corrected for these values and parameter values of the Langmuir Isotherm model were compared among corrected and uncorrected data using a 2-factor ANOVA. Parameter values were significantly influenced by correcting for initial total copper concentrations ($p < 0.05$), but the absolute change in values was typically less than 5%.

The detection limit of Cu^{2+} titrations was calculated as the DOC concentration at which CC_1 and CC_2 values were significantly different from zero ($p < 0.05$). All samples of allochthonous DOM were above this detection limit with CC_1 and CC_2 values of 38 (SD = 4.3, n = 3) μmol C-g^{-1} and 320 (SD = 52, n = 3) μmol C-g^{-1} respectively at DOC

Table 2.1. Literature values of conditional stability parameters (Log(K_1), Log(K_2)) and Cu^{2+} complexing capacity (CC_1, CC_2; mol C-g^{-1}) for whole water and fulvic acid fractions of dissolved organic matter (DOM).

Range						
Fraction	log(K_1) (1/mole)	CC_1 (µmol C-g^{-1})	K_2	CC_2	n	Ref.
Whole	7.5 – 13.1	9 – 29	5.3 - 6.5	101 – 299	5	a
Whole	4.6 – 9.0	6 – 6100	-----	-----	10	b
Whole	7.26	210	5.1	2890		c
Fulvic	3.6 – 7.3	1900 – 6900	-----	-----	9	b
Fulvic	7 – 8.5	100 – 650	5.4 - 6.6	440 – 1900	18	d

a.) (Curtis *et al.*, unpublished data) b.) (Buffle 1990). c.) (MacRae *et al.* 1999b). d.) (McKnight *et al.* 1983).

concentrations as low as 3.71 C-mg L^{-1} (Figure 2.2). In contrast, samples of autochthonous DOM were below the detection limit for DOC concentrations less than 27.2 C-mg L^{-1} (Figure 2.3). The highest CC_1 and CC_2 values measured at DOC concentrations above the detection limit were 6.6 (SD = 0.1, n = 3) μmol C-g^{-1} and 28 (SD = 38, n = 3) μmol C-g^{-1} respectively, which is more than a factor of 6 less than allochthonous DOM (Figure 2.2 and 2.3).

The reproducibility of parameter values from the Langmuir Isotherm model was calculated as a 95% confidence interval (shown in figures 2.2 and 2.3) and a standard coefficient of variation (CV). The assumption of normality was investigated from ten replicate titrations of allochthonous DOM with skewness and kurtosis values not significantly different from zero ($P > 0.1$). Reproducibility was better for allochthonous DOM, decreasing from a maximum CV of 16% to less than 1% for all parameter values with increasing DOC concentration (Figure 2.2). The reproducibility of parameter values for autochthonous DOM decreased from 58% to less than 1% with increasing concentrations of DOC above the detection limit (Figure 2.3).

Sensitivity of the Langmuir Isotherm model to the titration end point of complexed Cu^{2+} (CuL, Cu-μmol C-g^{-1}) and free labile Cu^{2+} (Cu^{2+}, μmol L^{-1}) was determined by sequentially removing each titration data point from the model for samples of allochthonous and autochthonous DOM. The resulting parameter values were plotted against the free labile Cu^{2+} concentration associated with the titration end-point that was modeled (Figure 2.4). $Log(K_1)$ and $Log(K_2)$ values decrease logarithmically as a function of increasing concentrations of complexed and free labile Cu^{2+} that were modeled as the titration end point. Similarly, CC_1 and CC_2 increased as a function of increasing titration end point. This effect was typically greater for allochthonous DOM and particularly for $Log(K_1)$ values,

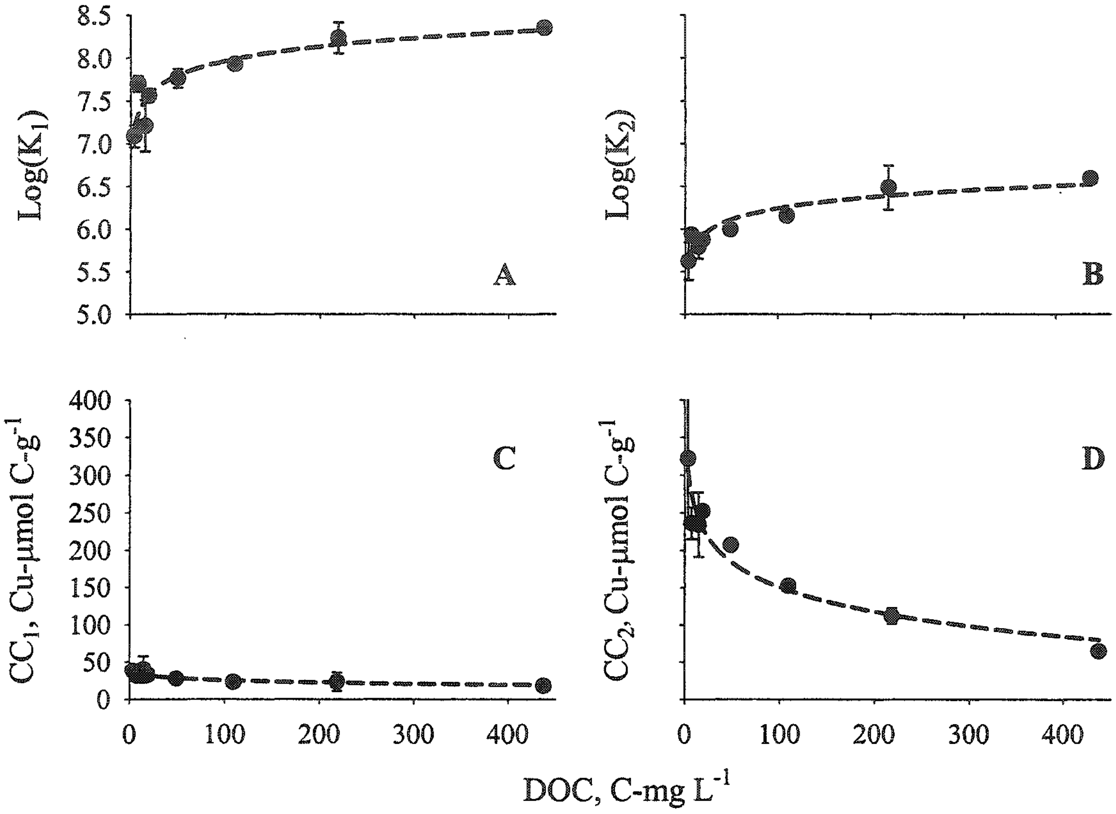

Figure 2.2. Parameter values of the Langmuir Isotherm model for allochthonous dissolved organic matter (DOM) calculated at a titration end point of 1.0 µmol L^{-1} of free labile copper (Cu^{2+}) and plotted as a function of increasing dissolved organic carbon (DOC) concentration. The 95% confidence limit is shown for all data points (cannot be seen for all data points). Logarithmic regression is significant in each case ($p < 0.05$). A and B. Conditional stability parameters ($Log(K_1)$, $Log(K_2)$). C and D. Complexing capacity (CC_1 and CC_2).

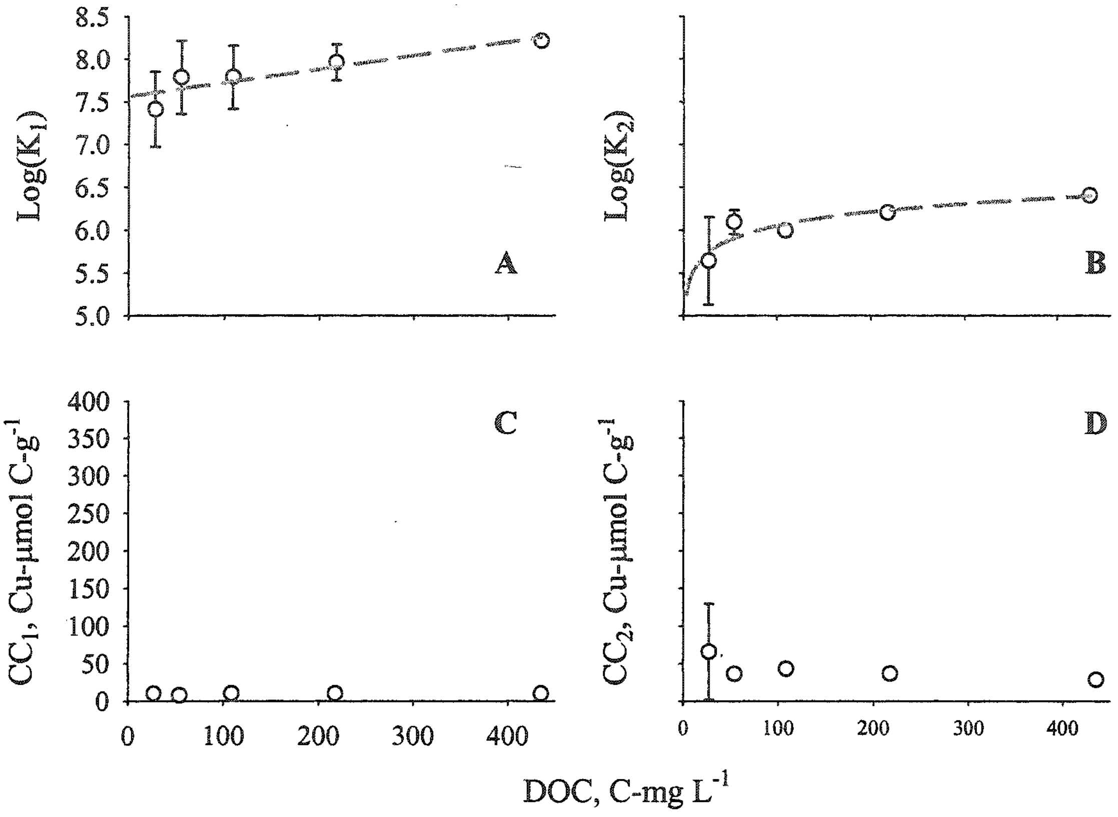

Figure 2.3. Parameter values of the Langmuir Isotherm model for autochthonous dissolved organic matter (DOM) calculated at a titration end point of 1.0 µmol L^{-1} of free labile copper (Cu^{2+}) and plotted as a function of increasing dissolved organic carbon (DOC) concentration. The 95% confidence limit is shown for all data points (cannot be seen for all data points). A. Conditional stability parameters ($Log(K_1)$). Linear regression is significant ($p < 0.05$). B. $Log(K_2)$. Logarithmic regression is significant ($p < 0.05$). C and D. Complexing capacity (CC_1 and CC_2).

