COPPER COMPLEXATION BY NATURAL ORGANIC MATTER (NOM) AS RELATED TO SAMPLE ORIGIN
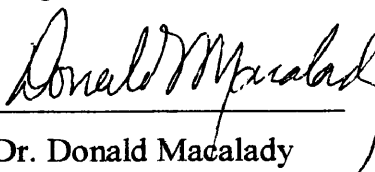
By

Magnus Sköld

A thesis submitted to the Faculty and Board of Trustees of the Colorado School of Mines in partial fulfillment of the requirements for the degree of Master of Science (Environmental Science and Engineering).

Golden, Colorado

Date 6/5/99

Signed: _____

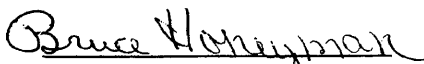Magnus Sköld

Approved: _____

Dr. Donald Macalady

Thesis Adviser

_____

Dr. Bruce Honeyman

Thesis Co-Adviser

Golden, Colorado

Date 24-VI-99

_____

Dr. Philipe Ross

Professor and Head,

Department of Environmental

Science and Engineering

ii

ABSTRACT

Copper is a required nutrient but may be toxic to aquatic organisms at elevated concentrations. Organic matter is ubiquitous in the environment and one of the most important copper complexants. It is well documented that organic matter in natural environments affects the transport and fate of copper as well as its toxicity. Despite the large volume of literature available on copper binding by organic matter, it is still unclear how the molecular structure and chemical composition of organic matter affect copper binding. The binding mechanism also remains unsolved.

In this work, the dependence of the origin of organic matter samples on copper binding is studied using ion-selective electrode titrations. Potentiometric (pH) titrations and UV-analyses were used to elucidate chemical structures such as acid titrable groups and aromaticity, respectively. Results from attempts to relate copper binding to differences in chemical structure show that copper binding properties are a function of the source of natural organic matter. For the samples studied here copper binding is not related to the concentration of acid titrable groups between pH 3.0 and 10.0. Experiments, however, seemed to exhibit a correlation between percent bound copper and UV adsorption.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENTS

OVERVIEW

Natural organic matter (NOM) is among the most important metal complexants in natural waters and it strongly affects the speciation of copper, which in turn is important for the toxicity, bioavailability, and fate of copper. Studies on interactions between organic matter and copper are important for predicting copper speciation in the environment. The purpose of this work has been to investigate differences in copper binding properties of solution phase natural organic matter samples as influenced by their origin. Three objectives were established for this purpose:

i. To quantify variations in copper binding by natural organic matter from a variety of sources.

ii. To investigate relationships between copper binding capacity and the total carboxylate or phenolic group concentrations in organic matter.

iii. To examine connections among other structural characteristics, such as aromaticity, of NOM samples and copper binding.

For each of the three objectives a hypothesis were established. They are, respectively:

i. Copper binding properties of NOM are a function of the source of the NOM.

ii. Acid-titrable functional groups do not correlate with copper binding.

iii. The abundance of aromatic functional groups measured as ultraviolet absorbance at 280 nm is a determining characteristic for explaining variations in the abundance of copper biding sites in NOM, i.e. there is a strong relationship between percent bound copper and the aromaticity of the NOM under a given set of conditions.

CHAPTER ONE. INTRODUCTION

The importance of metal binding by natural organic matter (NOM) has been investigated extensively (Buffle, 1980, McKnight et al., 1983 and Aiken et al., 1985). Despite all the effort, no model has been developed that is able to accurately predict and explain metal speciation under the various conditions prevailing in nature, e.g. pH, organic carbon concentration, competition by metals, ionic strength etc. The extreme complexity of organic matter is the principal reason. The most commonly investigated metal is copper. There are several reasons for this emphasis on copper. One is that there are numerous analytical techniques available for studying complexation of copper by NOM. Secondly, copper is ubiquitous in the environment and thirdly, copper is a micronutrient but also a potential toxicant for aquatic organisms (Amdour et al, 1991, Hutchinson and Sprague, 1987). In the following review of some of the existing literature, the emphasis is on copper although some reports on other metals have been included for comparison.

1.1 NOM

1.1.1 ORIGIN

Natural organic matter is the degradation and leaching product of organic materials such as dead leaves, plant litter and animal residues. The term humic substances is commonly used when referring to the subgroups humic and fulvic acids as well as humin. The material may originate from aquatic or terrestrial plants and organisms. Malcolm (1985) reviewed literature and found that soil and aquatic humic substances have different composition but that it has been assumed that soil organic matter is the primary source for aquatic organic matter in streams. He also states that the assumption is unfounded and points out that there are many potential sources for stream humic substances including groundwater, decaying vegetation and litter, canopy drip, sewage, soil and autochthonous material (material produced where it is found). Thurman and

Malcolm (1983) found that recent plant and soil organic matter contributed 90% of the organic carbon to the total dissolved organic carbon (DOC) in the Suwannee River. The average radiocarbon age was less than 30 years. They also observed a flushing effect during the wet season. Litter accumulated during the fall is swept away by heavy rainfall during fall or snow melting during spring, which increases the DOC concentration in the stream. Material originating from terrestrial systems (pedogenic NOM) can be expected to dominate over aquogenic materials (i.e. material originating from the decomposition of aquatic organisms) in most small lakes and rivers. The opposite is true for large lakes and the ocean (Macalady and Ranville, 1998).

The degradation of organic material is mediated by microorganisms that use the material either as carbon, energy or nutrient source or a combination thereof. The most easily degradable compounds like sugars may be broken down within minutes. But there are compounds that are extremely hard for microorganisms to break down. Most of them have an extremely complex composition with a fairly high aromatic group concentration. This complex composition of the remaining NOM makes it difficult to completely characterize all NOM structures. Despite this difficulty, substantial progress in understanding the properties of these materials has been achieved although only a limited number of compounds have been identified and isolated.

1.1.2 CLASSIFICATION

In an attempt to characterize NOM, the substances have been classified into several classes of compounds that can be separated into two main groups, geopolymers and biopolymers. Biopolymers are predominantly polysaccharides and polypeptides, whereas geopolymers, more commonly referred to as humic substances, can be divided into three subclasses depending on their solubility in acidic and basic solutions; fulvic acid, humic acid and humin. Humic acid (HA) is the fraction soluble in basic solution but insoluble in strong acids while fulvic acid (FA), is soluble under all pH conditions. Humin is the fraction that remains insoluble under all pH conditions (Saar and Weber, 1982). Figure 1
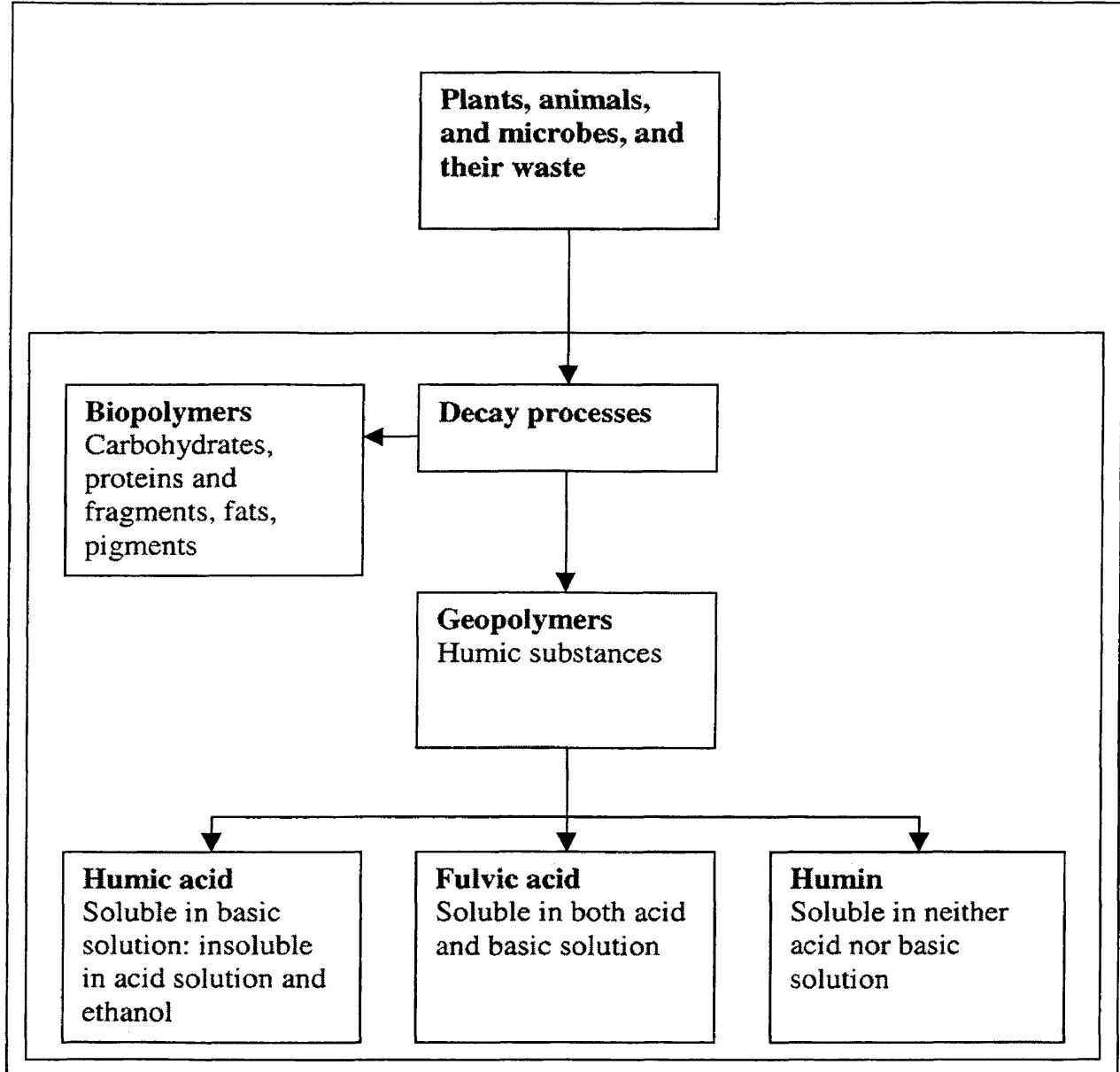
Figure 1. Origin of humic substances and relationships among them. Adapted from Saar and Weber (1982).

shows the classification of humic substances and the relationship between them. These classes are operationally defined and different separation techniques give slightly different results. In most reports on metal binding to organic matter it is presupposed that the term NOM refers to geopolymers.

1.1.3 CHEMICAL COMPOSITION AND STRUCTURE

Natural organic matter varies a lot in elemental and functional group composition depending on origin and fraction (Malcolm, 1985). The major element is carbon followed by oxygen, hydrogen and nitrogen. NOM also contains traces of sulfur and phosphorous. Table 1 shows mean elemental composition of humic and fulvic acid from fifteen rivers. Consistent with the high oxygen concentration, aquatic humic materials contain large numbers of functional groups. There is approximately one oxygen containing functional group per three to six carbons. However, there are differences in carboxyl content depending on aquatic environment. NOM from bogs, marshes and swamps has the lowest concentration of carboxyl groups of all aquatic organic matter. This reflects the more reducing conditions that prevail in these types of environments (Thurman, 1985). Sahu and Banerjee (1996) also suggested that seasonal changes might result in varying abundance of oxygen containing NOM in river water.

Table 1. Mean elemental composition of humic and fulvic acid from fifteen rivers (% mass). Adapted from Thurman (1985), referring to Thurman and Malcolm (unpublished data).

Sample	Chemical Element						
	C	H	O	N	P	S	Ash
FA	51.9	5.0	40.3	1.1	0.2	0.6	1.5
HA	50.5	4.7	39.6	2.0	--	--	5.0

The percentage of hydrophobic organic substances in groundwater is less than in surface water, reflecting that adsorption of these structures to particles probably has occurred (Thurman, 1985). Thurman also reported that groundwater organic matter contains fewer aromatic substances and less oxygen than humic substances from surface waters.

The carbon skeleton of humic and fulvic acids is mainly aliphatic but contains a lot of aromatic carbons. Malcolm (1985) reports that 16-20% of the carbon in stream fulvic acid is aromatic and that stream humic acids contains about 30% aromatic carbon. Fulvic acids are generally more highly charged per mass and have a higher average molecular mass than humic acids. As a result FA are more soluble than HA (Macalady, 1998), see also figure 1.

Organic carbon concentration in visually uncolored water in the USA ranges from 1.5 to 10 mg C/l with an average of 2.2 mg/l (Malcolm, 1985). Figure 2 shows the classification of organic carbon in uncolored surface water in the USA. Half of the organic carbon consists of humic substances, with FA being the dominant fraction. Hydrophilic acids is the fraction of humic substances that is not retained by an XAD resin at pH 2, i.e. the substances are more hydrophilic than both FA and HA that are soluble at different pH's but retained by an XAD resin. Visually colored surface water varies much more both in carbon concentration and composition. Carbon concentration ranges from approximately 5 mg C/l to more than 50 mg C/l. Humic substances represent 80% of the total DOC in Suwannee River water in Georgia as compared to 48% humic substances of the 33 mg C/l DOC Pleasant River water in Maine.
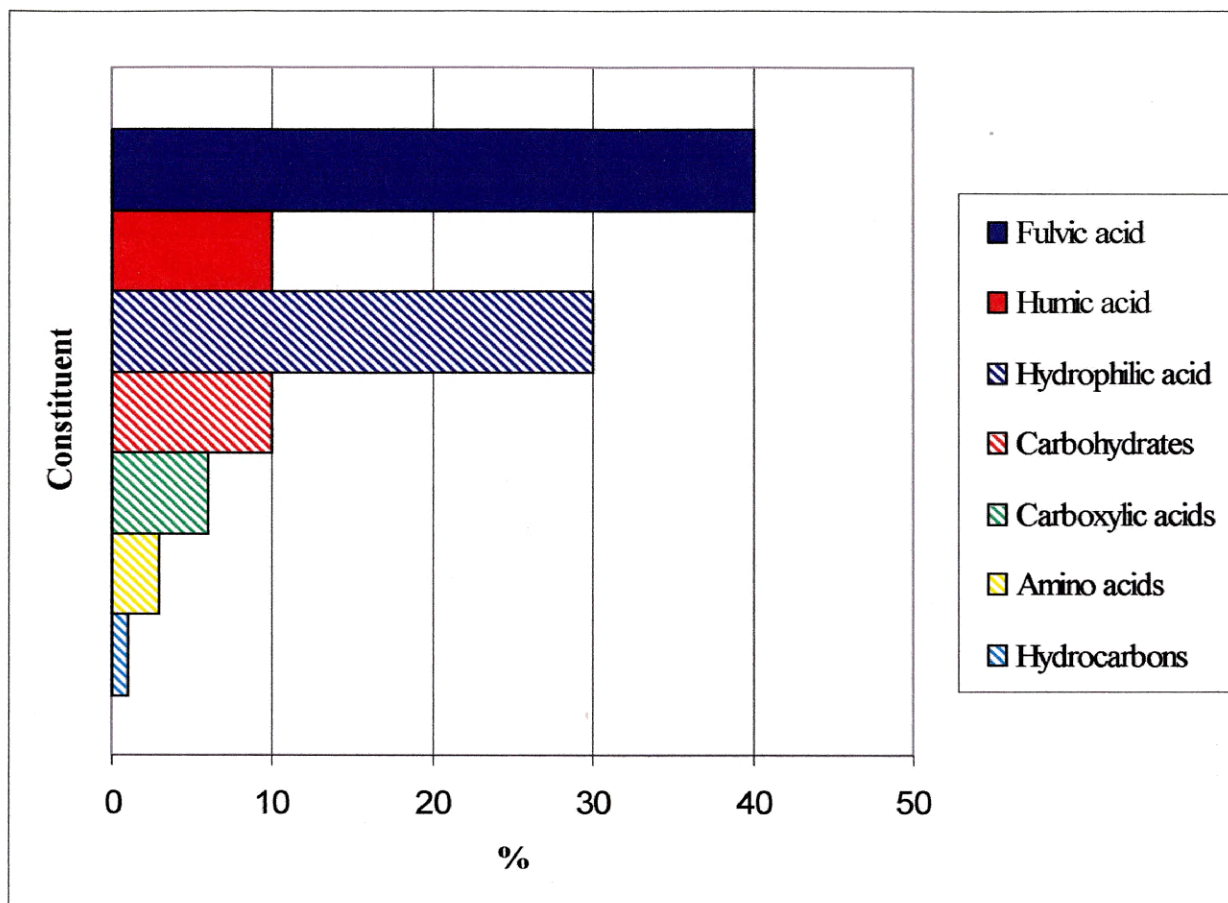
Figure 2. Distribution of dissolved organic carbon (DOC) per dry mass basis in an average river with a DOC of 5 mg C/l. Adapted from Thurman (1985).