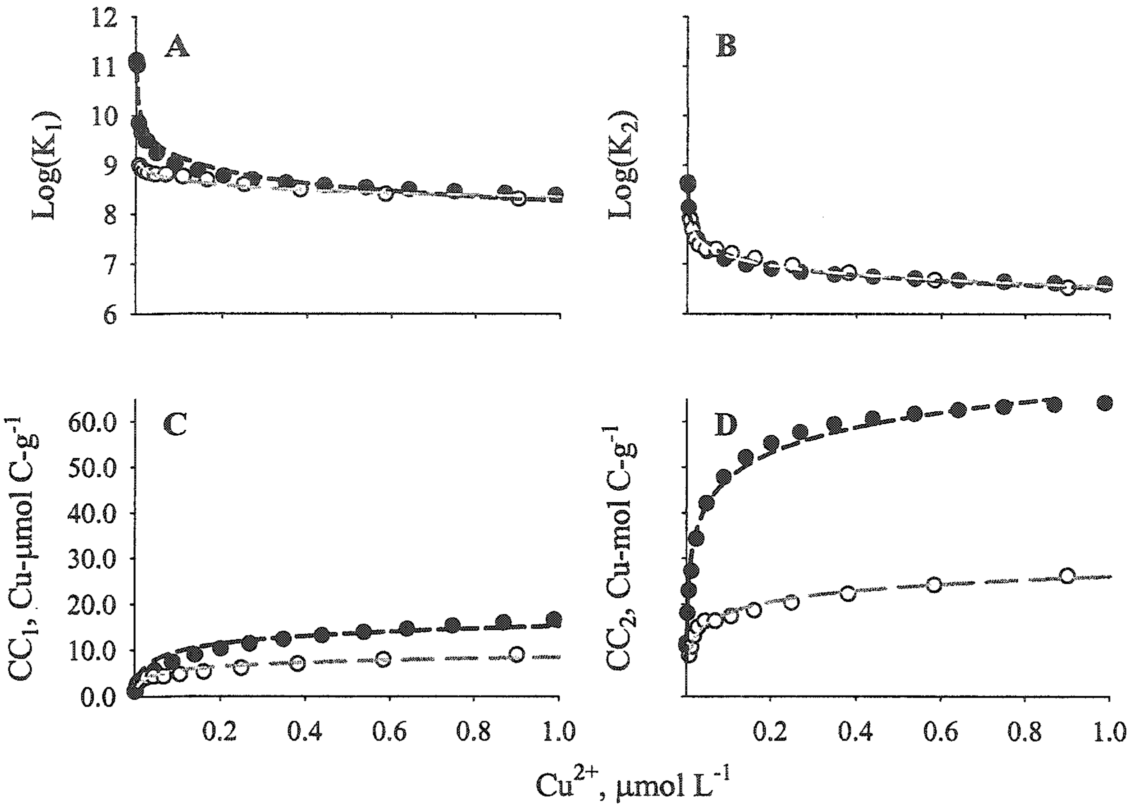

Figure 2.4. Parameter values of the Langmuir Isotherm model calculated at titration end-points that correspond to increasing concentrations of complexed copper (Cu^{2+}) and plotted against corresponding concentrations of free labile Cu^{2+}. Data shown for allochthonous (closed circle) and autochthonous (open circle) sources of dissolved organic matter (DOM) respectively at 438 and 435 C-mg L^{-1}. Logarithmic regression is significant in each case ($p < 0.05$). A and B. Conditional stability parameters (Log(K_1), Log(K_2)). C and D. Complexing capacity (CC_1 and CC_2).

which decreased from 11.2 to 8.3 as a function of increasing CuL and free labile Cu^{2+} concentrations included in the model (Figure 2.4).

The effect of DOC concentration on Cu^{2+} complexation was determined from titration data with an operational end-point of 1.0 μmol L^{-1} of free labile Cu^{2+} for all samples. Parameter values vary significantly ($p < 0.05$) as a function of DOC concentration, but generally only for allochthonous sources of DOM with $Log(K_1)$ values increasing from 7.1 to 8.4 and $Log(K_2)$ values increasing from 5.6 to 6.6 (Figure 2.2 and 2.3). CC_1 values for allochthonous DOM decreased from 37.8 to 17.7 μmol C-g^{-1} as a function of DOC concentration and CC_2 values decreased from 322 to 64.7 μmol C-g^{-1}, as compared to no effect of DOC concentration for autochthonous DOC (Figure 2.2 and 2.3). This trend is consistent with empirical titration curves.

Changes in Cu^{2+} binding density (CuL, Cu-μmol g^{-1}) that were operationally measured from titration data at 1.0 μmol L^{-1} of free labile Cu^{2+} were consistent with the DOC concentration-dependence of CC_1 and CC_2 values for allochthonous and autochthonous DOM. Cu^{2+} binding density decreases significantly from approximately 140 to 69.3 μmol C-g^{-1} as a function of increasing DOC concentration for allochthonous DOM ($p < 0.05$, Figure 2.5), whereas there is no effect for autochthonous DOM ($p > 0.05$). Copper binding densities are also significantly higher for allochthonous DOM, corresponding to higher CC_1 and CC_2 values.

Comparison with literature values (Table 2.1) indicates that DOC concentration could potentially explain a significant percentage of the reported variation in parameter values of the Langmuir Isotherm model. For example, the range in $Log(K_1)$ values is 3 to 4 $Log(K_1)$ units. Conservatively 20 to 30% of this range in $Log(K_1)$ values could result from variation

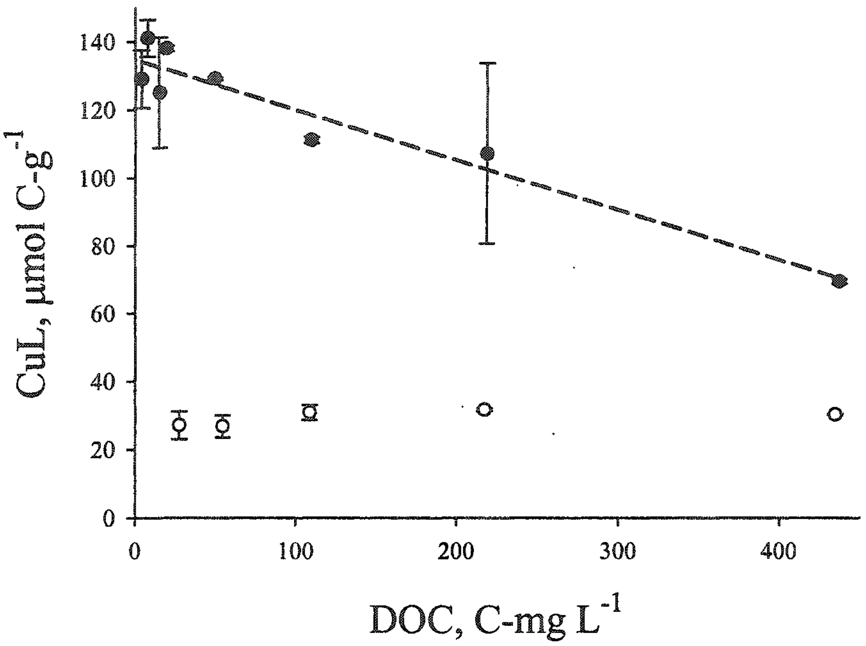

Figure 2.5. Density of complexed Cu^{2+} (CuL, Cu-µmol C-g^{-1}) calculated at 1.0 µmol L^{-1} of free labile copper (Cu^{2+}) for increasing dissolved organic carbon (DOC) concentrations of allochthonous and autochthonous dissolved organic matter (DOM). The 95% confidence limit is shown for all data points. Linear regression is significant for allochthonous DOM ($p < 0.05$).

in the concentration of allochthonous DOC (1 to 50 C-mg L^{-1}). Similarly for Log(K_2), up to 60% of the typical range in reported values could result from variation in DOC concentration. CC_1 and CC_2 values are approximately ten times lower than the majority of literature values, probably due to differences in experimental conditions, and are therefore difficult to compare.

2.5 Discussion

2.5.1 Organic Acidity

Measurements of charge density (CD) and acid dissociation (pK_a) parameters for dissolved organic matter (DOM) are limited by dissolved organic carbon (DOC) concentration. DOC concentration is the limiting factor because the total charge density (CD_T) of DOM is low compared to background proton (H^+) activity throughout the relevant range of organic acid dissociation, which is between 2.1 and 3.1 for allochthonous and autochthonous DOM. Therefore, error in measuring background proton activity, which is generally dictated by the accuracy limitations of a pH electrode, results in poor resolution of organic acid dissociation (Kinniburgh *et al.* 1999). The overall result is an indistinguishable signal of CD at ambient DOC concentrations for the vast majority of aquatic systems (Curtis 1998).

Total charge density (CD_T) and acid dissociation (pK_a) parameters measured at DOC concentrations of approximately 435 mg L^{-1} are consistent with literature values. To my knowledge, values for CD_T and pK_a for autochthonous DOM have not been reported previously in the literature. The reproducibility of these values is better for allochthonous DOM because of higher CD_T, which results in a higher signal to noise ratio with respect to background proton activity. Therefore, the DOC concentration required to measure organic acidity is inversely dependent on CD_T, which means that lower concentrations of DOC are required for DOM of higher CD_T.

DOM with a higher CD_T will tend to occur in aquatic systems that are characteristic of allochthonous DOM and lower CD_T will occur for autochthonous-like systems. However, pK_a values are similar among sources of DOM and are consistent with the majority of reported values in the carboxylic group range (Lydersen 1998), which have been estimated to constitute 60 to 90% of organic acids (Morel 1983; Perdue and Lytle 1983b). This consistency among pK_a values supports the conclusion that there is very little detectable variation in the bulk composition of organic acids among surface freshwaters (Tipping and Hurley 1992).