1.1.4 ENVIRONMENTAL IMPORTANCE

NOM affects several environmentally significant reactions. One of the most important characteristics of organic matter is that hydrophobic organic contaminants tend to sorb to organic matter in soils. The amount of organic contaminant that partitions onto soil is often directly related to the organic carbon concentration in the soil. In addition, NOM is important for the carbon cycle in ecosystems and the redox properties of NOM affects the recycling of iron in surface waters as well as redox reactions of organic contaminants (Macalady and Ranville, 1998). This thesis focuses on the ability of NOM to bind metal ions, which is an extremely important feature for metal availability and toxicity in aquatic environments. This quality also enables NOM to act as either a sink for metals by sorption onto particles or a facilitator of transport in natural aquifers by binding to dissolved NOM (Macalady and Ranville, 1998).

1.2 METAL TOXICITY

Copper is a required micronutrient for most organisms and very low concentrations may result in deficiencies (Amdour et al., 1991), conversely it may have adverse toxic effects at elevated concentrations (in the order of µmole/l). Copper has been shown to be toxic to many species; for example, embryo and adult zebrafish (Palmer et al., 1998), dapnidae (Giesy et al., 1983) and rainbow trout (Hollis et al., 1997).

Fishes have effective defenses against ingested metals but have no mechanism to tolerate free metal ions contained in water pumped past their gills (Florence et al., 1992). Metal ions can exert their toxicity directly at the gill surface or by passing through the gills and giving rise to adverse effects internally. The sorption of positive metal ions on the negative fish gills is a faster reaction than the slow transport processes that result in internal harmful effects (Hollis et al., 1997). Therefore, longer experiments are needed to determine metal accumulation in fish than for acute toxicity.

Copper is believed to affect sodium uptake in trout. Trout plasma has a higher sodium concentration (140 mmole/l) than fresh water resulting in a concentration gradient and

diffusion of sodium from the fish to the water. To compensate for the loss, sodium is actively transported back into the fish. Copper ions in the water may bind to sites on the fish gill, and once internalized, interfere with the active sodium uptake. If NOM or other complexing or chelating agents are present in the water, the concentration of free copper ions is decreased by binding to the NOM and the toxic effect is reduced (Hollis et al., 1997).

It has been suggested in the literature that the fully hydrated copper ion is the only toxic form of copper, whereas other results have suggested that other copper species may contribute to copper toxicity. Ma et al. (1999) found that the rate of the reaction between copper and humic acid in the presence of calcium affected the bioavailability and toxicity of copper. Calcium was found to react faster with HA than copper but with time copper binding to HA increased, resulting in decreasing free copper concentration. They related copper toxicity to *Ceriodaphia dubia* directly to the free ion form. Welsh et al. (1993) showed that copper is acutely toxic to larval fathead minnow at 2 µg/l at pH 5.6 and 0.2 mg DOC/l and that 96 hr LC_{50} values (concentration resulting in 50% mortality) increased (toxicity decreased) with increasing pH and unfiltered lake DOC (to 182 µg Cu/l at pH 6.9 and 15.6 mg DOC/l). This is in line with observed increase in copper binding by NOM at increasing pH and organic matter concentration. Hutchinson and Sprague (1987) showed that toxicity of zinc, aluminum and copper decrease in the presence of organic matter. Erickson et al. (1996) suggested that about 20% of the copper bound to organic matter is available for toxicity. Some of the more weakly bound copper may become available at the surface of the fish gill by competition between the organic matter and the gill surface. Welsh et al. (1993) state that organic matter reduces acute metal toxicity but it is still unclear wether or not some organometallic species contribute to chronic toxicity. Binding constants for cadmium and copper on gill surfaces have been calculated showing that cadmium binds about 16 times more strongly than copper at fewer sites according to calculations using Langmuir isotherms (Playle et al., 1993). As NOM is more efficient in binding copper than cadmium, it seems likely that NOM is

more important for reducing copper than cadmium toxicity towards fish. Palmer et al. (1998) monitored copper toxicity to embryo and adult zebrafish. They concluded that, in addition to free copper, lipophilic, but not lipophobic, copper complexes such as Cu-n-hexadecylmalonic acid, contributed to copper toxicity measured as delayed hatching times and mortality for adult zebrafish.

Accumulation of cadmium and copper in rainbow trout (Hollis et al., 1997) over a period of seven days was shown to be due to the free ion forms. Giesy et al. (1983) found in their experiments that bioconcentration factors calculated as free copper were similar between different ponds and well water but that accumulation of copper was not strictly first order with respect to free copper ions.

Cadmium has also been suggested to interfere with ionoregulation in fish. Due to similarities in ionic radius cadmium competes with calcium for sites on fish gills, thereby blocking the pumping of calcium ions from surrounding water into the fish, necessary to compensate for the diffusive loss from fish plasma to water. Calcium concentration in fish plasma is considerably lower (2.2 mmole/l) than sodium and cadmium toxicity may be reduced by relatively high calcium concentration in the water because of less diffusive loss of calcium. Hollis et al. (1997) suggested that trout attempt to compensate for loss in calcium uptake by producing more, possibly stronger calcium binding sites on the fish gill. Such compensation has been reported for rainbow trout experiencing competitive inhibition of calcium uptake by zinc (Hogstrand et al., 1995).

1.3 METAL INTERACTIONS WITH NOM

NOM is among the most important complexing agents for metal ions in natural surface and interstitial waters (Buffle et al., 1977). Metal/NOM interactions strongly influence bioavailability (Palmer et al., 1998), toxicity (Welsh et al., 1993), mobility (Temminghoff et al., 1997), fate (Chapra, 1997) and accumulation in sediments (Buffle et al., 1977) of the metal. Metal/NOM interactions are selective, that is both the type of

metal and composition of the NOM influence the metal binding. Some important properties of metal/NOM interactions are discussed below.

1.3.1 BINDING TYPES

Two fundamentally different binding types have been suggested in the literature; non-specific and specific binding (Zhang et al., 1996). Non-specific binding is sometimes also referred to as territorial binding or counterion condensation. Specific binding interacts covalently with an electrostatic component with a specific ligand on the organic molecule whereas the non-specific bond is governed by electrostatic interactions between the positively charged metal ion and the negatively charged organic macromolecule.

Metals have different tendencies for the two binding mechanisms. Non-specific binding should be less important than is binding at a specific site for group 1B and 2B metals (Zhang et al., 1996). Elements in these groups have an electron pair in the d-orbital available for binding (Gamble et al., 1980). The alkaline metals on the other hand would have stronger tendency for electrostatic interactions. Lewis classification of transition metals into soft, intermediate and hard acids may also play an important role.

Carboxylic and phenolic groups have commonly been suggested to act as principal sites for metal binding (Gamble et al., 1980). Sites of ortho dicarboxylic acid and salicylic acid type have been suggested to provide sites where metals may form chelates, a very strong binding. As an example, salicylic acid has a log binding constant for a 1:1 copper/ligand complex of 10.6 at 25 °C and 0.1 mole/l ionic strength (Martell and Smith, 1973).

Strong acid sites with pK_a's around two have also been found as constituents of natural organic matter. Leenheer et al. (1995) suggested possible relationships among these aliphatic carboxylic structures and the metal binding properties of NOM. These strong acids would occur if α-ether or α-ester groups were in cyclic structures with two to three additional electronegative groups at adjacent positions on the ring.

The type of bonds between metal ions and the NOM molecule determines to a great extent how strong the bond is. Cleven (1984) used the mobility of metal ions to describe the strength of the bond. Based on the diffusion coefficient in aqueous systems in the presence of NOM as compared to an identical solution without NOM, he classified metals interacting with polyacids into three groups:

i.   Ions that have lost their original mobility because they are fixed to functional groups on the polyacid. The molecular diffusion coefficient of the metal is identical to that of the polyacid.

ii. Partially (de)hydrated with restricted mobility as compared to a polyacid-free but otherwise identical solution, with respect to pH, ionic strength etc. The behavior of these ions is mainly attributed to electrostatic field effects. Some authors consider them bound and some consider them free.

iii. Free metals, e.g. metals unassociated with the polyacid. The mobility is virtually the same as in a polyacid-free solution.

Gamble et al. (1980) discovered that for 50 mg C/l solutions of fulvic acid titrated with copper, standard Gibbs free energy decreased with increasing total copper concentration. The change was most marked at low copper concentrations. The results were interpreted in terms of the strongest chelate (or complex) forming first. Cleven (1984) states that for the association of metal ions with highly charged polycarboxylic acids, the electrostatic contribution to the free energy may be much higher than the covalent contribution. Generally, however, covalent bonds between metals and NOM are considered stronger than electrostatic interactions.

1.3.2 PARAMETERS INFLUENCING METAL BINDING

Type of NOM

Metal binding properties of fractionated aquatic and terrestrial organic matter have been studied extensively whereas whole water samples or plant extracts have not received

the same attention. The two fractions that have been considered most often are humic and fulvic acids. McKnight (1983) found similar copper binding properties for aquatic humic acids isolated from eighteen different environments and concluded that copper binding by aquatic humic acids was similar and that copper speciation could be modeled to within a factor of two by measuring parameters like pH, organic carbon concentration and concentration of important inorganic ions. Other authors have compared metal binding properties of humic and fulvic acids. In an attempt to compare the protonation reactions and copper binding strength and capacity for different peat humic and fulvic acids, little variation in binding strength and capacity per gram of organic matter was found among humic acids or among fulvic acids extracted at different pH values (Town and Powell, 1993). The major differences were between the fulvic and humic fractions, where HA had both higher conditional binding constants and higher complexation capacity than FA. It was also noted that FA had higher titrable acidity and a larger calculated fraction of strongly dissociating carboxyl groups (42-46% of total acidity with $pK_a < 2.3$) compared to HA (27-41% with $pK_a < 2.8$). Thus, the carboxylic groups in the humic acid bound more copper per carbon than the investigated fulvic acid. Sahu and Banerjee (1996) found that metal humates (copper, lead and cadmium) were more stable than metal fulvates when investigating HA and FA from river sediments.

Metal

Metals have varying tendencies to interact with organic matter. Transition elements tend to form covalent bonds using d-orbitals and therefore have a possible advantage over other divalent metal ions that only form electrostatic interactions [see section above on binding types]. As an example, copper interacts more strongly with NOM than calcium (Hering and Morel, 1988). Sahu and Banerjee (1996) studied three heavy metals and found that conditional formation constants of sediment-derived FA and HA followed the order Cu-L > Pb-L > Cd-L. They discussed the results based on ionic potential and Lewis' classification of hard and soft acids. The results of van den Hoop et al. (1995)

show that, in the presence of Ca at different concentrations, zinc has slightly higher stability functions than cadmium.

Competition between metal ions for binding sites

Divalent metal ions may compete with copper for sites on the NOM molecule to a much higher degree than do monovalent ions because of the higher charge. Calcium and magnesium are divalent ions that exist at relatively high concentrations in natural waters and may therefore compete with copper, despite their lower formation constants. Calcium is the most commonly used ion to study competitive effects with copper. Different degrees of competition have been reported. Hering and Morel (1988) found little or no competitive effect at 0.5 mole/l NaCl as background electrolyte. Van den Hoop et al. (1995) reached a different conclusion from experiments performed in 0.03 mole/l KNO$_3$. They suggested that the calcium competed for the same sites as zinc and cadmium and, subsequently, binding of cadmium and zinc in the presence of calcium can be modeled with calcium formation constants determined in the absence of any other metal ions. They also concluded that electrostatic contribution is not important in calcium binding in experiments where the calcium/ligand ratios were varied substantially. Cabaniss and Shuman (1988) found that copper binding decreased slightly when the calcium or magnesium concentration increased from 0 to 0.01 mole/l at 1 μ mole/l copper. Competition is expected to be more important if the competing metal ions form bonds with the same ligand type on the organic molecule.

Metal loading and organic carbon concentration

The metal to ligand ratio has been used to study the effect of electrostatic attractions on metal/NOM binding. Increasing metal association with NOM decreases the effective charge on the macromolecule. Subsequently, added metal ion may be associated less firmly if electrostatic attraction governs over the covalent contribution. Also, the higher concentrations of ligands, the more specific binding sites are available for metal ions having a potential for binding at those sites. The charge of the macromolecule also affects covalent binding. At low metal concentration the most thermodynamically favorable site

would for the most part be occupied first leaving remaining, less attractive binding sites for higher metal concentrations. Variations in single point stability constants calculated at different ligand/metal ratios are therefore expected and have been assigned to heterogeneiteis in the organic matter molecules (McKnight and Wershaw, 1994) as well as to changes in electrostatic attractions (Cleven, 1984). At high ligand/metal ratios, mean stability values for zinc and cadmium are greater than at low ratios (van den Hoop et al., 1995). Mathuthu and Ephraim (1993) found that stability functions for calcium increased with FA/metal ratios at low ionic strength but that the they were insensitive to ligand/metal ratios at high ionic strength because of screening by sodium ions. They argued that calcium binding to humic substances is primarily electrostatic.

pH

Hydrogen ion activity (pH) influences binding of metals to both organic and inorganic ligands. The importance of hydroxide and carbonate complexation increases with increasing pH due to higher concentrations of hydroxide and carbonate ions. Organic matter has more deprotonated functional groups at high pH, resulting in more negatively charged organic molecules. Many scientists have investigated copper binding to NOM as influenced by pH and it has been established that binding increases with pH over natural pH ranges (Cabaniss and Shuman, 1988, Kipton et al., 1996 and Sahu and Banerjee, 1996). Ephraim and Allard (1994) found that the fraction bound copper (bound Cu/total Cu) increased from 35% to 90% from pH 4 to 6 with FA/Cu molar ratio equal 3 and 0.1 M $NaClO_4$ ionic strength.

Ionic strength

According to Manning's counter-ion theory (Manning, 1979 and 1981), increasing counter ion concentration (ionic strength) would increase metal ion binding to polyacid molecules. However, the opposite has generally been found experimentally. McKnight and Wershaw (1994) found in their experiments that copper binding to a fulvic acid decreased with increasing ionic strength and Cabaniss and Shuman (1988) found a

significant decrease in copper binding with increasing ionic strength as well. The effect was more pronounced at pH 7 than at pH 5.14 or 8.44.

1.3.3 METAL BINDING MODELS

If a model that is able to accurately predict metal ion binding by natural organic matter could be developed, it would mean great progress. Toxicity, mobility and transport of the metal could ideally be estimated from a few simple measurements of the water and/or soil of interest. Much effort has been expended to reach this goal and several models have been formulated, see table 1 in Westall et al. (1995). A model must account for differences in binding strength and stoichiometry at various ligand/metal ratios, electrostatic interactions, variations in pH and ionic strength, competition by other metals and heterogeneities in the organic matter in order to be valid over a wide range of environmental conditions. For the purpose of this work the models have been divided into two types. The first type assumes a small number of discrete coordination sites in the manner of classical coordination chemistry and the second type includes a continuous distribution of binding sites.

Discrete binding site models

In the simplest model it is assumed metal binding to only one site of concentration L_T, no interactions between sites and 1:1 binding stoichiometry. The model can be described by the equilibrium (charges have been omitted for simplicity):

$$Cu + L \Leftrightarrow CuL \qquad [1]$$

With a conditional formation constant

$$K = [CuL]/[Cu][L] \qquad [2]$$

[Cu] represents concentration of free copper, [L] is the free ligand concentration and [CuL] is the conentration of copper bound to NOM. If it is believed that more than one

site exists, the total amount of bound copper can be calculated by summing the contribution from each of n sites. The contribution from each ligand is derived from

$$Cu + L_i \Leftrightarrow CuL_i \qquad \text{where } i>1 \qquad [3]$$

Electrostatic interactions due to the charge on the macromolecule have been corrected for in the electrostatic model (Turner et al., 1986). The model includes only one binding site but with the stability constant dependant on the amount of metal bound to the ligand. The apparent stability constant, K^*, is calculated from

$$K^* = K_{int}*exp\{-(M_t-M_{free})/L_{tot}\} \qquad [4]$$

where K_{int} is the intrinsic binding constant, M_t is the total metal concentration, M_{free} is the free metal concentration and L_{tot} is the total ligand concentration on the NOM molecule. The intrinsic binding constant is the binding constant at no interactions between the binding sites at zero ionic strength.

These models are only valid at fixed pH and the effect of the hydrogen ion activity must be included to correctly predict metal binding at varying pH. Variations in copper binding have been found to be of variable stoichiometry with respect to pH (Cabaniss and Shuman, 1988). But if, for simplicity, it is assumed that increasing metal binding is first order with respect to increasing hydrogen ion activity this feature can be modeled by the equation

$$Cu + HL \Leftrightarrow CuL + H \qquad [5]$$

Continuous distribution models

Continuous distribution models assume a very large number of sites. The form of the distribution may be unknown (Gamble et al., 1980), assumed to follow a Gaussian distribution (Bartshat et al., 1992) or follow a normal distribution (Perdue and Charles, 1983). Various metal to ligand binding stoichiometries (1:n) have been considered (Buffle, 1984) and are described by

$$Cu + nL_i \Leftrightarrow Cu(L_i)_n \qquad \text{where } n>1 \qquad [6]$$

Interactions between the binding sites, such as electrostatic interactions and stereochemical availability have been accounted for. These interactions have been incorporated by multiplying the intrinsic metal binding constant by a correction function as in the electrochemical model to get an apparent conditional formation constant. As it is likely that NOM has a variety of binding sites for copper, continuous distribution models may describe the chemistry of the system better than models with only one or two binding sites.

Lately one model that includes both site-specific binding and electrostatic interactions has been developed. This model, the NICA-Donnan model (Benedetti et al., 1996 and Kinniburgh et al., 1996) assumes equilibrium with a gel phase and the NOM/metal complex and metal binding is mathematically described by a bimodal Gaussian distribution. Due to higher molecular weight it is suggested that the model works better for HA than for FA. The model also "attempts to separate the effects due to generic heterogeneity experienced by all ionic species from those effects arising from effects specific to each particular ion" (Kinniburgh et al., 1996).

Turner et al. (1986) investigated several models and found that the simple two-site model fits copper-into-NOM titration data the best, based on both statistical and practical grounds. They noted however, that the binding parameters may not have any direct physical significance and may not be valid at different ligand to metal concentrations. For the scope of this work it was believed that this type of model would give enough insight to compare experimental data collected under identical conditions, changing only the origin of the NOM.

1.3.4 MODEL APPROACH USED IN THIS THESIS

The literature suggests that carboxylic and phenolic groups serve as principal copper binding sites (Gamble et al., 1980). The purpose of the model described here is to investigate if carboxylic or phenolic groups serve as principal binding sites with equal copper binding intensity. If they are similar in copper binding strength, the concentration

of acid titrable groups measured from pH titrations may serve as an estimate of ligand concentration in copper binding models. However, if no correlation is found between percent bound copper and carboxylic and/or phenolic group concentrations we may conclude that the sites are significantly different in copper binding strength. Another measurement of ligand concentration than the total concentration of carboxylic and phenolic groups must then be used to assess ligand concentration in copper binding models.

If it is assumed that one predominant site with fixed conditional formation constant (without correction for electrostatic interactions) exists on various organic matter at the same pH and ionic strength:

$$Cu + L \Leftrightarrow CuL \qquad\qquad [7]$$

a conditional constant, K, can be expressed as

$$K = [CuL]/([Cu]*[L]) \qquad\qquad [8]$$

where [Cu] is the free copper concentration, [L] is the free ligand concentration and [CuL] represents the concentration of copper bound to NOM. Using mass balance equations, the ratio of bound copper/free copper can be expressed as a function of free ligand concentration

$$[CuL]/[Cu_{tot}] = [L]/([Cu]*[L]) \qquad\qquad [9]$$

As

$$[L_{tot}] = [L] + [CuL] \qquad\qquad [10]$$

we can assume that [L] is close to [L_{tot}] (total ligand concentration) in a solution where the copper concentration is low compared to the total ligand concentration.

Figure 3 shows calculated percent bound copper as a function of total ligand concentration at a copper concentration where free ligands are in excess over occupied ligands. It is assumed that copper binding to NOM can be described by only one binding site. Results from experiments in this thesis is presented in section 3.4.1 to show how

well the model described above depicts the relationship between percent bound copper and acid titrable groups.
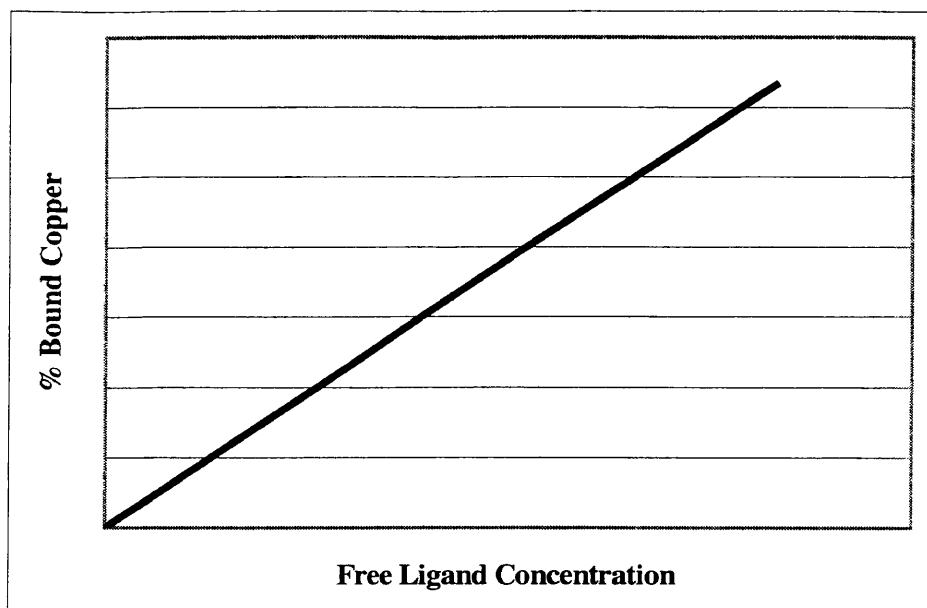
Figure 3. Bound copper vs free ligand concentration. It is assumed that copper concentration is low and that [CuL] is much smaller than [L]. The slope of the line depends on the formation constant and the scale on axis is arbitrary.

CHAPTER TWO.   MATERIALS AND METHODS

2.1 NATURAL ORGANIC MATTER (NOM)

In this thesis single-species extracts and river water samples were used as sources of NOM. The choice of samples was based on the wish to represent a range of ecological systems as sources of organic matter. As single-species extracts contain degradation and leachate products from only one plant species they are likely to be more different from each other than a mixture of degradation products from several species. River water consists of organic matter from terrestrial and aquatic sources where the terrestrial NOM has passed through physicochemical reactions during transport, such as sorption and redox reactions that affect and possibly homogenize the chemical composition of the material.

It was also desired to compare copper binding properties of NOM from dominant plant species in a catchment basin with the river water itself. Such a comparison may provide a slight insight into the geochemical processes that NOM is going through during transport from the place of origin to the stream. For that purpose two rivers whose catchment basins are dominated by one plant species were chosen. Table 2 summarizes the samples used in this work.

Single species extracts were prepared by allowing mixtures of deionized water and plant material to rest for one to one and a half months in a closed container in the dark and cold. The resulting "tea" was then filtered with a screening filter and the sample was bubbled with nitrogen and thereafter stored in the cold and dark for circa two years before use. The natural waters were filtered using a filter with pore size of 0.45 micrometer followed by nitrogen purging and aging in refrigerator for approximately two years. Oxygen concentration was limited in the NOM solutions but strictly anoxic conditions did not exist.

Table 2. Descripiton of the samples used in this work (Stewart, 1993 and Salmon, 1996).

Source of NOM	Biological Origin
Bamboo (genus unknown)	Grass distributed worldwide. Live and dead leaves and young plant shoots sampled at Dunedin, N.Z.
Cabbage Tree (*Cordyline austraulis*)	Many branched palm-like tree. Normally grows up to 12 m. Grows in swampy areas in N.Z. Old trees have trunk diameters of up to 1 m. Fallen dead leaves and stems were sampled in Dunedin, N.Z.
Kauri (*Agathis australis*)	Largest tree in N.Z. Reaches 30 m or more. Trunks have diameter of 3-7 m. Produces gum when cut. Largest trees are estimated to be 2000-4000 years old. Sampled from Dunedin Botanical Garden, N.Z. Sample consisted of live and dead leaves and twigs.
Manuka (*Leptospermum scoparium*)	Small tree or shrub. Grows to 4-8 m high. Occurs in shrublands and forests in N.Z. Trunk diameter around 60 cm. Sampled from cut tree, bark and wood chips only, in Dunedin, N.Z.
Red Beech (*Nothotagus fusca*)	Tree growing in N.Z. reaching 30 m high with 2-3 m through trunks. Wood is dark red when first cut. Used as timber. Sampled near Westport, N.Z. Fallen leaves and twigs, some sawdust.
Red Tussock (genus unknown)	Grass growing in tufts. Dominating species in the Central Otago grassland of N.Z. (Sutton Stream). Live plants with both living and non-living parts were sampled at the Dunedin Botanical Gardens, N.Z.
Sutton Stream	Stream in N.Z. starts in Central Otago, flows east and ends in the Atlantic Ocean.
Suwannee River	Starts in the Okefenokee Swamp in Georgia, runs south-west and flows out in the Gulf of Mexico. Sampled by the USGS in 1995. Sampling site unknown.
Swamp Cypress (*Taxodium distichum(L)*)	Dominating species in the Okefenokee Swamp, which is the source for Suwannee River.

The concentration of organic matter was measured as organic carbon concentration. NOM samples acidified to pH 3.0 with nitric acid, were analyzed on a Shimadzu TOC 500 total carbon analyzer in triplicates.

2.2 COPPER SPECIATION

2.2.1 CHOICE OF TECHNIQUE

There are many techniques available for measuring copper ion speciation in water samples. They are generally divided into separation and non-separation techniques. In separation techniques copper/NOM complexes are separated from dissolved copper ions by differences in chemical properties like molecular size and polarity. Ultrafiltration, liquid chromatography and equilibrium dialysis have been applied to separate free copper ions from complexed or bound ions. After the separation step the total copper concentration is measured by, for instance, mass spectrometry that can measure a very wide range of metals, a major advantage compared to most non-separation techniques. On the other hand separation techniques have two major disadvantages: (1) adsorption on membranes or chromatographic materials and (2) shifting of equilibria (Saar and Weber, 1982).

Fluorescence quenching, stripping voltammetry and ion selective potentiometry are widely used non-separation techniques. The basis for fluorescence quenching is that humic matter fluoresces, but its fluorescence is quenched by complexation to paramagnetic ions. In stripping voltammetry, an electrical potential is applied to the sample causing changes in oxidation state of the metal ion. As a result the metal ion is deposited on an electrode surface. The amount of metal ion absorbed on the electrode surface is quantified by measuring the current resulting from the release of the ions when the potential changes direction. For this study ion selective potentiometry (ISE) measures the electrode potential generated by free metal ions in the solution. Ion selective potentiometry was chosen, as it provides an accurate way to measure free copper in the presence of NOM without using much equipment. The concentration of free copper ions,

the parameter of most environmental concern, is measured directly and the bound copper can be calculated as the difference between free and total copper concentrations. The method has been used in numerous studies dealing with copper binding by natural organic matter (Buffle, 1980, Breault et al., 1996 and Midorikawa, 1990). It is important to notice two disadvantages with this technique. First, free copper ion concentration is measured on a log scale and the relative experimental error is fairly large even though the electromotive force (e.m.f.) can be measured accurately. The maximum experimental uncertainty is expected to be 1 mV, which is equivalent to 8% uncertainty in free copper concentration. Secondly, the minimum measured free copper concentration in this work was found to be 0.1 μmoles/l. The background concentration in most rivers is below this value and the results can not be extrapolated to the background concentrations without introducing some uncertainties.

2.2.2 COPPER TITRATIONS

Copper titrations were performed in a glass beaker with sufficient capacity for 100 ml solution. Copper ions in the form of copper perchlorate hexahydrate were added to the NOM solution with calibrated mechanical pipettes and cupric activity was monitored using an ORION Model 9629 ion**plus**™ cupric ion-selective electrode with a built-in reference cell connected to a ORION RESEARCH microprocess ionoanalyzer/901 voltmeter. Hydrogen ion activity was measured simultaneously with an ORION combination pH electrode connected to a Beckman Φ45 pH meter. The total added copper concentration ranged from 0.05 μmole/l to 3 mmole/l in each experiment. The titrations were performed at room temperature, 23 to 25 °C, pH 6.00 ± 0.02 with 50 mmole/l certified A.C.S. Fischer Chemical KNO_3 as background electrolyte. In order to minimize light interference with the electrode surface, the glass beaker was wrapped with aluminum foil. The NOM sample to be titrated was filtered using a 0.45 micrometer syringe filter followed by dilution with deionized water to achieve an NOM level of 10 mg C/l (except for Sutton Stream water that has an organic carbon concentration of only

5.7 mg C/l). After dilution the NOM solution was bubbled with oxygen-free nitrogen gas for at least one hour at a pH adjusted to 4.0 (with drops of 1.0 mole/l HNO_3) in order to drive off all inorganic carbon (CO_2). A nitrogen atmosphere was kept over the solution throughout the experiment and small amounts of diluted HNO_3 and NaOH were added during the course of each titration to keep the pH stable at 6.0.

Before and after each titration the cupric ion selective electrode and the pH electrode were rinsed in 0.01 mole/l HNO_3 for three to five minutes to dissolve any metal remaining on the electrode surface. The electrodes were stabilized in 0.1 mole/l KNO_3 for fifteen minutes before use.

Standard copper solutions with concentrations ranging from 10^{-5} to 1.0 mole/l were made every four weeks from reagent grade $Cu(ClO_4)_2 \cdot 6H_2O$ obtained from The G. Frederick Smith Chemical Co.. The copper standards were refrigerated and stored in plastic or glass bottles. No difference in electrode response could be measured between old and freshly made solutions. Acid and base solutions were made by diluting concentrated J.T. Baker reagent grade HNO_3 and Mallinckrodt analytical reagent NaOH to 1.0, 0.1, 0.01 and 0.001 mole/l. Acids and bases were stored at room temperature in glass and plastic flasks, respectively.