2.5.2 Copper Complexation

Higher Cu^{2+}complexing capacity (CC_1, CC_2) for allochthonous DOM is consistent with a higher CD_T, which supports two conclusions regarding the composition of DOM in aquatic systems. Firstly, there is a correlation between total organic functional group density and the density of organic Cu^{2+} binding sites (CuL, μmol C-g^{-1}). Secondly, allochthonous DOM is more efficient than autochthonous DOM at complexing Cu^{2+} (Richards *et al.* 2001) because of a higher density of higher affinity complexing sites. Both conclusions are conservative, given that differences between allochthonous and autochthonous DOM may be underestimated by CC_1, CC_2 and $Log(K_1)$, $Log(K_2)$ parameter values of the Langmuir Isotherm model.

The comparison of parameter values from the Langmuir Isotherm model among allochthonous and autochthonous sources of DOM is limited by two factors. The first limiting factor is the reproducibility of titration analyses to high affinity Cu^{2+} binding sites because analyses are limited by the lower limit of the linear calibration range for the cupric ion selective electrode (ISE). Depending on the composition of DOM that is being analyzed, ambient concentrations of DOC may be too low for comparisons of parameter values among samples.

The increase in precision of parameter values as a function of increasing DOC concentration is due to a higher total concentration of Cu^{2+} binding sites (Avdeef *et al.* 1983; Buffle 1988). By increasing the total concentration of Cu^{2+} binding sites, titration analyses become more sensitive to low concentrations of high affinity binding sites, which may be the most relevant to *in situ* processes (Coale and Bruland 1988; Buffle *et al.* 1990; Marr *et al.* 1999). Sensitivity to high affinity binding sites is particularly relevant to autochthonous-like DOM. Autochthonous DOM may exhibit high affinity Cu^{2+} complexing sites (Xue and Sigg 1993; Leal *et al.* 1999), but these sites are difficult to resolve because of such low densities, approximated by CC_1 and CC_2 parameters, and low ambient DOC concentrations (McKnight *et al.* 1994). Therefore, this research suggests that autochthonous DOM makes a negligible contribution to Cu^{2+} complexation processes in surface freshwaters, as compared to allochthonous DOM.

The second limiting factor to the comparison of parameter values is the sensitivity of the Langmuir Isotherm model to the operational end-point of experimental titration data. It is well documented that discrete ligand models do not reflect the complexing capacity and stability of Cu^{2+} complexes over the entire distribution of organic functional groups (Buffle *et al.* 1990; Town and Filella 2000). This issue has been previously investigated as the ratio of metal to ligand concentrations, which indicates that Log(K_1) values increase as the ratio of metal to ligand concentration asymptotically approaches zero (Perdue and Lytle 1983a). High affinity Cu^{2+} binding sites are preferentially consumed by Cu^{2+} ions (Town and Filella 2000), but concentrations are small by comparison to lower affinity binding sites when they are evaluated numerically (Buffle *et al.* 1990). This drastic difference in concentration skews the distribution of ligands modeled by Log(K_1) and Log(K_2) values to lower affinity binding sites so that real differences in high affinity sites are underestimated from parameter values of the model.

The distribution of ligands modeled by Log(K_1) and Log(K_2) values will be increasingly skewed towards lower affinity binding sites, as indicated by lower Log(K_1) and Log(K_2) values, as a function of increasing concentrations of complexed and free labile Cu^{2+} that are modeled. This trend is a fitting artifact of the Langmuir Isotherm model and will likely depend on proportional differences in high and low affinity binding sites for DOM. For example, higher proportions of low affinity binding sites will more drastically skew the distribution of ligands modeled by Log(K_1) values. Therefore, this effect is particularly relevant to allochthonous DOM because it exhibits higher affinity Cu^{2+} binding sites and a proportionally higher capacity of lower affinity binding sites, as compared to autochthonous DOM. This conclusion implies that the measured difference in binding affinity among allochthonous and autochthonous DOM is underestimated by discrete ligand modeling.

Sample concentration alleviates problems of reproducibility and it is generally accepted that the Langmuir Isotherm model is conditional to experimental conditions, including operational titration end-point criteria (Buffle 1990; Town and Filella 2000).

However, sample concentration imposes limitations to the interpretation of Cu^{2+} titration data at ambient DOC concentrations. Titration data are sufficiently reproducible to identify a statistically significant increase in Cu^{2+} binding affinity (($Log(K_1)$, $Log(K_2)$)) and a decrease in binding capacity (CC_1, CC_2) as a function of increasing DOC concentration (Figure 2.2 and 2.3). These trends are consistent with at least two non-exclusive possibilities.

Firstly, the overall trends are consistent with multidentate complexation of a central Cu^{2+} ion by organic ligands of DOM. Multidentate binding involves more than one ligand binding a central Cu^{2+} ion, which typically results in higher complex stability. This effect is consistent with the direct dependence of $Log(K_1)$ and $Log(K_2)$ parameter values on DOC concentration, as well as the inverse dependence of CC_1 and CC_2 parameter values (Morel 1983; Manahan 1994; Martell and Hancock 1994). A second possibility is the occlusion of Cu^{2+} binding ligands. Potential Cu^{2+} binding sites may be blocked or effectively deactivated by physicochemical changes in the composition of DOM as a function of DOC concentration. This effect could contribute to the observed decrease in complexing capacity for allochthonous DOM. However, more research is required to determine the contribution of either possibility to changes in Cu^{2+} complexation as a function of DOC concentration.

Regardless of the exact mechanism, increases in DOC concentration could potentially explain some of the variation in parameter values of the Langmuir Isotherm model that is reported in the literature (Table 2.1). This further explanation is because DOC concentration and organic acid composition have a combined effect on Cu^{2+} complexation in aquatic systems. Organic acids of the same source and composition complex Cu^{2+} differently at different DOC concentrations.

This effect of DOC concentration suggests that there are concessions to different analytical approaches of quantifying Cu^{2+} complexation by ligands of DOM. One approach is to concentrate samples by reverse osmosis, thereby minimizing the effect of DOC concentration on Cu^{2+} complexation and maximizing the precision of the analysis. However, results from this type of analysis poorly reflect Cu^{2+} complexation at *in situ* DOC concentrations. The alternative is to analyze samples at *in situ* DOC concentrations, which may result in poor precision and increased variability due differences in DOC concentration because titration data are typically normalized to carbon.

2.6 Conclusions

The precision and accuracy of proton (H^+) and copper (Cu^{2+}) potentiometric titration analyses of dissolved organic matter (DOM) is directly dependent on experimental conditions, including dissolved organic carbon (DOC) concentration, which could explain 20% to 60% of the reported variation in organic Cu^{2+} complexation. This effect varies for allochthonous and autochthonous DOM because of differences in composition. Allochthonous DOM exhibits a higher total charge density (CD_T), which is consistent with a higher density of higher affinity Cu^{2+} complexing sites. It is important to recognize that these results are conditional to experimental conditions, but differences between allochthonous and autochthonous DOM are likely underestimated in this research. Therefore, autochthonous DOM likely makes a negligible contribution to organic pH buffering and Cu^{2+} complexation in surface freshwaters.

3. Chapter 3. The Effect of Environmental and Experimental Transformation and Fractionation of Dissolved Organic Matter on Organic Copper (II) Complexation

3.1 Abstract

The effect of environmental and experimental transformation and fractionation of dissolved organic matter (DOM) on organic Cu^{2+} complexation was investigated. The effect of environmental processes was investigated as a function of (1) DOM source, and (2) exposure to transformation and fractionation processes, as approximated by cumulative hydrologic residence time (CHRT). The effect of experimental processes was investigated by individually treating the same samples across micro-reactors that maximized rates of three commonly identified transformation and fractionation processes; including photochemical decomposition, microbial decomposition and adsorptive fractionation of DOM. Results show that Cu^{2+} complexation is primarily dependent on allochthonous sources of DOM and inversely dependent on CHRT ($p < 0.05$). This trend is consistent with an inverse dependence of DOM colour and dissolved organic carbon concentration (DOC) on CHRT ($p < 0.05$), with values of DOC ranging from 10.1 to 5.2 mg L^{-1}. All three of the experimental transformation and fractionation processes investigated could potentially contribute to this trend, either by influencing DOM composition or DOC concentration. The latter may be the most important variable, as indicated by a proportionally greater dependence on CHRT, which results in a 60% decrease in the molar concentration of Cu^{2+} binding cites (CuL_M) from 1.7 to 0.7 μmol L^{-1} as a function of CHRT ($p < 0.05$).

3.2 Introduction

Dissolved organic matter (DOM) is a heterogeneous mixture of organic acids that can complex trace metals in surface freshwaters (Langford *et al.* 1983; Buffle 1990; Perdue 1998). Ligands of DOM contain organic functional groups that complex or bind trace metals, decreasing trace metal toxicity to biota (Sunda and Guillard 1976; Sunda and Lewis 1978; Playle and Dixon 1993). Thus, trace metal availability depends on organic complexing capacity, and complex stability. Complexing capacity is directly proportional to dissolved organic carbon (DOC) concentration by the density of binding sites, and complex stability directly depends on the binding affinity of ligands that constitute DOM (Buffle 1990).

The concentration and composition of DOM in aquatic systems is controlled by two factors, including (1) DOM source and (2) exposure to transformation and fractionation processes (Meili 1992; Fukushima *et al.* 1996; Curtis 1998; Bertilsson and Tranvik 2000). DOM is mainly loaded to aquatic systems from terrestrial (allochthonous) sources, which is characteristically dark in colour (Waiser and Robarts 2000; Imai *et al.* 2001) and high in organic functional group density (Chapter 2). However, small contributions of DOM are also derived from sources of primary productivity (autochthonous) (McKnight *et al.* 1994; McKnight and Aiken 1998). Therefore, DOM in surface freshwaters is an amalgam of DOM from allochthonous and autochthonous sources and DOM that has been transformed and fractionated by environmental processes. Three environmental processes that can transform or fractionate molecules of DOM over time include photochemical decomposition, microbial decomposition and adsorption (Moran and Hodson 1990; McKnight *et al.* 1992; Volk *et al.* 1997; Bertilsson and Tranvik 2000).