Two electrode readings were taken after each copper addition. After each addition the solution was stirred until equilibrium was reached. When the stirred reading was taken the beaker was removed from the stirrer and left untouched until the reading stabilized again. Equilibrium was assumed when the change in e.m.f. was less than 0.2 mV/minute over two subsequent minutes. The time to reach stable reading, stirred or non-stirred, varied from 3 to 15 minutes per reading, the longer time occurred at low copper concentrations. One copper-into-NOM titration generally took 5-6 hours.

With each NOM titration a blank titration was performed to calibrate the electrode and to confirm that the electrode response was Nernstian. The blank consisted of deionized water with 50 mmole/l KNO_3 as background electrolyte. The conditions were identical to the NOM titration with respect to pH, ionic strength, acids and bases used to adjust pH

along with copper solutions to control copper concentration. Fewer data points were used in the blank than in the NOM titrations. The copper concentration ranged from 0.05 μmole/l to 1 mmole/l. The electrode response from the blank was used to translate electrode readings to log free copper concentration in the succeeding NOM titration.

2.2.3 MODELING OF COPPER TITRATION DATA

A one-site and a two-site model were used to model copper titration data with a computer program. The code, FITEQL (Herbelin and Westall, 1996), mathematically finds the best fit of a model to experimental data by minimizing the weighted sum of squares (WSOS). A description of FITEQL can be found in Appendix A. As free copper concentration was measured on a log scale, the titration data was input FITEQL as log free and log total copper concentration. To estimate parameters in the two-site model two conditional formation constants with appurtenant total ligand concentrations were estimated by minimizing the weighted sum of squares between the model and the titration data by a semi manual iteration procedure. Conditional formation constants were first found by the computer code using arbitrary total ligand concentrations. Next, these constants were used to let the code find new total ligand concentrations, which in turn were used to find more accurate conditional formation constants. The iteration procedure was completed when there was no change in conditional formation constants or total ligand concentration during two subsequent iteration steps. FITEQL reports parameters with three decimals. The one-site model was fitted directly by FITEQL and no iteration "by hand" was necessary to find one conditional formation constant and one total ligand concentration.

2.3 ACID/BASE TITRATIONS

Acid/base titrations used 50 mmole/l KNO_3 as background electrolyte and a temperature of 25 °C. Prior to each titration 1.0 mole/l HCl was added to adjust the pH to below 3, followed by bubbling with oxygen free nitrogen gas for at least one hour in

order to drive off inorganic carbon. A slight increase in pH was noted while purging the solution with nitrogen gas.

The experiments were performed in a glass vessel. The NOM samples were filtered with a 0.45 μm filter followed by dilution to an approximate NOM concentration of 50 mg C/l, whereas Sutton Stream water was titrated at original filtered concentration, 5.7 mg C/l. An ORION Ross combined pH electrode connected to an ORION Expandable ionAnalyzer EA 940 voltmeter measured pH and a computer program of in-house design (Titrator) was given instructions for the addition of acid and base. The same program was used for all NOM titrations and a second file was used for blank titrations. "Titrator" defined equilibrium as a potential drift less than 1.5 mV/30 seconds.

Incremental volumes of 0.1 mole/l NaOH as governed by the computer program were added from pH 3 to 11 while monitoring pH. Subsequently, the sample was titrated back to pH 3 with 0.1 mole/l HNO_3. Approximately every third titration consisted of a blank, composed of 50 mmole/l KNO_3 in deionized water. The blank was subtracted from each NOM titration when calculating acid/base titrable groups.

2.4 UV SPECTROMETRY

Analyses with ultraviolet light were performed on liquid samples in a quartz cell using a Perkin Elmer Lambda 11 UV/VIS spectrometer. The samples were scanned four cycles with wavelengths ranging from 220 to 400 nm. The samples were diluted with deionized water to concentrations yielding a maximum absorbance less than one. The organic carbon concentration in the samples ranged from 5.7 to 14 mg C/l in the experiments, with Kauri NOM having an organic carbon concentration of 180 mg C/l. The absorbance at 280 nm normalized to organic carbon concentration was used for further analyses of the samples. It was assumed that absorbance at 280 nm represents a surrogate for concentrations of aromatic moieties in the NOM sample (Skoog and Leary, 1992).

2.5 INORGANIC ANALYSES

The samples were analyzed for concentrations of inorganic constituents (metals) in order to investigate whether or not differences in copper binding among NOM samples could be explained by competition for binding sites by inherent metal concentrations. The NOM solutions were filtered with a 0.45 μm syringe filter prior to analysis using a Perkin Elmer Optima 3000 ICP/ES.

CHAPTER THREE. RESULTS AND DISCUSSION

3.1 ORGANIC MATTER

3.1.1 DOC CONCENTRATIONS

The carbon concentrations of 0.45 μm filtered samples ranged from 50 to 150 mg C/l except for two extremes, Kauri extract (1820 mg C/l) and Sutton Stream water (5.7 mg C/l). The relative standard deviation for one triplicate analysis set was generally between 1 and 2 percent. When samples were analyzed on several occasions the difference among analyses were higher, about 10 percent. Table 3 summarizes results of DOC analyses.

Table 3. Measured dissolved organic carbon (DOC) concentrations in samples used in this study.

Sample	DOC (mg/l) Concentration (range)	
Bamboo Extract	68	(61-68)
Cabbage Tree Extract	130	(124-144)
Kauri Extract	1820	(1780-1850)
Manuka Extract	110	(98-140)
Red Beech Extract	140	(116-151)
Red Tussock Extract	120	(96-147)
Suttton Stream water	5.7	(5.4-5.7)
Suwannee River water	49	(42-50)
Swamp Cypress Extract	83	(75-102)

3.1.2 NATIVE METAL CONCENTRATIONS

The importance of metal ions for competition with copper for binding sites on natural organic matter has been described in chapter one. ICP/ES analyses of undiluted NOM samples reveal that the total inherent metal ion concentrations in the samples are low compared to added copper concentrations. The measured metal ions may be in the form of free ions or as complexed or incorporated in the structure of the organic molecules. Table 4 shows elemental analyses normalized to the organic matter concentration used in copper speciation experiments, 10 mg C/l.

The inherent copper concentration ranges from 0.01 μmole/l to 0.4 μmole/l at NOM concentrations used in copper titrations. Only two samples contain levels above the starting concentration in copper titration experiments. Measured copper binding may be affected at very low concentration but inherent copper should not have a major impact on binding above micromolar levels in 10 mg C/l.

Other metal ions that bind relatively strongly to organic matter include cadmium, zinc and lead. The samples contain zinc in micromolar concentration or lower. Zinc binds less strongly to NOM than does copper [see chapter one] and would not be a strong competitor for copper binding sites. Sutton Stream is the sample that contains the most cadmium. Still the concentration, 0.03 μmole/l, is too low to be important in these experiments. Lead is below the detection limit, 0.1 μmole/l, in undiluted samples, in all but one sample. In a hypothetical sample with organic carbon concentration of 100 mg C/l, a total lead concentration of 0.01 μmole/l at 10 mg C/l would be detected.

Magnesium and calcium may compete with copper due to their high abundance in natural environments. Iron could be included in this group as well, but the concentration is insignificant. The summed concentrations of calcium and magnesium reach up to 0.6 mmole/l at 10 mg C/l in some samples. McKnight and Wershaw (1994) found that 0.1 mmole/l $Ca(NO_3)_2$ ionic strength decreased free copper with around 0.4 log units in the presence of 26 mg C/l Suwannee River FA at 0.1 μmole/l free copper

Table 4. ICP analyses of metal ions in samples used in this work. Concentrations in µ or nmole/l are normalized to 10 mg C/l, the NOM concentration used in copper-into-NOM titrations. Maximum experimental uncertainty is estimated to be 20 %.

Sample	Element						
	Cu (nM)	Cd (nM)	Pb (nM)	Zn (nM)	Ca (µM)	Mg (µM)	Fe (µM)
Bamboo	220	0.18	170	1900	390	140	9.1
Cabbage Tree	28	1.9	< 8	410	390	100	0.40
Kauri	1.3	0.11	< 0.5	14	60	15	0.0023
Manuka	23	1.6	< 10	410	37	15	0.15
Red Beech	185	3.2	< 8	280	34	11	0.34
Red Tussock	35	11	< 9	58	560	38	0.16
Sutton Stream	420	32	< 100	1300	76	58	5.6
Suwannee River	65	6	< 21	80	5.1	5.4	2.1
Swamp Cypress	8.3	3.2	< 12	10	380	250	< 0.011

concentration. The same experiment with $NaNO_3$ as background electrolyte at various concentrations did not affect copper binding. Hering and Morel (1988) found no effect on copper binding with or without 10 mmole/l calcium present in experiments with 220 mg C/l Suwannee Stream HA.

To summarize, calcium and magnesium may have an effect on copper binding at very low copper concentrations in the experiments performed in this work. Other metal ions are not believed to affect copper speciation significantly above micro molar levels. The main purpose of this work is to investigate variations in metal binding due to the origin of NOM. All samples contain trace amounts of metals and it is the difference in inherent metal ions that may influence the variance in copper binding. Thus, the small effect of inherent metal ions does not change the overall picture that copper binding is dependent on the origin of NOM, fig 6. Moreover, the two extremes in copper binding, Kauri and Manuka NOM, have very similar metal ion concentrations at the same organic carbon concentration. Also, Bamboo NOM has the highest metal concentration but still is one of the strongest copper binders.

3.2 COPPER SPECIATION MEASUREMENTS

Copper-into-NOM titrations were the principal means to investigate copper binding by various sources of organic matter. Before the experiments were performed a copper titration method was developed. The titrations show significant difference in copper binding between various NOM samples. Free copper concentration at the same pH, ionic strength and NOM concentration varied by over one log unit for the samples examined.

3.2.1 CHOICE OF EXPERIMENTAL CONDITIONS

In the method development process two background electrolytes at different concentrations were tested (KNO_3 at 50 mmole/l and $NaClO_4$ at 1 mmole/l). Nitric acid

versus hydrochloric acid to adjust pH was applied as well as different organic matter concentrations.

The electrode response slope was greater than the Nernstian when 1 mmole/l $NaClO_4$ was used as background electrolyte and HCl used with NaOH to adjust pH in blank titrations. The slope was between 31 and 32 mV/log free copper concentration in a blank titration as compared to the Nernstian 29.6 mV/log. The correlation coefficient for the linear $log(Cu^{2+})$ vs millivolt plot was lower than when 50 mmole/l KNO_3 and HNO_3 were used in the blanks. In the following copper-into-NOM titrations with NOM concentrations ranging from 5.7 to 100 mg C/l, the electrode response indicated that free copper concentration (calculated from the electrode response in the blank titrations) would be considerably higher than the added copper concentration above pCu_{tot} equal 4.5. An example is shown in figure 3. This is obviously impossible considering that all NOM samples contained very low amounts of copper and therefore almost no copper could be added during the experiments. The effect was more pronounced at higher organic carbon concentration in spite of expected increased copper binding. At higher organic matter concentration more hydrochloric acid is needed to adjust pH and the concentration of chloride ion increases.

Another important parallel observation is that there is a large variance in apparent free copper concentration depending on whether the copper/NOM solution was stirred or not (figure 4). The electromotive force (e.m.f.) in NOM titrations using HCl and $NaClO_4$ increased above the blank at high copper concentration while stirring. The e.m.f. decreased very slowly when the solution was let to rest but both the stirred and non-stirred values were above the electrode response in the blank. The difference in e.m.f. between stirred and non-stirred readings was sometimes higher than 40 mV, i.e. equivalent to more than one order of magnitude variance in free copper concentration. If it were true that chloride interferes with the electrode, increased chloride concentration would explain the unreasonable high apparent free copper concentration and also the amplified difference between stirred and non-stirred values.
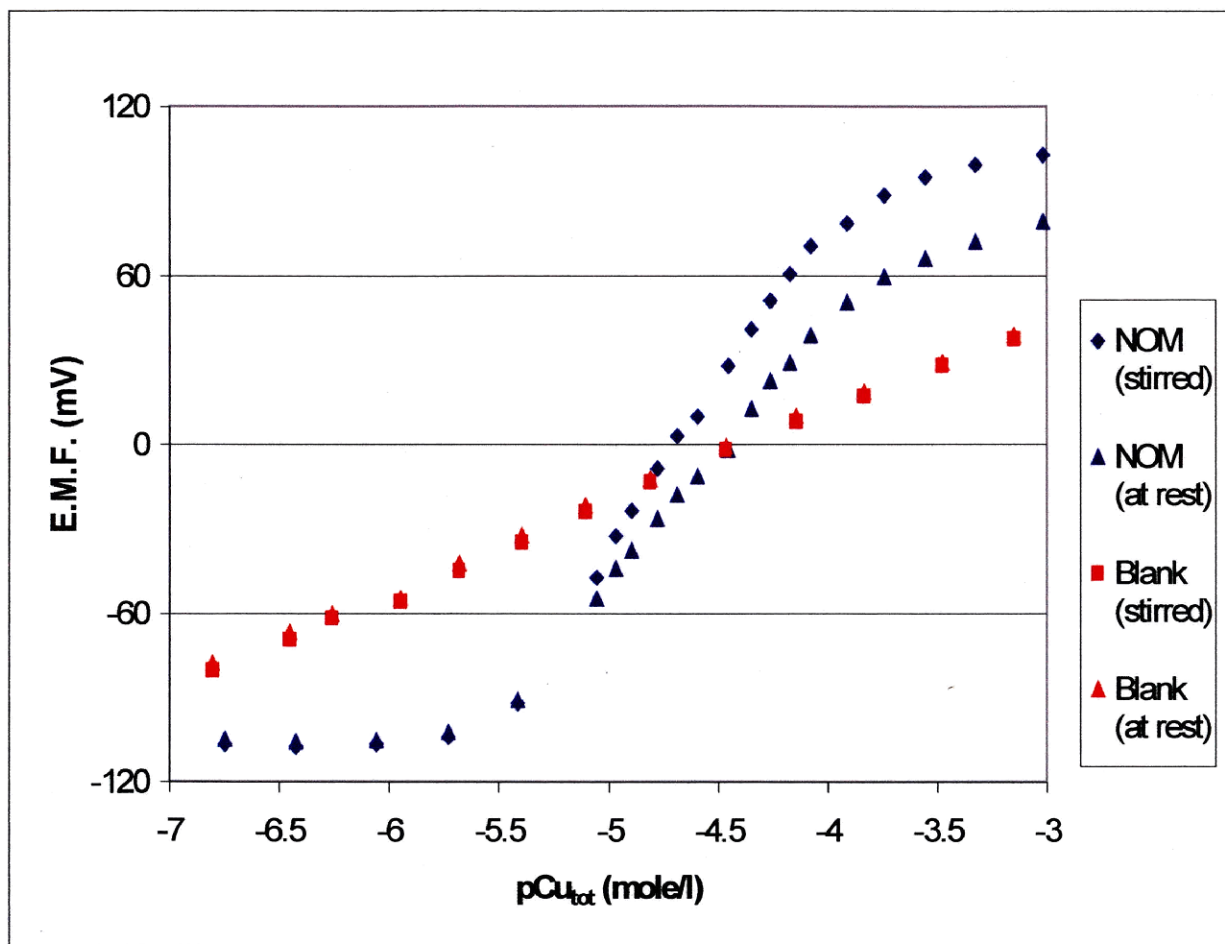
Figure 4. Copper titration of blank and Red Tussock NOM at pH 6, 1 mmole/l NaClO₄ and 61 mg C/l. Hydrochloric acid and sodium hydroxide were used to adjust pH. The stirring rate was rapid.

When various organic matter samples were titrated into 0.1 mole/l copper solution in the absence of chloride a difference between the stirred and non-stirred values was noticed as well. The disparity was less pronounced than in copper-into-NOM titrations in the presence of chloride but was still noticable. The stirred values for one out of three samples indicated an initial increase in free copper concentration. Thus, it seems from experiments in this work that organic matter may variably affect the electrode under some experimental conditions.

There was no sign of interference on the electrode in the absence of chloride at the higher ionic strength in copper-into-NOM titrations. The difference between stirred and non-stirred solutions with HNO_3 and 50 mmole/l $NaClO_4$ was consistently close to 0.5 mV. It has been shown in the literature that high concentrations of chloride ion may interfere with the electrode surface (Westall et al., 1979). The chloride concentration in titrations using HCl as pH adjuster was circa 0.2 mmole/l, which is considerably lower than what Westall and co-workers suggested. Their results show no or little effect at chloride concentrations below 10 mmole/l. They suggested that chloride ion stabilizes monovalent copper at the electrode surface. The electrode responds to Cu(I) and the nernstian slope would double. As a result of the initial experiment nitric acid and potassium nitrate were used in copper-into-NOM titrations instead of HCl and $NaClO_4$.

Based on literature and experiments 10 mg C/l was chosen as an appropriate NOM concentration in copper binding experiments. The higher NOM concentration the lower free copper concentration. Thus, an increase in NOM concentration would decrease the total copper range due to the detection limit of the method. The chosen NOM concentration provides significant copper binding for most of the samples but still allows total copper down to pCu_{tot} equal 5.5. In addition, 10 mg C/l is close to common concentrations in many rivers and lakes.

The choice to fix the pH at 6.0 in copper titrations was based on several factors. First of all, it is common to find pH 6.0 in aquatic environments. Secondly, copper binding by NOM increases with pH, thus the higher pH the greater the expected difference in free

copper concentration among the samples. At higher pH copper hydroxide complexes becomes more thermodynamically favoured, making speciation calculations necessary, which introduces another source of uncertainty. Preliminary copper titrations at pH 7.0 indicated formation of copper hydroxides. The electromotive force varied drastically between data points at high copper concentration and the solution became turbid and colored. Chemical speciation calculations using the computer code MINTEQA2 (Allison et al., 1993) suggested that tenorite, CuO, would form at pH 6.0 when pCu_{tot} is above 4.5 mole/l. That was not supported by experiments. On the contrary, no sign of precipitation was noticed at pH 6.0. With tenorite precipitation suppressed, calculations predicted that 98.5 % of the total copper would be present as free copper ions and only 1.5 % in the form of hydroxides. As the experimental uncertainty was higher than 1.5 percent, the free copper concentration in the titrations was not corrected for the formation of hydroxides suggested by MINTEQA2. If hydroxides were present in the NOM titrations they should be present in the blank as well, making corrections redundant.

3.2.2 DETECTION LIMIT AND REPRODUCIBILITY

Blank titrations in 50 mmole/l KNO_3 using HNO_3 and $NaOH$ as pH adjusters established that the electrode was sensitive to free copper ion concentration from 0.1 μmole/l at the lower end and up to at least 1mmole/l at the higher end. The electrode response was considered Nernstian over this concentration range when the slope was 29.6 ± 0.5 mV/log free copper concentration. Titrations started at 0.05 μmole/l total copper in order for the electrode to have time to adjust to the NOM/copper solution. Only data points with free copper concentrations higher than 0.1 μmole/l were considered in the analyses. Blank titrations performed in connection to each copper titration of organic matter were consistent over the period of this work and the performance of the electrode in blank titrations was not affected by whether the electrode was calibrated (with a blank titration) previous to or after the NOM titration. Also, in all copper titrations of organic matter the electrode response reached an asymptote equal to the response in the blank

titration suggesting that possible NOM coatings on the electrode surface was not a problem of concern in this series of titrations.

Replicate titrations of Swamp Cypress NOM under identical conditions confirmed the reproducibility of the procedure. The first titration was done in the beginning of the series and the second was performed as the last titration. As shown in figure 5, the difference between the two titrations was less than 1 mV at free copper concentrations above 0.1 μmole/l and below 0.5 mV above pCu_{free} equal 1μmole/l. Even the dip in free copper at high concentration is reproducible. This feature was found only for a couple of the samples.

3.2.3 COPPER/NOM BINDING EXPERIMENTS

The titrations discussed below were all performed under the same conditions. The carbon concentration did not change among the experiments except for Sutton Stream water that had lower carbon concentration than the other samples. The same ionic strength was used and pH was constant. As a result, the experimental set-up provides a direct way to compare free (or bound) copper as a function of the source of NOM.

The titration curves of the nine different NOM samples shown in figure 6 have very similar features. The percent bound copper decreases steadily with increasing total copper concentration - free copper concentration approaches the asymptote described by the no binding (blank) curve. The relative difference between the curves is fairly consistent and no curves cross one other. Consequently, one value of total copper can be used to represent copper binding by the various NOM samples. Total copper concentration of pCu_{tot} equal 5.5 mole/l was chosen for comparison because it provides the lowest value where free copper is above the detection limit of the method (0.1 μmole/l) in all experiments (figure 6). At low copper concentrations the difference in free copper in the presence of various organic matter is more pronounced, making it easier to notice differences. It seems likely that the difference in free copper is greater below 0.1 μmole/l
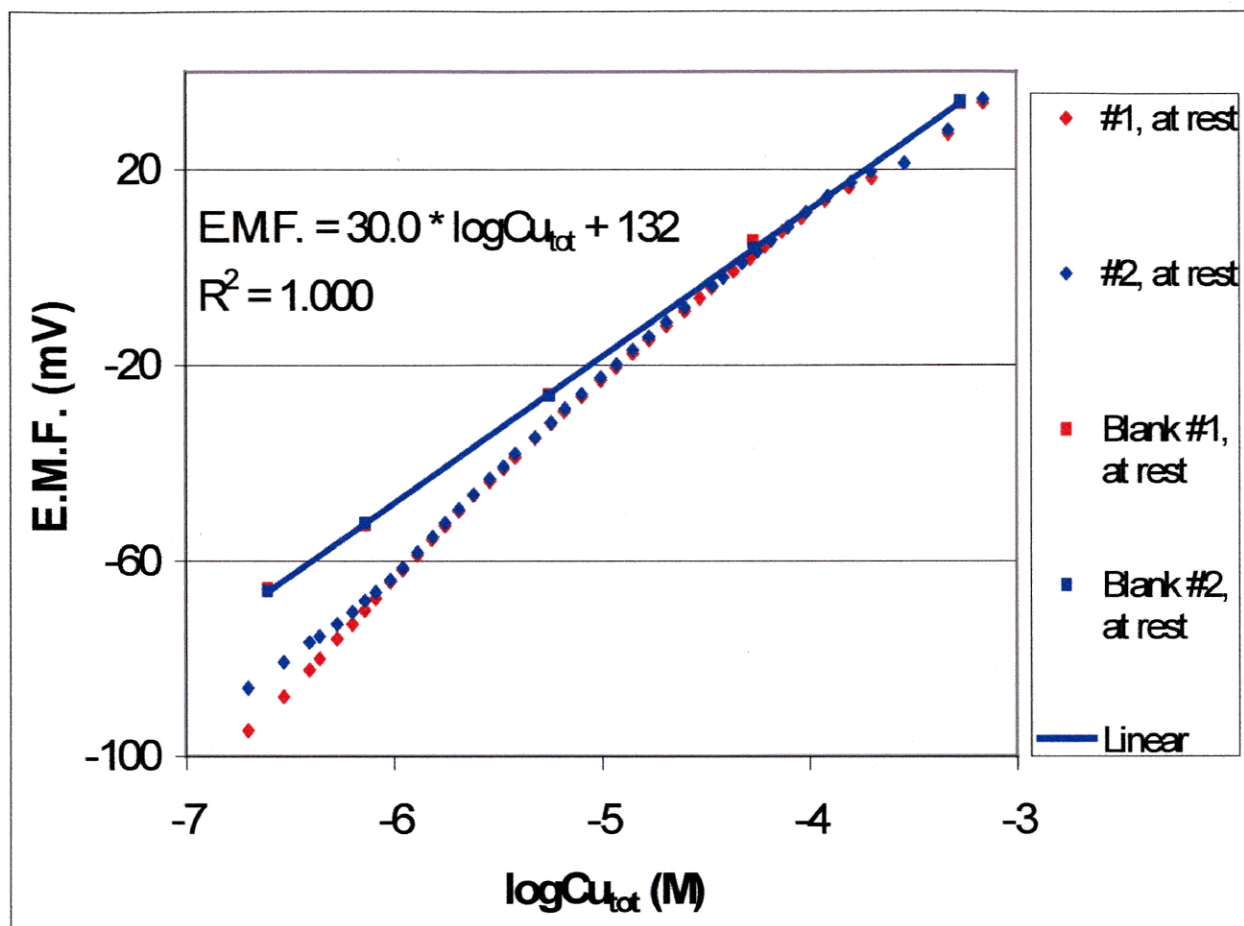
Figure 5. Replicate titrations of Swamp Cypress extract with appurtenant blank titrations at 10 mg C/l, pH 6 and 50 mmole/l KNO_3 as background electrolyte. Nitric acid and sodium hydroxide was used to control pH. Given in figure is the equation (slope equal 30 mV/log free copper) and R^2-value for one of the blank titrations.
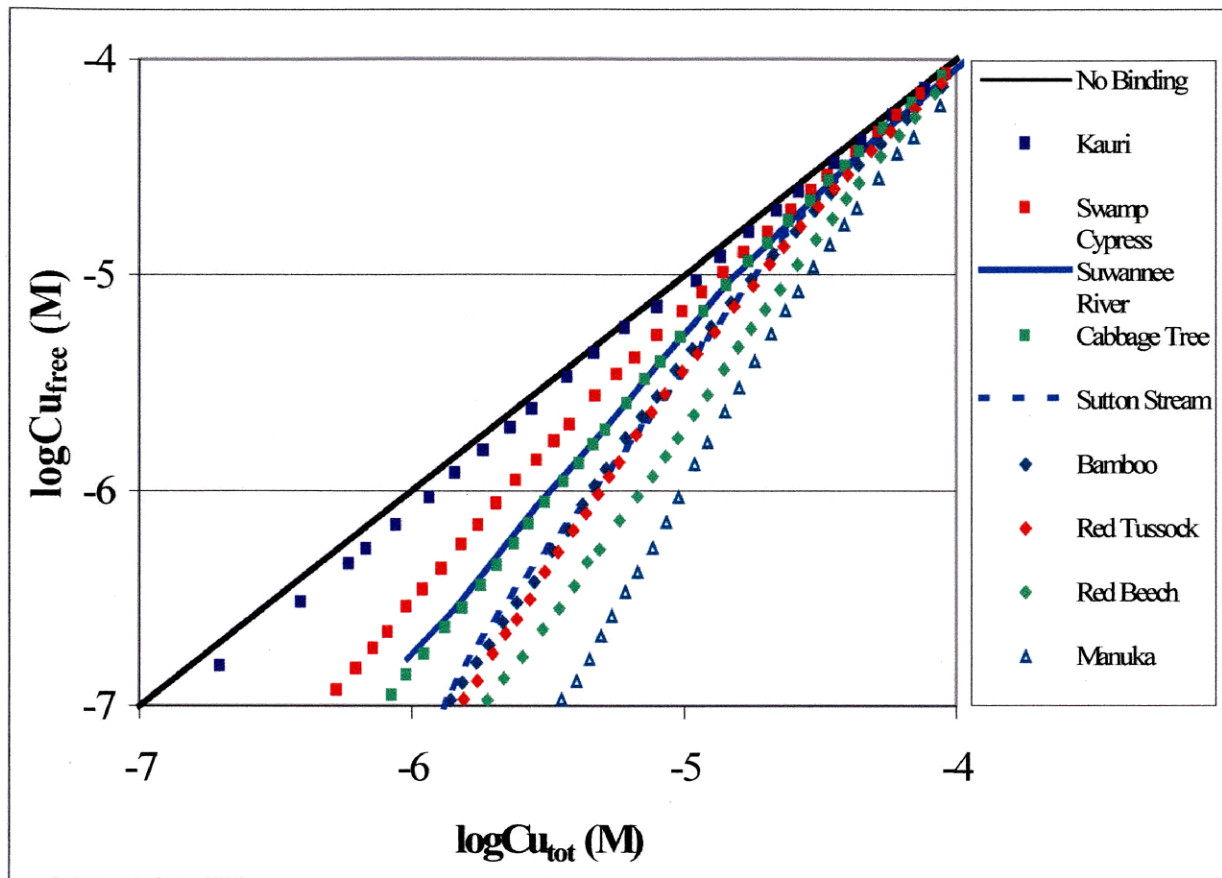
Figure 6. Copper titration of nine NOM samples from different origins at 10 mg C/l, pH 6 and 50 mmole/l KNO_3. Sutton Stream water has been corrected for organic matter concentration by increasing ligand concentration in the one-site model 10/5.7 times.
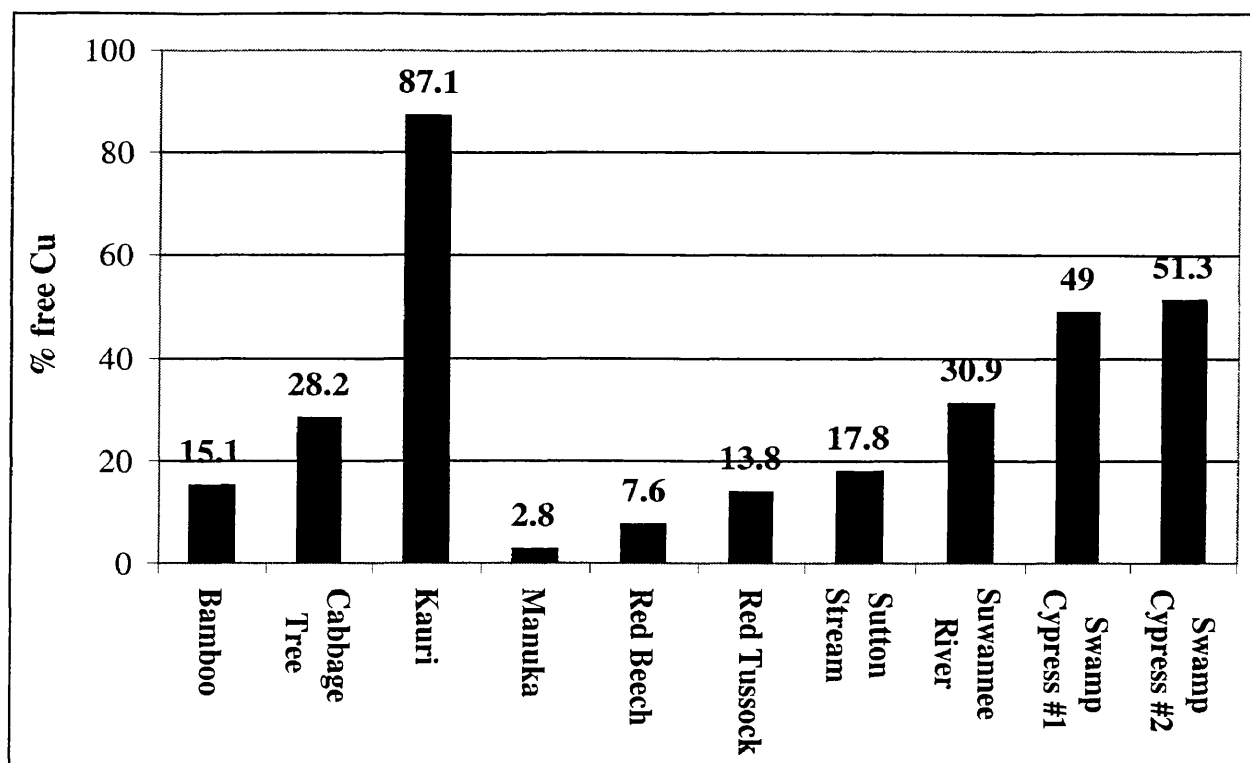
Figure 7. Free copper concentration at pCutot equal 5.5 mole/l in copper titrations of various organic matter samples at pH 6, 50 mmole/l KNO$_3$ and 10 mg C/l.

free copper but it can not be proved by the experiments presented herein. Raw data for all copper titrations is presented in Appendix B.