It has been determined for some properties of DOM that environmental transformation and fractionation varies as a function of exposure (Curtis and Schindler 1997; Curtis 1998). This relationship was determined by an inverse relationship between hydrologic residence time (HRT), which is calculated as the quotient of lake volume and outflow, and measures of DOC concentration and colour, as compared by a specific absorbance coefficient (SAC). An inverse correlation between colour and the toxicity of trace metals (Richards *et al.* 2001) suggests a similar relationship between HRT and trace metal complexation by DOM in surface freshwaters.

Organic trace metal complexation is commonly measured for Cu^{2+} by potentiometric titration analyses, which provides a direct empirical measure of complexing capacity and complex stability (Buffle *et al.* 1977; Cabaniss and Shuman 1988; Buffle 1988; Holm and Curtiss III 1990; MacRae *et al.* 1999b). Limitations to this approach include a low resolution of high affinity binding sites at *in situ* ligand concentrations, and instrumental variation due to matrix effects, such as interfering cations and variation in Cu^{2+} ion activity (Midgely and Torrance 1991; Hales *et al.* 1999). These limitations are minimized by operationally standardizing experimental conditions. For example, increasing DOC concentration by reverse osmosis (RO) increases the sensitivity of potentiometric titrations to high affinity binding sites (Chapter 2). Similarly, exchanging interfering cations with a cation exchange resin (CER) and adjusting ionic strength minimizes instrumental variation (Avdeef *et al.* 1983; Buffle 1988; Midgely and Torrance 1991).

Potentiometric data are conditional to experimental conditions and are typically interpreted by modeling for pairs of parameter values that empirically represent the complexing capacity (CC) and binding affinity (Log(K)) of organic ligands (Buffle 1990).

Parameter values typically correspond to a low CC_1 – high $Log(K_1)$ pair, termed high affinity sites for discussion purposes, and a high CC_2 – low $Log(K_2)$ pair, termed low affinity sites (Buffle *et al.* 1990; Town and Filella 2000). However, it is important to recognize that these pairs of parameter values do not represent specific ligand classes (Town and Filella 2000), but rather the bulk Cu^{2+} complexing properties of DOM for empirical comparisons of titration curves among samples (Perdue and Lytle 1983a). These pairs of parameter values inevitably predict Cu^{2+} binding by multiple ligands because DOM is a heterogeneous mixture of organic acids with a continuous distribution of binding affinities (Perdue and Lytle 1983a; Buffle *et al.* 1990; Benedetti *et al.* 1995). The rationale for this approach is to avoid statistical over parameterization of titration curves that may occur with more complicated models (McKnight *et al.* 1983; Coale and Bruland 1988).

The objective of this research is to determine how environmental and experimental transformation and fractionation of DOM affects organic Cu^{2+} complexation. The effects of environmental transformation and fractionation are determined by measuring Cu^{2+} complexation in surface freshwater sampled from aquatic systems of increasing cumulative hydrologic residence time (CHRT), which accounts for the pre-exposure of DOM to transformation and fractionation processes. The effects of experimental transformation and fractionation are determined by treating sub-samples in micro-reactors that maximize rates of photochemical decomposition, microbial decomposition and adsorption processes. Patterns in sensitivity to experimental transformation and fractionation are used to infer whether individual mechanisms are consistent with environmental transformation and fractionation. Cu^{2+} complexation is measured by potentiometric titration analyses and empirically investigated with a two-ligand Langmuir Isotherm for comparisons among reference and treated samples. The results depend on experimental conditions, which are operationally standardized among samples.

3.3 Site Description

Samples were collected from a headwater creek (unnamed) and three downstream lakes (Duck, Wood and Kalamalka Lakes) in the Okanagan Lake watershed of the upper Columbia River (Southern Interior of British Columbia, Canada, Figure 3.1). Cumulative hydrologic residence time (CHRT) increases with flow-path because the hydrology of the lake chain is surface discharge dominated, and the climate at the elevation of the lakes is semi-arid. Thus, the lakes are analogous to batch reactors positioned in series.

3.4 Methods

3.4.1 Sample Collection

The headwater creek sample, with a CHRT less than 1 day, is dominated by allochthonous dissolved organic matter (DOM). Autochthonous DOM was produced in an acrylic mesocosm (1 m^3) as described in Chapter 2, and was therefore not included in regression analyses according to CHRT with the rest of the samples because the mesocosm is not representative of a natural system. Instead, autochthonous DOM was compared to other DOM samples as the lower extreme of colour and Cu^{2+} complexation (see chapter 2)

Approximately 20 L of surface water were collected into polyethylene containers from each sample site and from the mesocosm in August 2001. Lake samples were collected from shore, well away from the entrance of any tributaries to attain a representative sample

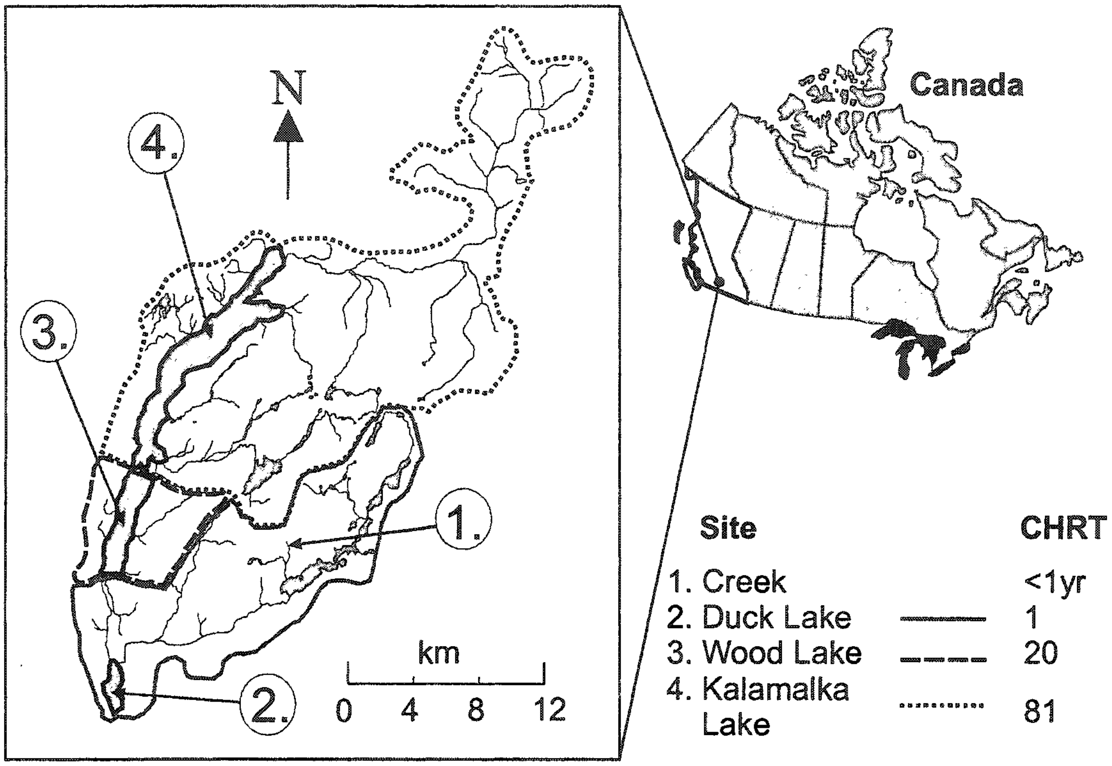

Figure 3.1. Map of the study area showing hydrologic flowpath (1 through 4), drainage basins and cumulative hydrologic residence time (CHRT) for each system that was sampled.

of the surface mixing layer for each lake. All samples were filtered through GF/C Whatman glass fiber filters to remove seston and the 20 L filtered samples were split into four subsamples of 5 L each; one reference and three experimental subsamples. Each set of experimental subsamples was individually treated in one of the three types of micro-reactors designed to maximize rates of photochemical decomposition, microbial decomposition and adsorption processes.

3.4.2 Experimental Transformation and fractionation

Photochemical decomposition of DOM was experimentally investigated in the laboratory by exposing subsamples to light emitted from a high-pressure mercury vapor lamp. Glass tubes (295 mL) were used as reactor vessels to filter lower wavelengths of ultraviolet radiation (UVR) emitted from the lamp (0% transmittance below a wavelength of 280nm), which are not common in the natural environment (Wetzel *et al.* 1995). Furthermore, the top of each tube was covered during exposure to minimize contamination. Exposure time was experimentally determined prior to sample treatment to ensure complete reaction of the photo-labile fraction of DOM (24hrs) and the precision of the treatment process was measured by replicate analyses (Coefficient of variation < 5% for dissolved organic carbon (DOC), n = 11). In addition, the initial sample volume in each tube was conserved by the addition of ultra-pure water at the end of the exposure period for measurements of DOC concentration and colour, as determined by a specific absorbance coefficient at a wavelength of 350nm (SAC_{350}). Treated samples were collected in polyethylene sample bottles and stored in the dark at 4°C.

Microbial decomposition of DOM was experimentally investigated in the laboratory by treating subsamples in plug-flow biofilm reactors following a similar construction to Kaplan and Newbold (Kaplan and Newbold 1995). Samples were diluted by a factor of 8 to maintain oxic conditions in all reactors, nutrient enriched with N and P (1.5 and 0.1 mg L^{-1} respectively with NO_3 and PO_4 salts) to mimic eutrophic conditions, aerated, and filtered through in-line Whatman GF/C glass fiber filters to remove seston, however colloids and bacterial size fractions are generally not removed by this process.

Reactors were constructed from 750 mL amber glass bottles that were darkened by covering with tin foil and packed with glass beads (d = 1.5 mm), resulting in a potential biofilm surface area of approximately 2.1 m^2. Sample residence time was approximately 11 hr with a flow rate of approximately 21 mL/hr, and the treated sample was collected for a period of 12 weeks. Dissolved oxygen (DO) and DOC concentrations were measured once a week to monitor microbial activity within each reactor and to ensure aerobic conditions. Dissolved oxygen was measured in reactor inlet and outlet water. Dissolved oxygen typically decreased between 50% and 60% across reactors, indicating microbial respiration within the reactors. DOC in water from the reactors varied widely. Treated samples were collected daily in polyethylene sample bottles and stored in the dark at 4°C.