Figure 7 shows free copper at pCu_{tot} equal 5.5 mole/l in the titrations. Four aspects of copper binding as a function of the source of NOM are pointed out based on the information provided by figure 6 and 7.

i.   All but one of the samples binds 50% or more of the copper at $10^{-5.5}$ mole/l total copper concentration. It is no new information but it is still worthwhile pointing out that NOM is very important for copper speciation in natural environments at 10 mg C/l and pH 6.0. Copper toxicity decreases significantly [see chapter one] when bound to NOM and natural organic matter should be considered when predicting toxic effects of copper.

ii.   Replicate titrations of Swamp Cypress NOM closely match each other. The difference in free copper between any two other titrations is much larger. The variation in copper binding by various organic matter samples is striking. The free copper concentration at 3.2 μmole/l total copper varies from 3 to 87 % depending on the origin of NOM. It is important to realize that organic matter is not a homogeneous group of substances and that copper speciation varies substantially in the presence of various types of organic matter. These variations result from differences in chemical composition and structure between the samples.

iii.   The two river waters exhibit free copper concentrations in the middle range of the nine samples. The result is not surprising considering that the extracts contain a less diverse mixture of substances than river waters as they contain degradation and leachate products from only one species. Hence, river waters as a group may be expected to be more uniform in copper binding properties than single species extracts for two reasons. They contain more diverse chemical substances and would statistically accommodate more similar substances with copper binding properties and also because geochemical processes may tend to make them more homogeneous. This is not the same as saying that river waters have identical

composition and copper binding properties. Several more river waters need to be investigated in order to fortify this notion.

iv.   An interesting feature of the experimental data is that the dominating species in the tributary of two rivers have similar copper binding properties as do the river waters. The free copper concentration in the presence of Sutton Stream corrected for organic carbon concentration follows the free copper in the Red Tussock titration very closely and the Suwannee River water titration resembles the one of Swamp Cypress, figure 8. Only two pairs is of course too little data to draw any conclusions from but it may inspire further research related to structural changes in NOM by natural processes and how metal binding is affected by the changes. Effects of processes occurring during transport may be important for copper binding properties.

The prominent differences in copper binding are of course assumed to be related to the structure and/or chemical composition of NOM. Literature suggests different types of binding sites but as far as the knowledge of the author goes, no proofs have been provided showing that one type dominates over the others. The collection of samples presented in this work, with large variations in copper binding, may serve as a means to relate copper binding properties to structural groups of NOM.

3.3 MODELING OF COPPER TITRATION DATA

The purpose of modeling titration data was to use the models as a tool to quantify and understand variations in copper binding among organic matter samples from various sources. If two similar conditional formation constants would be found for various samples, it was believed it would support the theory that phenolic and carboxylate groups provide two different binding sites. Also, in case of very closely matching conditional formation constants from sample to sample, one could relate copper binding differences
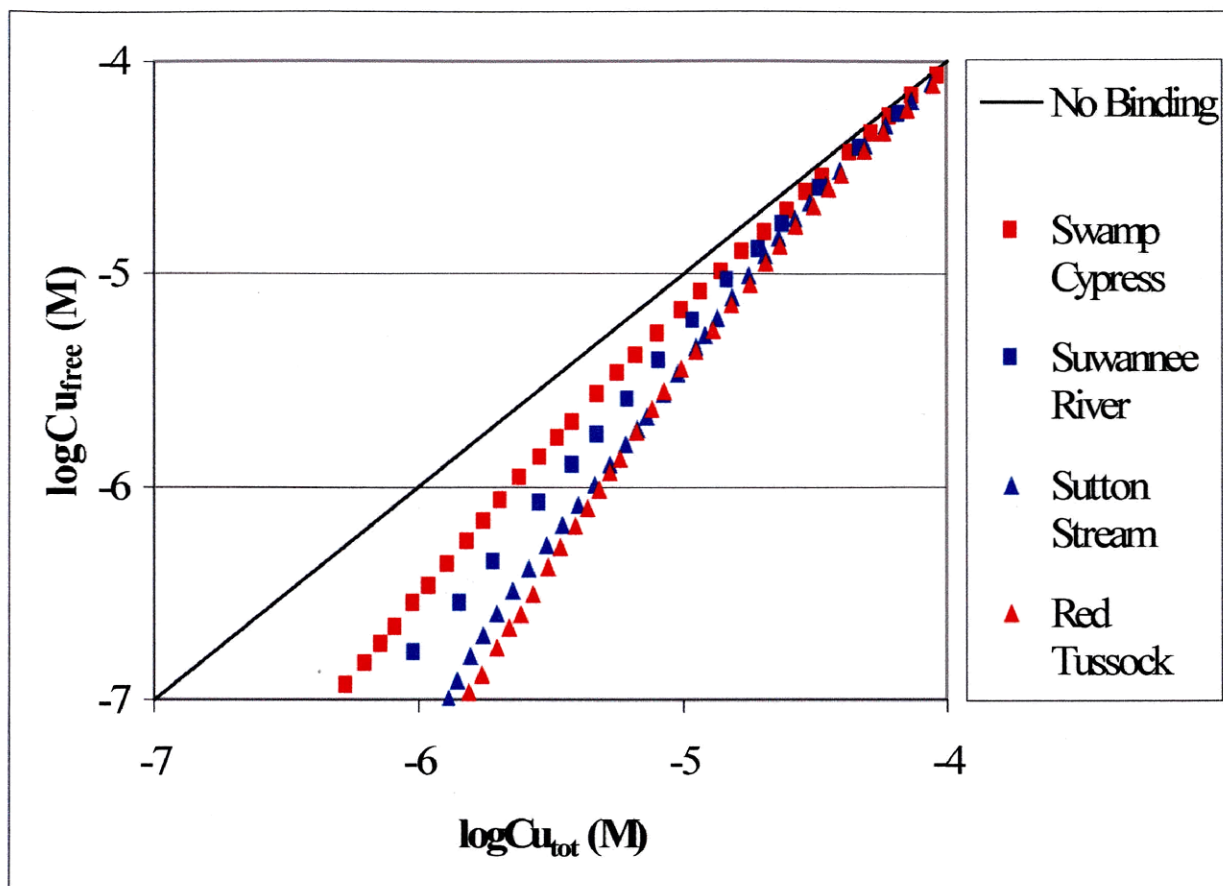
Figure 8. Comparison of copper binding properties between river water and dominant species in catchment basin.

to differences in model-calculated ligand concentrations.

3.3.1 COMPARISON OF MODELS

Two simple models were applied to model the titration data. They are described by the following equations:

One-site model:

$$Cu + L \Leftrightarrow CuL \qquad [11]$$

$$\text{with} \quad K = \{CuL\}/(\{Cu\}*\{L\}) \qquad [12]$$

Two-site model:

$$Cu + L_1 \Leftrightarrow CuL_1 \qquad [13]$$

$$\text{with} \quad K = \{CuL_1\}/(\{Cu\}*\{L_1\}) \qquad [14]$$

$$Cu + L_2 \Leftrightarrow CuL_2 \qquad [15]$$

$$\text{with} \quad K = \{CuL_2\}/(\{Cu\}*\{L_2\}) \qquad [16]$$

As seen from the equations above no corrections for electrostatic interactions are included in the models.

The two-site model generally fit the data better than did the one-site model. The results of the modeling are represented by fitting parameters in table 5. The ligand concentrations are not normalized to organic carbon concentration as the chemical significance of the parameters is not fully understood and because the titrations were all performed at the same organic carbon concentration (10 mg C/l) with the exception of Sutton Stream water (5.7 mg C/l).

Goodness of fit is found by minimizing WSOS/DF, which is the sum of squares divided by an estimate of the experimental error and by the degrees of freedom. When error estimates were provided to FITEQL the iteration did not converge because WSOS/DF approached zero and the iteration terminated. Default values were used instead and as a result WSOS/DF can only be used to find best fit of a model and to

Table 5. Results from modeling of titration data with FITEQL using one and two site models. Total ligand concentrations represent values at 10 mg C/l. Equations describing the models can be found on page 46.

	One-Site Model			Two-Site model				
				Site One		Site Two		
Parameter	$\log K$	L_{tot}	WSOS/DF	$\log K_1$	$L_{1,tot}$	$\log K_2$	$L_{2,tot}$	WSOS/DF[*]
Sample								
Bamboo	5.6	1.2E-5	0.61	4.8	1.2E-5	6.6	3.6E-6	0.007
Cabbage Tree	5.6	7.7E-6	0.12	5.2	6.7E-6	6.6	2.0E-6	0.022
Kauri	4.2	9.4E-6	0.02	3.6	1.4E-5	4.7	2.4E-6	0.021
Manuka	5.8	2.3E-5	2.35	5.0	2.2E-5	6.8	7.8E-6	0.029
Red Beech	5.7	1.7E-5	0.56	5.5	1.6E-5	7.1	2.4E-6	0.282
Red Tussock	5.7	1.1E-5	0.33	5.3	1.0E-5	7.0	2.5E-6	0.015
Sutton Stream	5.8	5.2E-6	0.21	5.7	4.9E-6	27	4.6E-7	0.184
Suwannee River	5.5	8.2E-6	0.17	4.4	1.1E-5	6.1	4.0E-6	0.024
Swamp Cypress #1	5.3	6.4E-6	0.08	4.9	6.5E-6	6.7	9.6E-7	0.013
Swamp Cypress #2	5.6	3.8E-6	0.04	5.3	3.4E-6	6.6	7.6E-7	0.024

[*] Default values for experimental error were used.
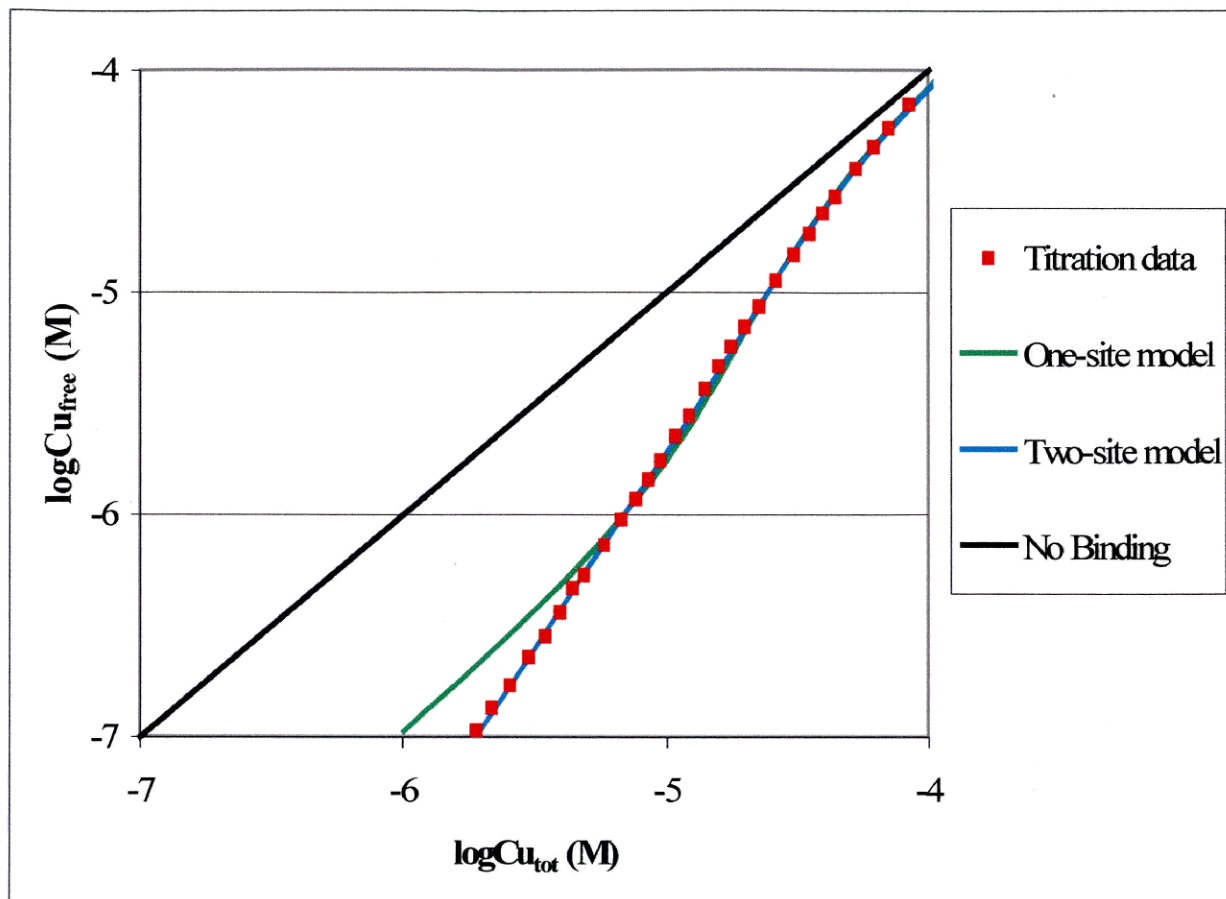
Figure 9. Fit of one and two-site model to Red Beech copper titration data between 0.1 μmole/l and 0.1 mmole/l free copper concentration. The experiment was performed at 10 mg C/l, pH 6 and 50 mmole/l KNO_3 ionic strength.

compare fit of model to various experimental data. No statistical analyses of fitted parameters could be used. If estimates of experimental error could be used, WSOS/DF values below one would suggest that the model contains too many parameters. Values much above one would mean that the experimental data is more complicated than the model and that the model needs to be developed further. In this work WSOS/DF was only used to determine the best fit of a model and compare the fit of the two-site model to the one-site model on mathematical grounds. As an example, the fits of the two models to Red Beech titration data are shown in figure 9. The two-site model fits the data almost perfectly over the whole copper concentration range whereas the one-site model diverges from the titration data at low copper concentrations.

The one-site model results in conditional formation constants within 0.5 log units for eight of the samples, Kauri NOM excepted. The goodness of fit for the one-site model decreases among the samples with increasing metal binding, compare WSOS/DF in table 5 with copper binding in table or figure 6 or 7. Copper titration of Kauri, which is the weakest copper binder of the samples investigated in this work, is fitted equally well with the one and the two-site model. The one-site model fits the second worst copper binder, Swamp Cypress, almost as well as the does the two-site model. However, the one-site model is not sufficient when fitting titration data of a NOM sample with strong copper binding properties, e.g. Manuka.

3.3.2 SIGNIFICANCE OF MODELS

Before discussing the significance of the two models it is important to remember that the fitting parameter WSOS/DF is only valid for comparing titration data and to find the best-fit of a model to experimental data. The reasonableness of the model can not be assessed by WSOS/DF as the experimental error in this work could not be used, see above. Also, no corrections term for electrostatic interactions between the binding sites were included in the model; for equations used in the models see page 44. As a result, no

claims can be made that the conditional formation constants fitted with FITEQL represent actual equilibrium constants.