Adsorptive fractionation of DOM was experimentally investigated in the laboratory in batches by adding a slurry of concentrated iron hydroxide particles ($Fe(OH)_{3(s)}$) to 5 L subsamples in polyethylene bottles. The concentrated $Fe(OH)_{3(s)}$ slurry was produced synthetically by adjusting the pH of an iron (III) chloride solution ($FeCl_3$; 0.36 M) to pH 7 (Morel 1983). The resulting $Fe(OH)_{3(s)}$ floc was then repeatedly washed until the conductivity and DOC concentration of the wash water was the same as ultra pure water.

The volume of concentrated $Fe(OH)_{3(s)}$ slurry added to each sample was determined experimentally ($Fe(OH)_{3(s)}$ 3 g L^{-1}) to maximize adsorptive fractionation (Luider *et al.* in Press). Samples treated with the slurry were stirred for ten minutes, filtered through Whatman GF/C glass fiber filters, and then stored in clean polyethylene bottles at 4°C in the dark. The precision of the process was measured by replicate analyses of DOC scavenging, with a coefficient of variation less than 5% for DOC concentrations (n = 3).

3.4.3 Sample Concentration and Exposure to the Cation Exchange Resin

Reference and treated samples were concentrated using a bench-top stainless steel reverse osmosis (RO) concentrator (Limnological Research Corp., Kelowna, B.C.) with a stainless steel Delcon centrifugal pump, a stainless steel holding tank, and a Filmtec FT30 US Filters thin composite RO membrane housed in a stainless steel cartridge. Operating pressure was between 700 and 1200 Kpa and the cartridge was cooled with a water jacket to keep samples at approximately room temperature (21°C). Concentrated sample was drained into 500 mL polyethylene bottles and stored at 4°C.

Concentrated samples were exposed to an Amberlite IR-118H cation exchange resin (CER) to replace potentially interfering cations with H^+ and to fully protonate organic acids. The resin was replenished with 4 mol L^{-1} HCl and washed ten times with ultra pure water between each sample treatment. Leaching of organics and inorganics from the resin was negligible after the tenth wash with ultra pure water. The resin was added to samples until further additions failed to lower pH (generally about pH 2).

3.4.4 Sample Analysis

DOC concentration was measured in all samples with a Shimadzu TOC-5000A Total Organic Carbon Analyzer. Dissolved inorganic carbon was removed by acidifying samples ($pH < 2$) with select grade hydrochloric acid (2N HCl) and sparging with oxygen for seven minutes before analysis. The coefficient of variation (CV) for instrumental response was consistently less than 2% and the detection limit of the instrument was 0.05 C-mg L^{-1} at an injection volume of 750 μL.

Organic colour was measured spectrophotometrically at 350nm with a Shimadzu UV-2101PC ultra violet (UV)-Visible scanning spectrophotometer in a 1cm quartz cuvette. A specific absorbance coefficient (SAC_{350}) for DOM was calculated according to equation 3.1 and reported in units of cm^2 C-mg^{-1} (Curtis and Schindler 1997).

$$SAC = \frac{(2.303 \times ABS_{350})/(pathlength)}{(DOC)/(1000)} \qquad \text{Eq 3.1}$$

where, pathlength is equal to 1cm and DOC is in units of mg L^{-1}. The coefficient of variation for this method was less than 9%, as determined by replicate analyses on split sample ($n = 12$).

Copper (Cu^{2+}) complexation by DOM was measured using the same methodology and experimental conditions described previously in Chapter 2. Complexing capacity (CC_1, CC_2) and conditional stability ($\log(K_1)$, $\log(K_2)$) parameters were calculated from titration data using a 2-ligand Langmuir Isotherm discrete binding model. In addition, the molar concentration of Cu^{2+} binding sites (CuL_M, μmol L^{-1}) was operationally calculated at 1.0E-06 mol L^{-1} of free labile Cu^{2+} and at *in situ* DOC concentrations. Confidence limits (95%) for each of the parameter values and for CuL_M were approximated from the results of Chapter 2 as a function of DOC concentration.

3.5 Results

3.5.1 Environmental Transformation and Fractionation of Dissolved Organic Matter (DOM)

Titration curves for reference samples were empirically ranked from 1 to 5 according to the degree of Cu^{2+} complexation, which was determined by the density of Cu^{2+} binding sites (CuL, μmol C-g^{-1}) for a given concentration of free labile Cu^{2+} (μmol L^{-1}). Using these criteria, the empirical rank order for reference titration curves was the same as the rank order for cumulative hydrologic residence time (CHRT; Figure 3.2a). For example, the titration curve for allochthonous dissolved organic matter (DOM), which corresponds to the lowest CHRT (< 1 day), consistently showed the highest density of Cu^{2+} binding sites (CuL, μmol C-g^{-1}) for any given concentration of measured free labile Cu^{2+} (μmol L^{-1}). This ranking indicated that allochthonous DOM exhibited the highest CuL, reaching a maximum of 164 μmol C-g^{-1} at the operational titration end-point of 1.0 μmol L^{-1} of free labile Cu^{2+}. In contrast, the titration curve for autochthonous DOM corresponded to the lowest extreme of Cu^{2+} complexation, with a maximum CuL of 68.5 μmol C-g^{-1}.

With respect to the Langmuir Isotherm model, higher CuL (μmol C-g^{-1}) corresponds to higher complexing capacity (CC_1, CC_2), and lower buffered concentrations of free labile Cu^{2+} correspond to ligands of higher binding affinity ($Log(K_1)$, $Log(K_2)$). The complexing capacity of high affinity binding sites (CC_1) and the binding affinity of low affinity sites

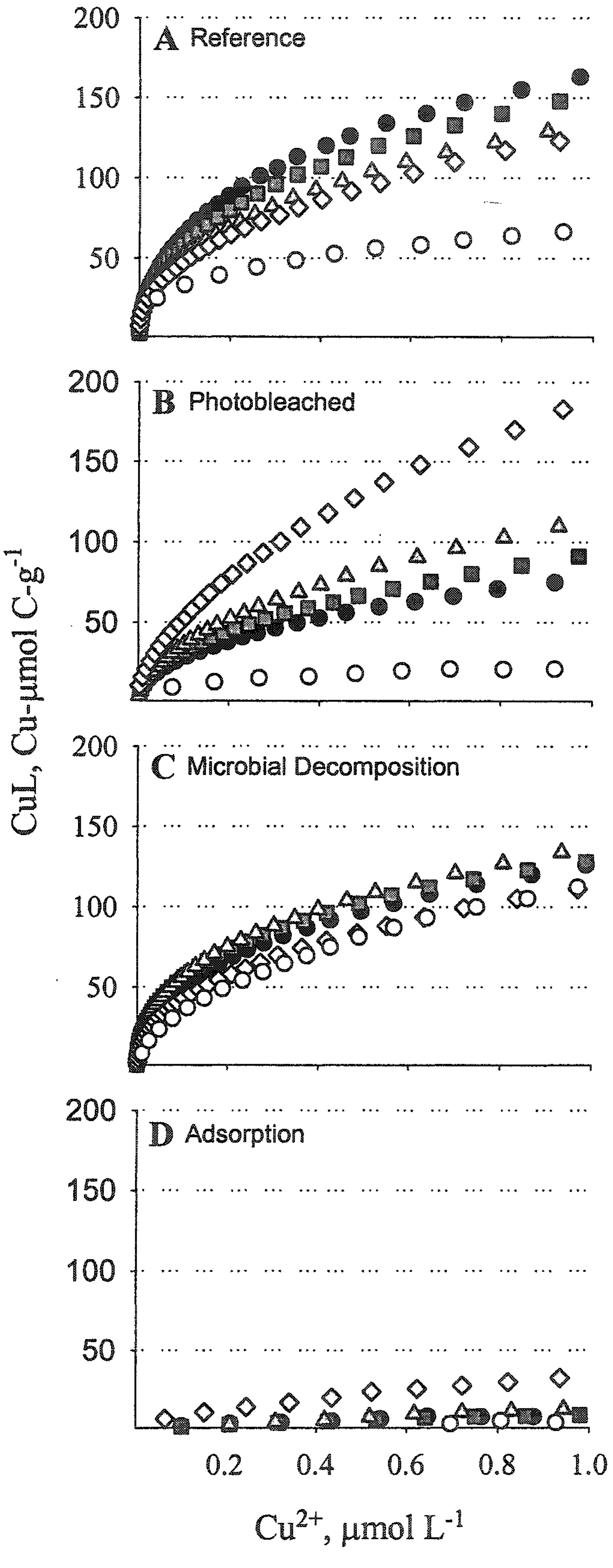

Figure 3.2. Potentiometric titration curves for allochthonous (closed circle) and autochthonous (open circle) dissolved organic matter (DOM) and for DOM of increasing cumulative hydrologic residence time (CHRT; square-1yr, triangle-20yr and diamond-81yr respectively). Curves are shown for reference (A) and treated samples (B, C and D).

(Log(K_2)) were inversely dependent on CHRT ($r^2 = 0.99$ and $r^2 = 0.91$ respectively; $p < 0.05$; Table 3.1), which was consistent with the empirical rank order for reference titration curves. CC_1 values decreased by 19% ranging from 39 to 32 μmol C-g^{-1} and Log(K_2) values decreased by 4% ranging from 6.3 to 6.1 as a function of CHRT (Table 3.1). Furthermore, the upper and lower extreme in CC_1 and Log(K_2) parameter values corresponded to allochthonous and autochthonous DOM respectively. Log(K_1) and CC_2 parameter values were independent of CHRT.

Dissolved organic carbon (DOC) concentration and DOM colour, measured by a specific absorbance coefficient (SAC_{350}), were inversely dependent on CHRT ($r^2 = 0.99$ and $r^2 = 0.98$ respectively, $p < 0.05$), which was consistent with dependence of organic Cu^{2+} complexation on CHRT (Figure 3.3). DOC concentration decreased by 48% from 10.1 to 5.2 C-mg L^{-1} ($p < 0.05$) as a function of CHRT (Figure 3.3), which is a greater proportional decrease as compared to CC_1 and Log(K_2) qualitative variables.

The molar concentration of Cu^{2+} binding cites (CuL_M), which is calculated as product of Cu^{2+} binding density and DOC concentration, is inversely dependent on CHRT ($r^2 = 0.99$, $p < 0.05$; Figure 3.3), and is strongly correlated to SAC_{350} ($r = 0.99$). Values of CuL_M, decreased by 60% from 1.7 to 0.7 μmol L^{-1} as a function of increasing CHRT, which approximately corresponds to the sum of the proportional decrease observed in parameter values (CC_1 and LogK_2) and DOC concentration.

Table 3.1. Dissolved organic carbon (DOC) concentration and parameter values of the 2-ligand Langmuir Isotherm for reference samples from study lakes spanning a gradient in cumulative hydrologic residence time (CHRT, yr).

CHRT, yr	DOC (C-mg L^{-1})	CC_1 (μmol C-g^{-1})	Log(K_1) (1/mole)	CC_2	Log(K_2)
Allo/ <<1	10.1	39	7.9	180	6.3
1	9.2	35	8.1	200	6.2
20	6.9	32	8.2	180	6.1
81	5.2	32	8.1	190	6.1
Auto	1.4	31	7.8	82	6.0
Mean	6.6	34	8.0	160	6.1
Standard Deviation	3.5	3.3	0.2	47	0.1

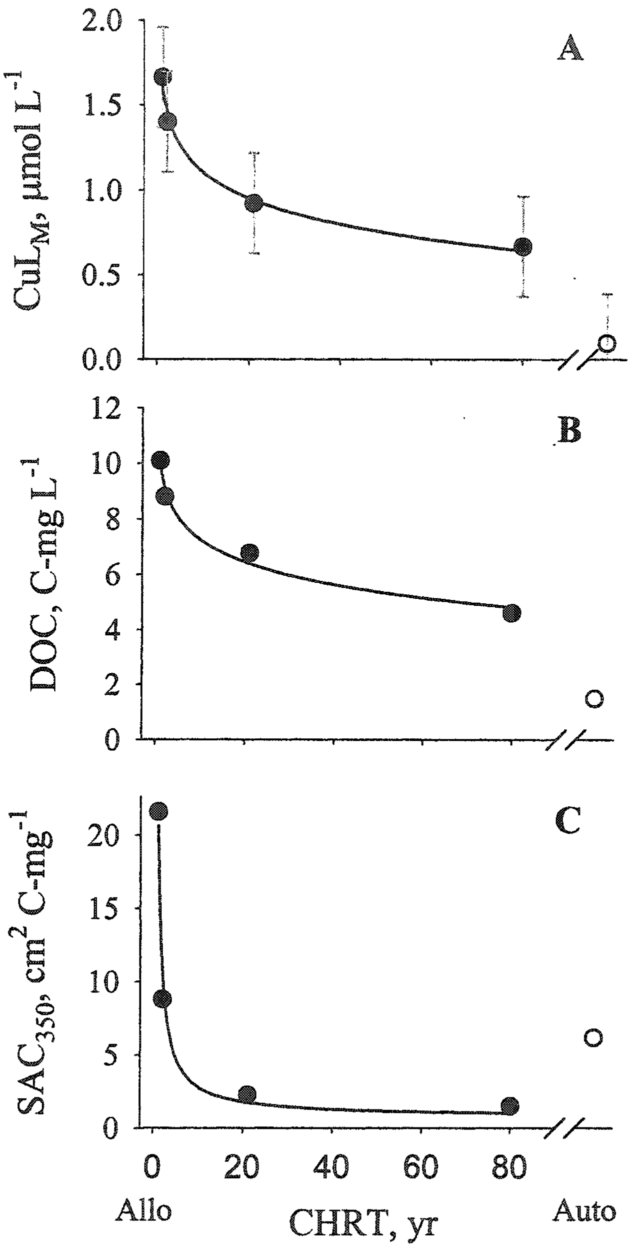

Figure 3.3. Molar concentration of Cu^{2+} complexing sites (CuL$_M$)(A), dissolved organic carbon (DOC) concentration (B) and specific absorbance (SAC$_{350}$)(C) as a function of increasing cumulative hydrologic residence time (CHRT; $p < 0.05$) for reference samples. The 95% confidence interval is shown for CuL$_M$.

3.5.2 Experimental Transformation and Fractionation of Dissolved Organic Matter (DOM)

3.5.2.1 Photochemical Decomposition

Titration curves for photobleached samples were empirically ranked from 1 to 5 as done for the reference curves. Using these criteria, the empirical rank order is exactly opposite to reference curves, with the exception of autochthonous DOM, which consistently represents the lower extreme of Cu^{2+} complexation (Figure 3.2b). For example, the titration curve for allochthonous DOM, which exhibits the lowest CHRT, consistently shows the second lowest density of Cu^{2+} binding sites (CuL, μmol $C\text{-}g^{-1}$) next to autochthonous DOM for any given concentration of free labile Cu^{2+}. In contrast, the titration curve for the sample of DOM corresponding to the highest CHRT exhibits the upper extreme of Cu^{2+} complexation, with CuL reaching a maximum of 188 μmol $C\text{-}g^{-1}$.

Parameter values of the Langmuir Isotherm for photobleached samples are consistent with the order of empirical titration curves for photobleached samples (Figure 3.2b) and were compared to reference samples by calculating the percent change. Therefore, negative scores correspond to a proportional decrease in parameter values and vice versa for positive scores (Figure 3.4a). The effect of photochemical decomposition on $Log(K_1)$ values ranged from –2% to -10% and is independent of CHRT, whereas changes to CC_1, $Log(K_2)$ and CC_2 values are all dependent on CHRT (Figure 3.4a). CC_1 and $Log(K_2)$ values decreased by as much as -40% asymptotically as a function of CHRT, which is consistent with the environmental trend measured in reference samples (Figure 3.3). For example, the largest reproducible decrease in CC_1 and $log(K_2)$ values is for allochthonous DOM, which exhibits the lowest CHRT and the least exposure to transformation and fractionation processes. In contrast, CC_2

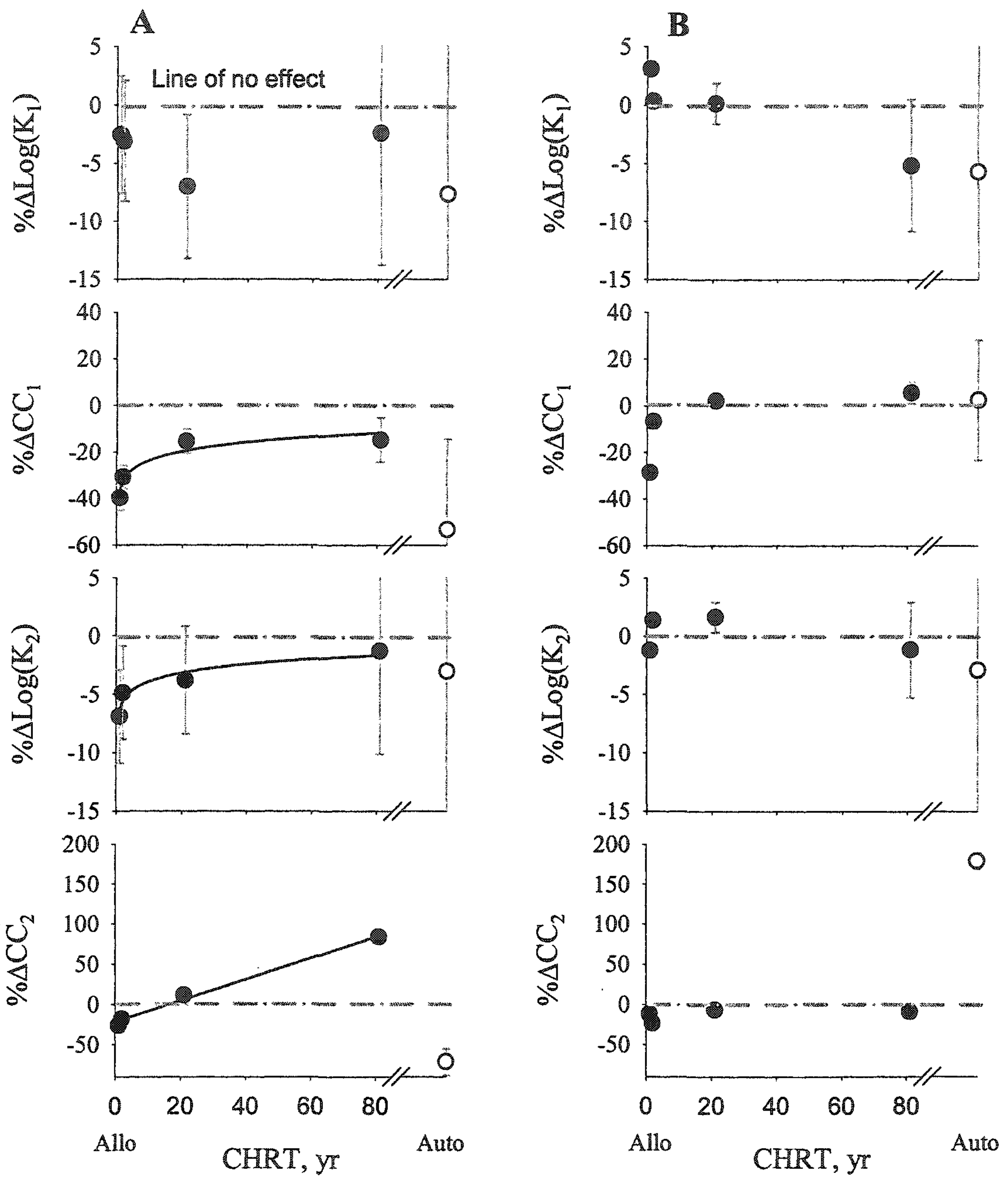

Figure 3.4. Percent change of treated to reference values for parameters of the 2-ligand Langmuir Isotherm as a function of cumulative hydrologic residence time (CHRT). Allochthonous and autochthonous sources of dissolved organic matter (DOM) are presented at extreme ends of CHRT scale. Data shown for photobleached DOM (A) and DOM exposed to high rates of microbial decomposition (B). Line of no effect is shown in each graph (horizontal grey dash-dot line). Trend lines only shown for significant regression analyses ($p < 0.05$). The 95% confidence interval is shown for all data points.

values increased by as much as +84% directly as a function of increasing CHRT.