When replicates of Swamp Cypress were modeled, fitted parameters turned out to be covariant for both the one and two-site model. Better agreement among fitted parameters presented in table 5 was expected from the very good conformity between the two titrations. The conditional formation constants vary 0.4 log units in the two-site model and 0.3 log units in the one-site model. The fit of the model to titration data with lower log K seems to be corrected for by increasing the related ligand concentration. The ligand concentration in the one-site model was nearly doubled when the conditional formation constant decreased 0.3 log units. The result from modeling the two replicate titrations with the two-site model suggested even stronger covariance. A change in K_1 with 0.4 log units resulted in halving the ligand concentration of that site. The second and stronger site decreased 90 percent. Also, during the fitting procedure, similar fits were found from different parameters. The results are not included in this report.

McKnight et al. (1983) titrated aquatic fulvic acids with copper at pH 6.25 and modeled the data with a two-site model. They found stronger conditional formation constants than in this thesis. The log mean values of the two constants in their work were 6.0 and 8.0. The two conditional formation constants from the two-site model in this work are 4.5 to 5.0 and 6.6 to 7.1, respectively. This is one to one and a half log units lower than McKnight and co-workers' conditional formation constants for FA. The fitted ligand concentrations in this work are in the same range if normalized to organic carbon concentration, as the ones McKnight et al. found in their experiments. Fulvic acid is a major component in surface water (figure 2). The sites available in fulvic acid should exist in river water as well, assuming that the structure of FA is not altered during extraction. Comparing modeling results herein and results in McKnight et al. (1983) shows that the conditional formation constants differ but the ligand concentration is similar. Thus, the modeling results suggest that for similar concentration of binding sites FA in McKnight and coworkers' research provides stronger binding sites than the river

waters and extracts herein. Geochemicaly, the opposite would be expected for the two river waters, i.e. the same sites would exist in the river water but at lower concentrations than in FA. One possible explanation for the surprising results is that McKnight et al. was able to investigate lower copper concentration than in this thesis and for mathematical reasons found higher conditional formation constants.

One way to estimate the significance of estimated conditional formation constants is to change the best-fit constants and minimize WSOS/DF by changing only the ligand concentrations. This approach is not a statistical method. It is only meant to give the reader an opinion about the significance of fitted parameters. This exercise was done for the Manuka extract copper titration. The results are given in table 6 and figure 10. The conditional formation constants that fit the experimental data the best was decreased by the user 0.1 and 0.5 log units and the ligand concentrations were recalculated with FITEQL using the altered log K's as fixed values. As seen from WSOS/DF in table 6 and figure 10, changing the best-fit constants 0.1 log units does not change the fit of the model substantially. However, changing the conditional formation constants 0.5 log units has a visible effect on the fit and the weighted sum of squares per degrees of freedom increases as well. As a larger formation constant value seems to be corrected for by lower total ligand concentration to some extent, comparison between data using calculated parameters is made very difficult.

3.4 RELATIONSHIP BETWEEN CHEMICAL STRUCTURE AND COPPER BINDING PROPERTIES

As the difference in copper binding by NOM is related to source of NOM, variations in molecular structure and/or chemical composition must account for the differences. Two different analytical techniques were used to investigate the structure of the samples. Attempts to relate copper binding to the results are presented below.
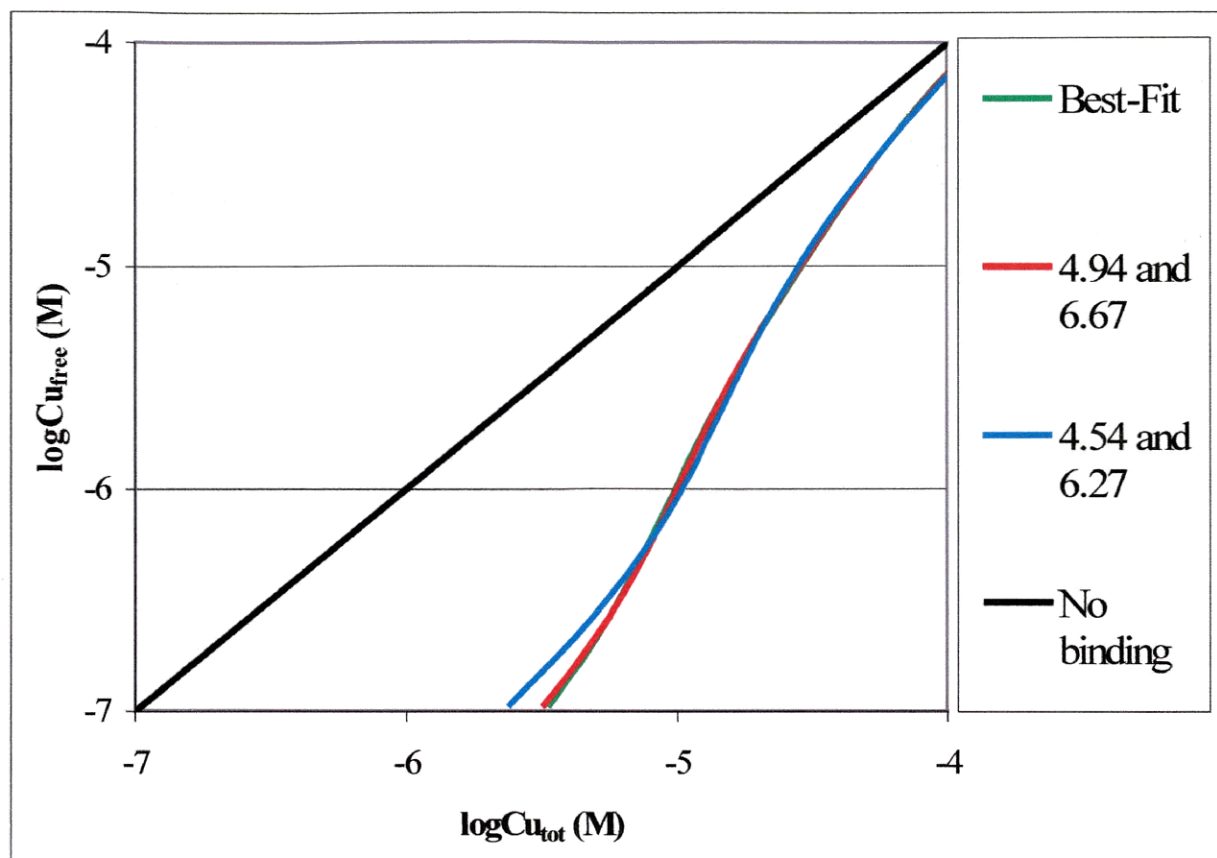
Figure 10. Best-fit of two-site model to Manuka NOM copper titration compared to model with altered conditional formation constants 0.1 or 0.5 log units.

Table 6. Fit of the two-site model to the Manuka NOM titration with altered conditional formation constants. Ligand concentrations are given in μmole/l.

	Site One		Site Two		
	$logK_1$	$logK_2$	$L_{1,tot}$	$L_{2,tot}$	WSOS/DF
Best-Fit	5.0	6.8	220	78	0.029
Change −0.1	4.9	6.7	220	88	0.042
Change −0.5	4.5	6.3	220	130	0.34

3.4.1 ACID TITRABLE GROUPS

In the most common theory about copper binding to NOM it is assumed that the metal binds to specific functional groups on the organic matter molecules (e.g. Kipton et al., 1996). Carboxylate and phenolic groups have been suggested to serve as binding sites. In an attempt to correlate copper binding to functional group concentration potentiometric titrations were used to calculate acid and base titrable groups in different pH ranges.

The titrable groups between pH 3.0 and 7.0 served as an estimate of carboxylic groups. The lower cut-off, pH 3.0, was used because of experimental error related to the pH electrode at low pH's. The inflection point in an acid/base titration of NOM appeared close to pH 7 (figure 11). As a result, this pH was used as cut-off for the upper limit. Other groups that are titrated with carboxyl groups are assumed to be insignificant.

The acid/base titrations show hysteresis, i.e. the acid and base titrations do not fall on the same points. Bowles et al. (1987) discuss this phenomena and suggest that hydrolysis of esters produces alcohols and carboxylic acids that consume base during the titration. They suggested another, less likely, possibility for hysteresis. Because of slow access of base to certain acid sites protonation may be diffusion limited. By keeping their Suwannee River FA at elevated pH for several days, a significant hysteresis effect was found. Production of acids and alcohols would result in lesser amount of acid needed to titrate the NOM solution back to a certain pH, which is the case in figure 11. The purpose of acid/base titrations in this thesis is to calculate the concentration of acid titrable groups. The results from the acid and the base titration overlaps and the hysteresis effect seems not to be strong enough to affect the results. The hysteresis effect may be minimized by rapid acid/base titrations.

Phenolic groups were estimated as the acid/base titrable groups between pH 7.0 and 10.0. Phenol has a pK_a of 9.89 at 20 °C (Martell and Smith, 1973) and most substitutes on the aromatic structure stabilizes the phenolate ion, making substituted phenols more acidic than phenol. As an example, 4-hydroxybenzoic acid has a second pK_a of 9.46 (Schwartzenbach et al., 1993).
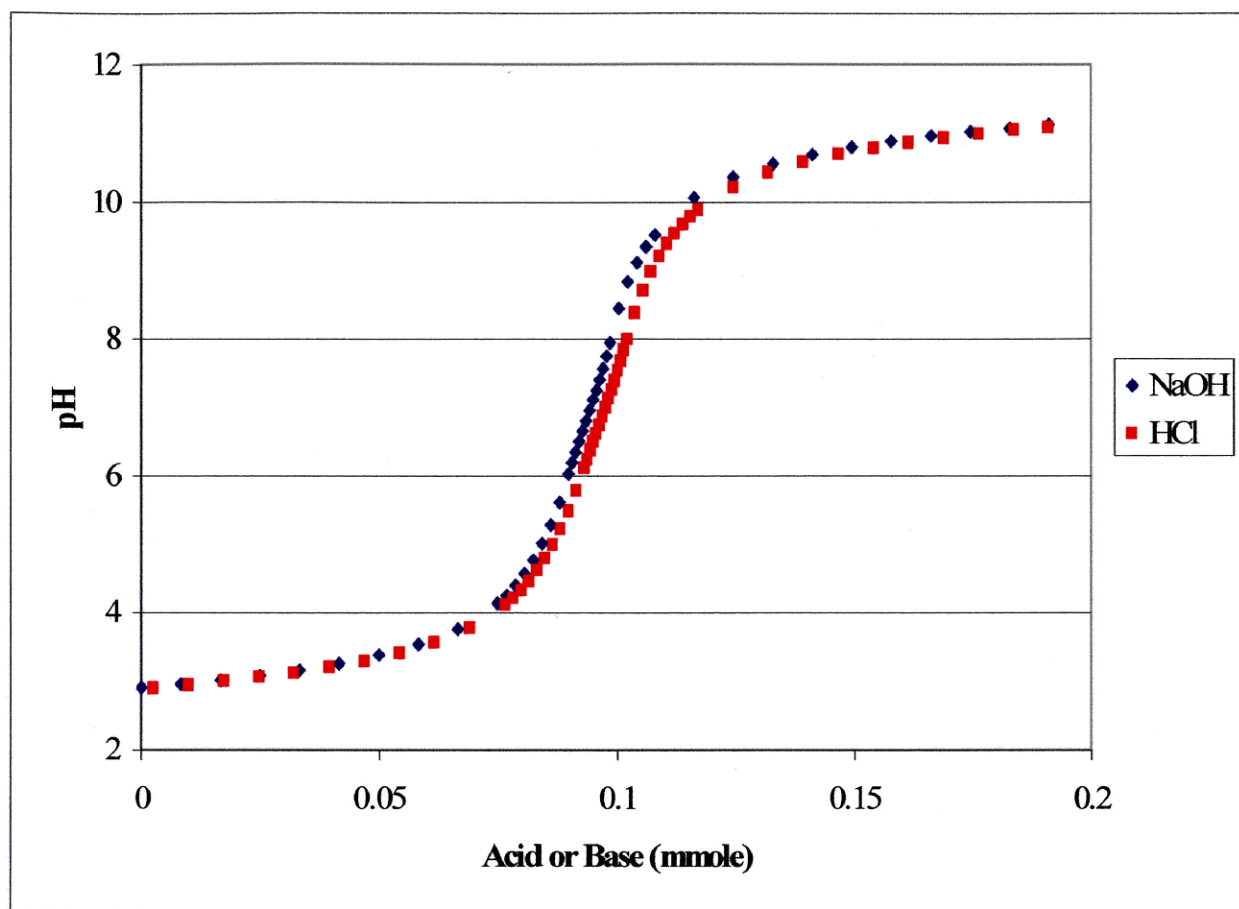
Figure 11. Acid/base titration of Manuka extract in 50 mmole/l KNO_3 ionic strength and at an organic carbon concentration of 55 mg C/l. The sample was titrated with sodium hydroxide followed by hydrochloric acid.

Table 7. Results from acid/base titrations. Concentrations of acid titrable groups are given in μeq/mg C. Reported results include titration with base and with acid. An average value of the two titrations is given. MD = missing data.

	pH range						
	pH 3 to 7			pH 7 to 10			pH 3 to 10*
Sample	NaOH	HCl	Average	NaOH	HCl	Average	Average
Bamboo	26.3	MD	26.3	88.7	MD	88.7	115
Cabbage Tree	22.7	24.3	23.5	4.9	6.5	5.7	29.1
Kauri #1	16.7	19.7	18.2	1.5	1.4	1.4	19.7
Kauri #2	20.0	17.1	18.6	1.3	0.9	1.1	18.2
Manuka #1	8.2	8.0	8.1	5.0	5.3	5.2	13.3
Manuka #2	9.6	10.4	10.0	4.4	4.5	4.5	14.5
Red Beech	8.0	7.7	7.8	6.6	7.1	6.9	14.7
Red Tussock	5.9	6.7	6.4	23.8	23.7	23.7	30.1
Sutton Stream	-0.5	2.7	1.1	19.0	19.1	19.1	20.2
Suwannee River	6.7	7.8	7.2	4.4	4.0	4.2	11.5
Swamp Cypress #1	MD	9.9	9.9	MD	43.0	43.0	53.0
Swamp Cypress #2	8.4	8.4	8.4	40.5	42.7	31.5	49.9

*Calculated as the sum of the averages of acid titrable groups between pH 3.0 to 7.0 and 7.0 to 10.0.

Strong dissociating acid functional groups were calculated between pH 3.0 and 4.0. Leenheer and co-workers (1995) have suggested that strongly acidic functional groups may provide strong copper binding sites. They suggest that pK_a of these structures is below 3.0. Titrations in this work were not performed below pH 3.0 and as a result, the stronger acids measured here are not the same as the ones Leenheer and co-workers suggest to be responsible for copper binding.

The results from the acid/base titrations are presented in table 7. Three replicates were included in the experiments. The replicates agree within about 15 percent when the blank is subtracted from the NOM titration, which means that experimental errors do not explain the variations in functional group concentrations. The carbon concentration of Sutton Stream water was too low to give reliable concentrations of acid titrable groups. Table 7 includes the total concentration of calculated acidic groups which can be used to relate the concentration of acid titrable functional groups to carbon concentration in the samples. If the concentration of NOM expressed in mass of carbon is converted to moles, a molar ratio between acid titrable functional groups and carbon can be found by dividing the concentration of NOM (in moles of carbon) by acid titrable groups (expressed as equivalents). A sample containing 10 µeq/mg C has a functional group/carbon molar ratio of close to 1:8. A functional group concentration of 50 µeq/mg C is equivalent to a molar ratio of 1 to 1.7. The results agree with literature suggesting that there is on average one oxygen containing functional group per three to six carbons. A larger variation among single species extracts compared to river water was expected for the same reasons as discussed in section 3.2.3. One sample, Bamboo extract, has a total concentration of acid titrable groups of 115 µeq/mg C. The molar ratio for this sample would be 1:0.7, i.e. every carbon atom needs to host on average 1.4 acid titrable groups. This value is unreasonable high and the experimental results need to be confirmed.