The DOC concentration of photobleached samples was compared to reference samples in the same way as parameter values of the Langmuir Isotherm (Figure 3.5a). Changes in DOC concentration range from -60% to -37% as a function of CHRT ($p < 0.05$). Furthermore, the final concentration of DOC in all photobleached samples is similar (4.8 C-mg L^{-1}, SD = 1.3) to the DOC concentration measured in high CHRT systems (5.2 C-mg L^{-1}).

3.5.2.2 Microbial Decomposition

Titration curves for DOM exposed to nutrient enriched microbial decomposition were empirically ranked from 1 to 5 as done for reference curves. However, the empirical rank order is unclear because the curves tend to overlap (Figure 3.2c). In addition, the interpretation of the titration curves is suspect because of significant losses in DOC concentration across the cation exchange resin (CER) pre-treatment step. Losses of DOC with the CER pre-treatment step range from -37.1 to -71.1 C-mg L^{-1} as a function of CHRT ($p < 0.05$), which corresponds to -28% and -62% respectively (Figure 3.6). This effect is not observed for any other set of reference or treatment samples, where only negligible losses of DOC are observed.

Parameter values of the Langmuir Isotherm for samples exposed to nutrient enriched microbial decomposition are suspect because of the DOC losses in the CER pre-treatment step, but are consistent with the order of empirical titration curves (Figure 3.2c). The parameter values were compared to reference samples using the same criteria as photobleached samples. The effect of microbial decomposition on parameter values of the Langmuir Isotherm is independent of CHRT in all cases (Figure 3.4b). However, the

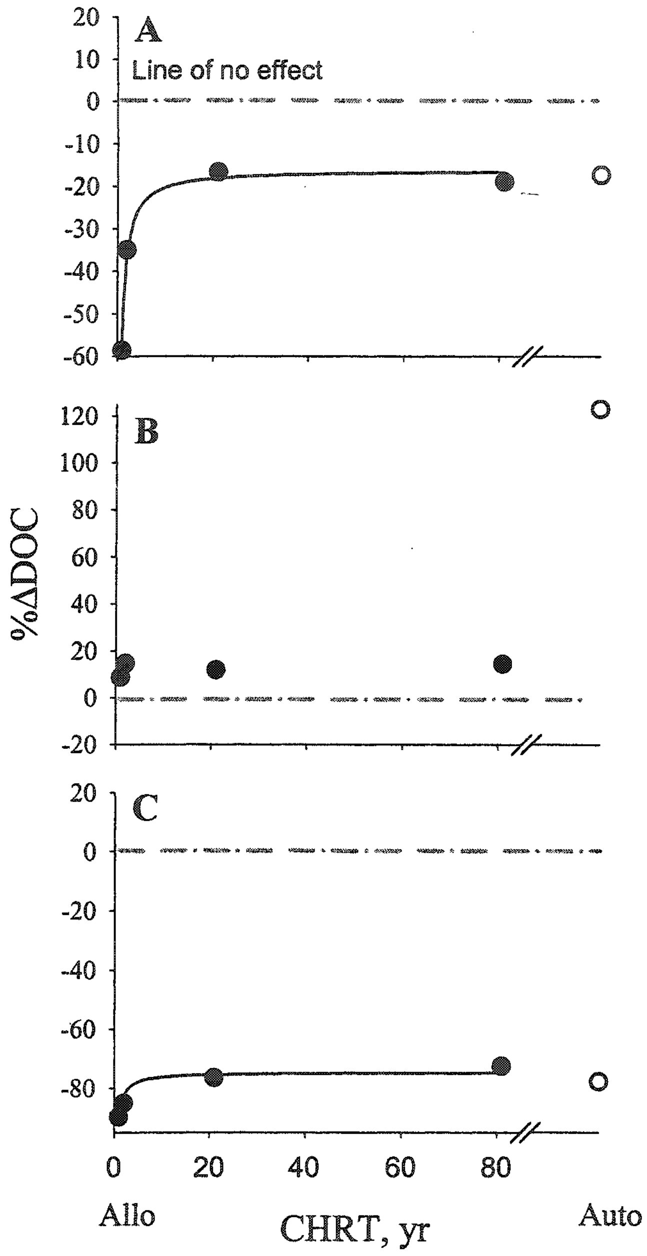

Figure 3.5. Percent change of treated to reference values for dissolved organic carbon (DOC) concentration as a function of cumulative hydrologic residence time (CHRT) and for allochthonous (closed circle) and autochthonous (open circle) sources of dissolved organic matter (DOM). Data shown for photobleached DOM (A), DOM exposed to high rates of microbial decomposition (B), and DOM exposed to high rates of adsorption fractionation (C). Line of no effect is shown in each graph (horizontal grey dash-dot line). Trend lines only shown for significant regression analyses ($p < 0.05$).

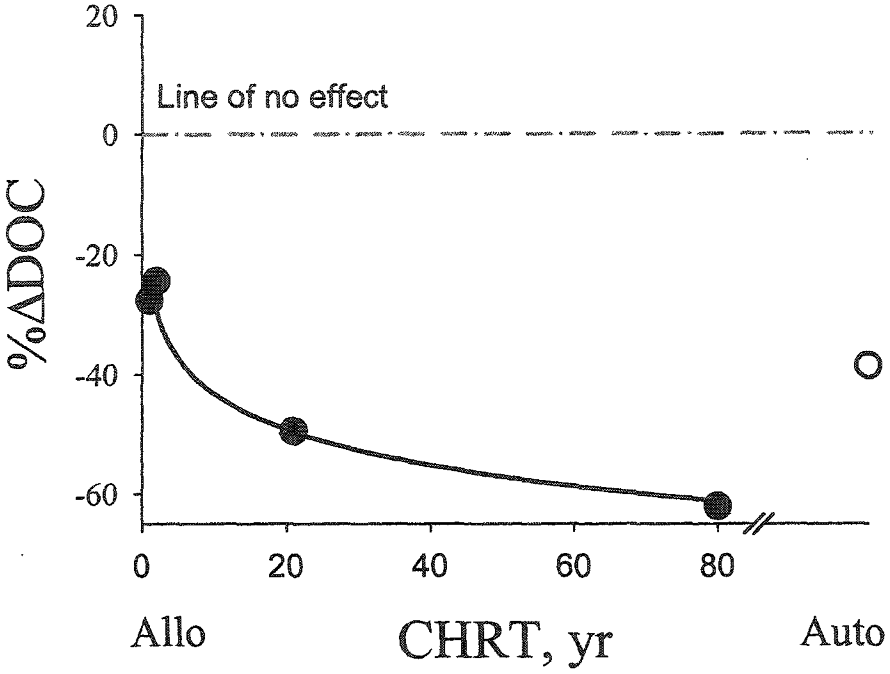

Figure 3.6. Net loss of dissolved organic carbon (DOC) with the cation exchange resin pretreatment step as a function of cumulative hydrologic residence time (CHRT; $p < 0.05$) for samples that were exposed to high rates of microbial decomposition. Line of no effect is shown (horizontal grey dash-dot line).

effect of microbial decomposition is positive for $Log(K_1)$ values of allochthonous DOM and negative for $Log(K_1)$ values of autochthonous DOM, with values of +3% and –5% respectively. Furthermore, CC_1 values decrease for allochthonous DOM by –29% and increase for autochthonous DOM by +3%. This trend is consistent with comparisons of reference and treated empirical titration curves (Figure 3.2).

3.5.2.3 Adsorption

Titration curves for samples treated with $FeOH_{3(s)}$ show a significant decrease in CuL (Figure 3.2d), but are suspect because DOC concentrations were below recommended concentrations (See Chapter 2), between 2 and 9 C-mg L^{-1} for all samples. Low DOC concentrations are due to significant scavenging of DOM by $FeOH_{3(s)}$ particles, which range from -90% to -79% directly as a function of CHRT (Luider *et al.* in Press). No parameter values were produced for these titration curves because Cu^{2+} complexation was near the lower extreme of detectable limits and because the fit of the Langmuir Isotherm to experimental data was not significant ($p > 0.05$).

3.6 Discussion

3.6.1 Environmental Transformation and Fractionation of Dissolved Organic Matter (DOM)

The rank order of Cu^{2+} titration curves for reference samples, and the dependence of CC_1 and $Log(K_2)$ values on cumulative hydrologic residence time (CHRT), supports the conclusion that Cu^{2+} complexation varies systematically in surface freshwaters as a function of two factors. These two factors include (1) dissolved organic matter (DOM) source, and

(2) exposure to transformation and fractionation processes with increasing CHRT. The dependence of organic Cu^{2+} complexation on DOM loading is consistent with a critical review of published data on organic Cu^{2+} complexation (Town and Filella 2000), and appears to depend primarily on DOM from allochthonous sources (Richards *et al.* 2001). Although high affinity binding sites are present in autochthonous DOM, the density of these sites is very low. A similar trend is observed for low affinity binding sites of autochthonous DOM, and autochthonous dissolved organic carbon (DOC) concentrations are comparatively low in surface freshwaters (Zumstein and Buffle 1989; Meili 1992; Jonsson *et al.* 2001). Therefore, the contribution of autochthonous DOM to Cu^{2+} complexation is likely negligible compared to allochthonous sources.

The dependence of organic Cu^{2+} complexation on CHRT is conservative because of an inverse dependence of Cu^{2+} binding density on DOC concentration, as determined in chapter 2. Cu^{2+} binding density decreases for allochthonous DOM as a function of increasing DOC concentration via reverse osmosis (RO) concentration. Therefore Cu^{2+} binding density may be underestimated for allochthonous DOM because samples were concentrated prior to potentiometric analyses. In addition, the dependence of organic Cu^{2+} on CHRT is consistent with other analytical measures of DOM, such as specific absorbance (SAC_{350}) and DOC concentration. This relationship supports conclusions that optical measures of DOM, such as SAC_{350}, can provide good approximations of organic Cu^{2+} complexation (Saar and Weber 1980; Gamble 1980; Ryan and Weber 1982).

Comparisons of qualitative measures of organic Cu^{2+} complexation to systematic variability in DOC concentration support the conclusion that the effects of increasing CHRT are greater on DOC concentration. There is relatively little change in CC_1 and $Log(K_1)$

parameter values with increasing CHRT, as compared to the change in DOC concentration. The result is a systematic decrease in the molar concentration of Cu^{2+} binding sites (CuL_M) as a function of increasing CHRT. Therefore, an inverse relationship between DOC concentration and CHRT augments systematic decreases in organic Cu^{2+} complexation due to changes in DOM composition. The implication of this trend to Cu^{2+} complexation in surface freshwaters is that DOM composition is of secondary importance to DOC concentration, which is consistent with current Cu^{2+} speciation prediction models (Breault *et al.* 1996; Christensen *et al.* 1999; Bryan *et al.* 2002).

3.6.2 Experimental Transformation and Fractionation of Dissolved Organic Matter (DOM)

3.6.2.1 Photochemical Decomposition

Changes in CC_1 and $Log(K_2)$ values for photobleached samples indicate that photochemical decomposition of DOM could contribute to the dependence of organic Cu^{2+} complexation on CHRT for two reasons. Firstly, the net effect of the photochemical decomposition treatment is a decrease in CC_1 and $Log(K_2)$ values, which is consistent with the effect of CHRT. Similarly, photochemical decomposition reduces DOC concentration, which contributes Cu^{2+} complexation by the molar concentration of Cu^{2+} binding sites (CuL_M). Increasing exposure of DOM to photochemical decomposition processes, such as photolysis, could therefore contribute to the inverse dependence of organic Cu^{2+} complexation on CHRT, which is consistent with previous research on increased metal bioavailability following DOM exposure to ultraviolet (UV) B radiation (Winch *et al.* 2002).

Secondly, the net effect of photochemical decomposition on CC_1 and $Log(K_2)$ values is inversely dependent on the pre-exposure of DOM to environmental transformation and fractionation processes, as approximated by CHRT. For example, the largest decrease in CC_1 and $Log(K_2)$ values for photobleached samples is observed in allochthonous DOM, which exhibits the least pre-exposure to environmental transformation and fractionation processes (CHRT < 1 day). Losses of DOC concentration with photochemical decomposition treatment follow a similar trend. Therefore, DOM that is pre-exposed to transformation and fractionation processes is more refractory to photochemical decomposition than unexposed DOM, with respect to Cu^{2+} complexation.

In contrast to changes in CC_1 values, changes in CC_2 values for photobleached samples are not consistent with the dependence of organic Cu^{2+} complexation on CHRT. Increases in CC_2 values of the Langmuir Isotherm with increasing CHRT suggest that the net effects of photochemical decomposition processes change as a function of increasing DOM exposure to environmental transformation and fractionation processes. Photochemical decomposition may therefore result in a higher density of low affinity Cu^{2+} complexing sites as DOM becomes increasingly refractory. This increase in binding site density is not consistent with the inverse dependency of organic Cu^{2+} complexation on CHRT. This discrepancy suggests that decreases in DOC concentration with increasing CHRT have a greater effect on CuL_M than increases in Cu^{2+} binding density that may occur as a result of photochemical decomposition.

3.6.2.2 *Microbial Decomposition*

Decreases in DOC concentration with the cation exchange resin (CER) pretreatment step suggest that the effects of microbial decomposition on DOM are directly dependent on CHRT. For example, the smallest decrease in DOC concentration is observed in allochthonous DOM, which exhibits the shortest CHRT (< 1 day). In addition, the fraction of DOC lost in the CER process likely corresponds to transformed or fractionated DOM because this effect is exclusive to microbially decomposed samples. Therefore, DOM that is pre-exposed to transformation and fractionation processes is more susceptible to microbial decomposition than unexposed DOM, as approximated by CHRT.

This rate dependence of microbial decomposition on CHRT is consistent with an identified coupling mechanism to photochemical decomposition processes because the byproducts of photolysed DOM can stimulate microbial productivity (Bushaw *et al.* 1996; Moran and Zepp 1997a). The exposure of DOM to photochemical decomposition processes increases directly as a function of CHRT, and therefore, so may the byproducts of photo-transformed and fractionated DOM. However, the extrapolation of this trend to surface freshwaters is limited by the controlled experimental conditions of the microbial decomposition treatment method.

Interpreting the effect of microbial decomposition on Cu^{2+} complexation is obviously limited by the decrease in DOC concentration with the CER pretreatment step because DOM was lost from the samples prior to potentiometric titration analysis. Therefore, analyses of Cu^{2+} complexation for microbially decomposed samples are suspect depending on the Cu^{2+} complexing properties of the lost DOM fraction. For example, analyses are conservative assuming that the lost fraction of DOM is similar to the remaining fraction with respect to organic Cu^{2+} complexation. Similarly, Cu^{2+} complexation may be under or overestimated in microbially decomposed samples if the lost fraction of DOC binds Cu^{2+} more or less effectively than the remaining fraction of DOM respectively.

3.6.2.3 Adsorption

The effect of adsorptive fractionation on Cu^{2+} complexation by DOM could not be measured directly because DOC concentrations remaining after the treatment process were below recommended levels for potentiometric titrations (See Chapter 2) due to significant scavenging by $FeOH_{3(s)}$. This decrease in DOC concentration suggests that the potential affect of adsorptive fractionation on Cu^{2+} complexation is high, but is apparently not fully expressed in surface freshwaters (Luider *et al.* in Press). Adsorptive fractionation of DOM could therefore significantly reduce organic Cu^{2+} complexation by effectively reducing DOC concentration, and particularly if the decrease in DOC concentration is proportional to a decrease in Cu^{2+} binding density (CuL, μmol C-g^{-1}).

3.7 Conclusion

This research supports the conclusion that the bulk Cu^{2+} complexing properties of DOM vary systematically in surface freshwaters as a function of the following two factors: (1) DOM from allochthonous sources, and (2) exposure to transformation and fractionation processes, as approximated by cumulative hydrologic residence time (CHRT). Photochemical decomposition, microbial decomposition and adsorptive fractionation processes apparently contribute to this trend either by influencing the composition of DOM or by contributing to a decrease in DOC concentration in surface freshwaters. The latter appears to be the most important variable in predicting organic Cu^{2+} complexation because the dependency of DOC concentration on CHRT is proportionally greater than Cu^{2+} complexation, as approximated by parameter values of the 2-ligand Langmuir Isotherm.

4. Chapter 4. Summary and Conclusions

Dissolved organic matter (DOM) plays an important role in surface freshwaters by buffering pH and trace metals, such as Cu^{2+}. DOM is composed of a heterogeneous mixture of organic acids, and a fraction of these organic acids act as ligands that can complex Cu^{2+} ions. The objective of this research was to determine if organic acidity and Cu^{2+} complexation vary systematically in surface freshwaters. This research is relevant to the field of ecotoxicology because of environmental problems due to acid rain and increasing metal contamination from anthropogenic sources, such as mining and smelting industries. Therefore, better understanding of how organic acidity and Cu^{2+} complexation vary in surface freshwaters is important from a management perspective.

My overall hypothesis was that organic acidity and Cu^{2+} complexation vary systematically according to DOM source and increasing hydrologic residence time (HRT). DOM is loaded from terrestrial (allochthonous) and aquatic (autochthonous) sources which each have characteristic properties. Moreover, it has been determined that the concentration and optical properties of DOM can be predicted from HRT by approximating exposure to transformation and fractionation processes, such as photochemical decomposition, microbial decomposition and adsorption processes. This approach was applied to organic acidity and the Cu^{2+} complexing properties of DOM.

Testing of this overall research hypothesis was divided into two parts. The first part of this research addressed the reproducibility of potentiometric titration analyses, as modeled by a two-ligand Langmuir Isotherm. Moreover, I concentrated samples of DOM in a reverse osmosis (RO) concentrator to test the effects of increasing dissolved organic carbon (DOC) concentration on potentiometric analyses of organic Cu^{2+} complexation. This analysis was applied to allochthonous and autochthonous sources of DOM, which are typically thought to represent the upper and lower extreme of organic acidity and Cu^{2+} complexation respectively.

I determined that analyses of organic acidity are limited by low concentrations of organic acids and by the accuracy of a pH electrode at *in situ* DOC concentrations in the majority of aquatic systems. Similarly, a low proportion of high affinity Cu^{2+} binding sites limits analyses of Cu^{2+} complexation. In addition, conditional stability parameters of the Langmuir Isotherm model decreased according to increasing titration end-point concentrations of complexed and free labile Cu^{2+} because of modeling artifacts. This effect was similar to the effect of increasing DOC concentration, which is consistent with a combination of two processes; multidentate complexation of Cu^{2+} and occlusion of Cu^{2+} binding ligands. Collectively, these results indicate that the precision and accuracy of potentiometric titration analyses is directly dependent on experimental conditions, such as DOC concentration, which could explain 20% to 60% of the reported variation in measures of organic Cu^{2+} complexation.

The second part of this research tested the overall research hypothesis. Organic acidity and the Cu^{2+} complexing properties of DOM were measured in samples from lakes spanning a gradient in cumulative hydrologic residence time (CHRT) and in allochthonous and autochthonous DOM end members. In addition, subsamples of DOM were treated across micro-reactors to investigate the contribution of photochemical decomposition, microbial decomposition and adsorption processes to systematic variation in DOM.

Reference and treated samples were concentrated in a reverse osmosis (RO) concentrator prior to potentiometric titration analyses. Sample concentration was investigated in the first part of this thesis, and therefore, I could measure organic Cu^{2+} complexation by high affinity binding sites and compare parameter values of the Langmuir Isotherm among reference and treated samples of DOM. However, DOC concentrations were too low for analyses of organic acidity. Analyses of organic acidity were therefore excluded from the results and the overall hypothesis of this research was applied primarily to Cu^{2+} complexation by ligands of DOM.

I determined that there is a 2.5-fold decrease in the molar concentration of Cu^{2+} complexing sites as a function of increasing CHRT ($p < 0.05$), which reflects a decrease in the Cu^{2+} binding properties of DOM and a decrease in DOC concentration. Treatment of DOM in the micro-reactors suggests that photochemical decomposition, microbial decomposition and adsorption processes could contribute to this trend. Therefore, the overall hypothesis of this research was accepted, which supports the conclusion that organic Cu^{2+} complexation will vary among surface freshwaters according to the loading of allochthonous and autochthonous DOM and CHRT. The majority of DOM is loaded from allochthonous sources, and there is an overall transition in surface freshwaters from DOM that is characteristic of allochthonous sources to DOM that is characteristic of autochthonous sources with increasing CHRT.

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