The concentrations of acid titrable groups were plotted against bound copper concentration in the copper titrations at pCu_{tot} equal 5.5 mole/l. As seen from figures 12 and 13, there is no correlation between copper binding and concentration of acid titrable
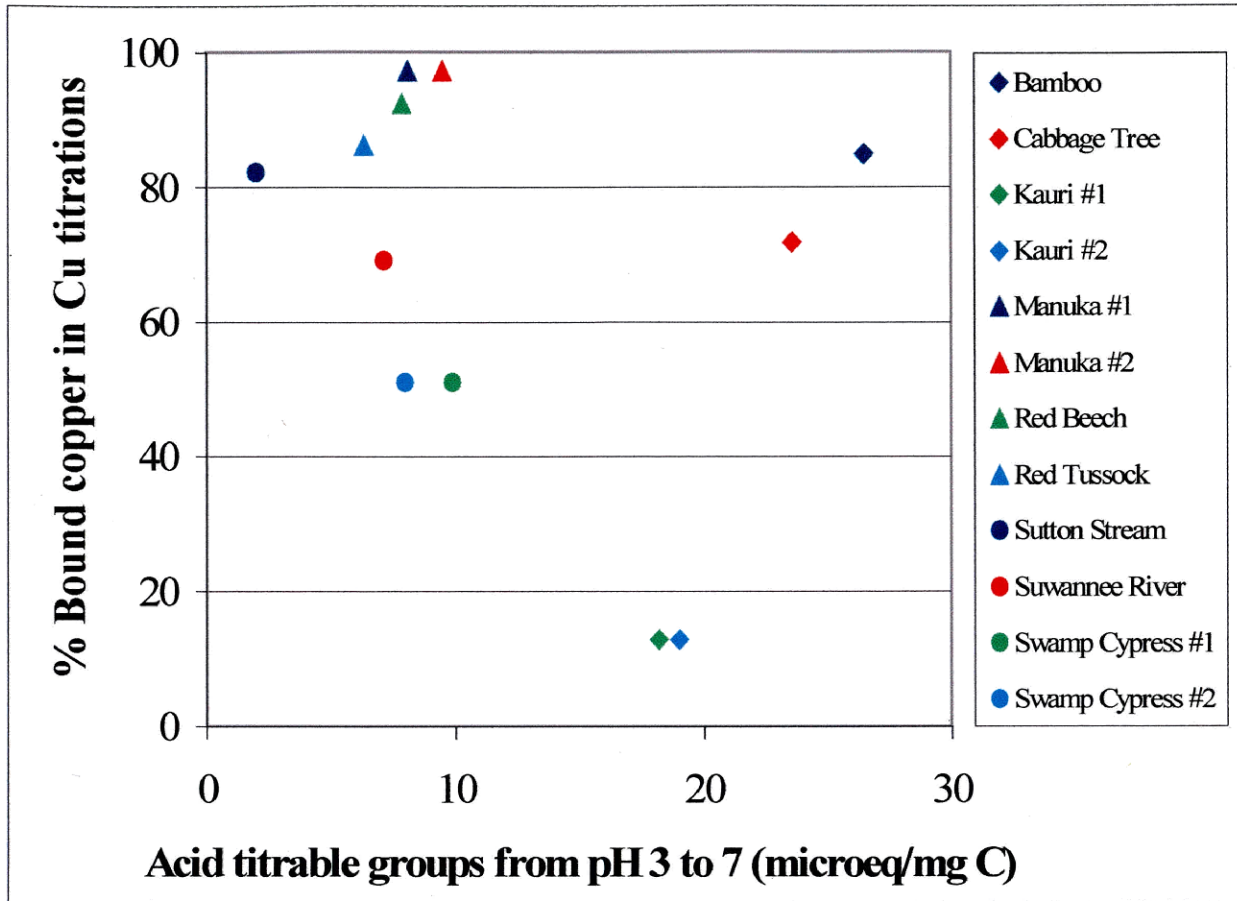
Figure 12. Bound copper at pCu_{tot} equal 5.5 versus acid titrable groups calculated between pH 3.0 and 7.0.
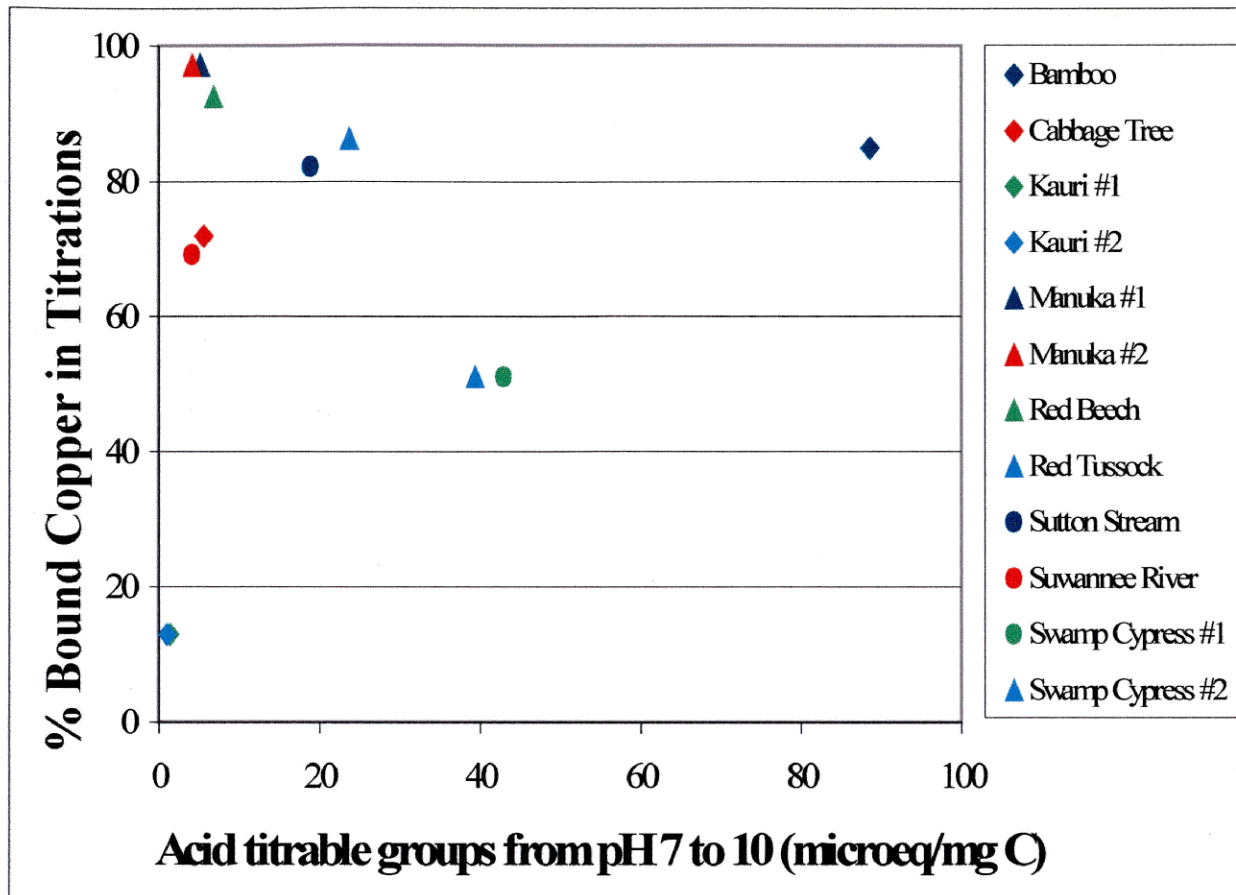
Figure 13. Bound copper at pCu$_{tot}$ equal 5.5 versus acid titrable groups between pH 7.0 and 10.0.

groups. There was no correlation between the stronger titrable groups (pH 3-4) and copper binding (not shown). The correspondence is equally bad in all three figures. As a consequence, a higher concentration of acid titrable groups does not mean that the sample binds copper more strongly. If we assume that on average all strong copper binding sites are taken at 10 μmole/l total copper concentration in the 10 mg C/l copper titrations, there is 1 μeq/mg C of copper bindings sites. If we further assume an average value of acid titrable groups of 30 μeq/mg C it means that NOM contains a considerable amount of acid titrable groups that are not involved in strong copper binding. Thus, only a portion of acid titrable groups act as strong copper binders (if they do at all). Estimating bound copper directly from a copper titration curve is of course not an optimal way to find the concentration of copper binding sites, but it serves the purpose for this discussion. The question still remains what the strong copper binding sites are and what sites bind copper by only weak electrostatic interactions. Finding the functionality responsible for copper binding would greatly improve modeling of copper binding by NOM.

3.4.2 UV ANALYSES

It was desired to investigate the effect on copper binding by aromatic versus aliphatic structures of the NOM samples. Aromatic and unsaturated structures provide regions of higher electron densities that may facilitate strong binding sites for copper. The literature suggests salicylic acid as a possible model for the types of sites where copper ions can form chelates with NOM. UV analysis was chosen, as it is a relatively simple technique to characterize molecular structure. The technique can not be used to find exact structures of NOM but can provide insight into major structural features of the molecules. Aromatic structures have absorption maximum around 260 to 280 nm whereas aliphatic structures have their maxima at shorter wavelengths. Benzene has its maximum absorption at 256 nm. Additional groups on the benzene ring increases the maximum absorption wavelength slightly, see table 8. Methyl substituents do not affect the maximum absorption wavelength to the same extent, as do hydroxyl groups. As the NOM samples

contain acid titratable groups it was assumed that the aromatic moieties of interest would have absorption maximum close to 280 nm.

Table 8. Maximum absorption wavelengths for some aromatic compounds. Adapted from Skoog and Leary (1992).

Compound	Absorption max (B band) (nm)
Benzene	256
Toluene	261
m-Xylene	263
Phenol	270
Aniline	280
Thiophenol	269

The UV absorption at 280 nm was normalized to organic carbon content in the sample under the assumption that Beer's law was valid, i.e. UV absorbance is directional proportional to the concentration of absorbing groups. The assumption was verified for Kauri NOM (data not shown). The normalized absorption was plotted against percent bound copper at $10^{-5.5}$ mole/l total copper. There seems to be a relationship between UV absorption and copper binding in figure 14. The higher UV absorbance per mg C the more copper is bound per mg C by NOM. The correlation coefficient is 0.623 and a statistical test shows that there is a correlation with 95 % confidence. However, the correlation was not significant at 99 % confidence level. The results imply that higher concentration of aromatic structures results in more and/or stronger sites for copper binding than do aliphatic structures.

The results do not exclude the probability that aliphatic structures may bind copper strongly. There are outliers indicating that there are other binding sites. A strong relationship between copper binding and UV absorption does not mean with 100 %
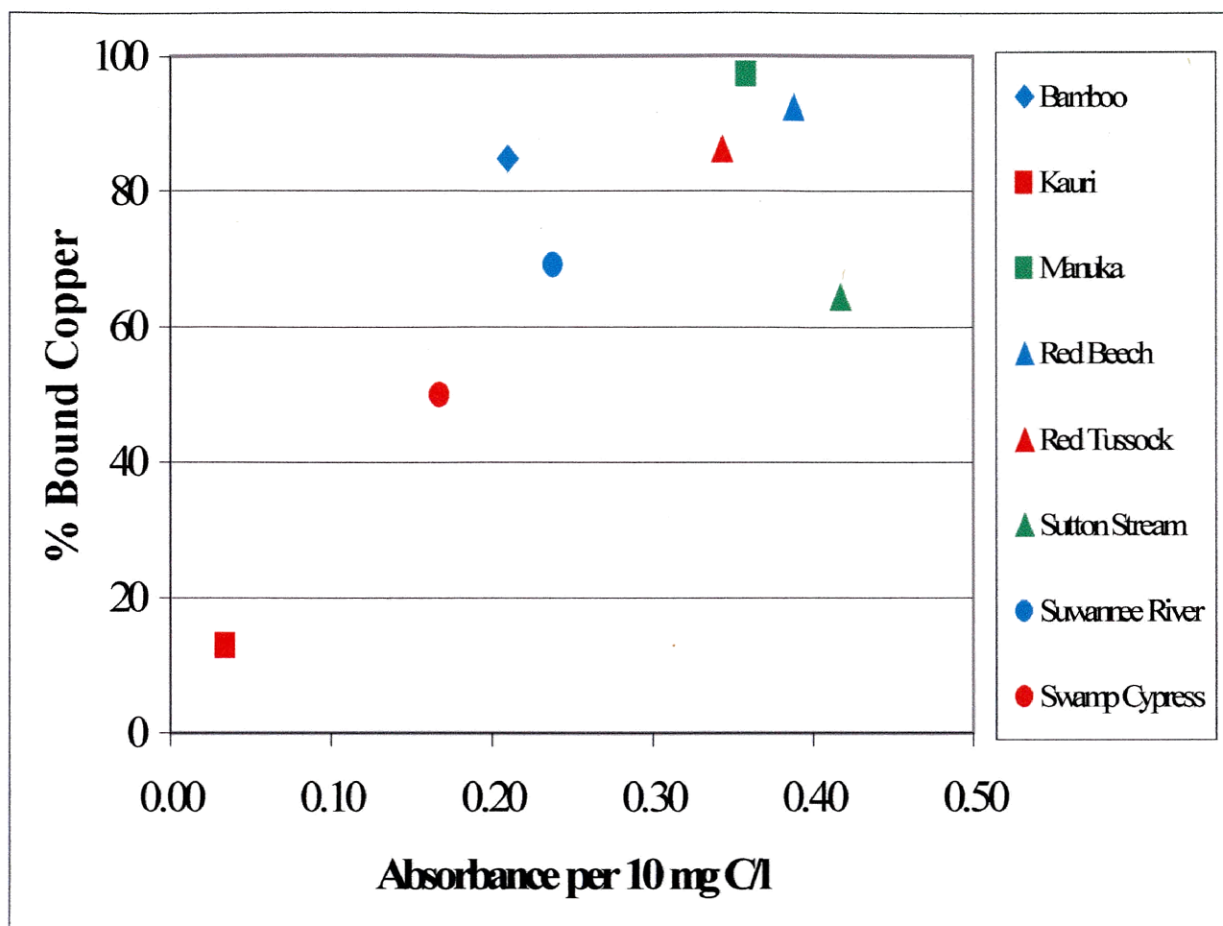
Figure 14. Correlation between bound copper in copper titrations at pCutot equal 5.5 and UV absorbance at 280 nm normalized to organic carbon concentration used in copper titrations. R^2-value equals 0.623 for eight samples.

certainty that the structures that absorb ultraviolet light are the same as the ones responsible for copper biding. It merely tells that the stronger copper binders have more unsaturated or aromatic structures than the NOM that binds copper less strongly. However, aromatic structures provide electron density that stabilizes copper/NOM complexes and it seems reasonable that aromatic structures provide stronger sites than do aliphatic. The results support research showing that metal humates are stronger than metal fulvates as humic acids are considered more aromatic than fulvic acids.

CONCLUSIONS

The following conclusions can be drawn from the experiments performed in this study.

i. Copper binding is a related to the source of NOM. Substantial differences in free copper in the presence of various organic matter samples under identical conditions were found.

ii. Modeling of titration data with a 1:1 stoichiometry one-site model and a 1:1 stoichiometry two-site model was not a feasible way to compare titration data. Fitted parameters using a least square technique resulted in covariant conditional formation constants and ligand concentrations.

iii. Acid titrable groups between pH 3.0 to 7.0, 3.0 to 4.0 or 7.0 to 10.0 did not correlate to copper binding by NOM from a variety of sources.

iv. Higher UV absorption at 280 nm appeared to correlate with stronger copper binding properties of organic matter.

SUGGESTIONS FOR FUTURE RESEARCH

This study has raised several questions that call for further research. The following topics are suggested:

i. The understanding of metal/NOM interactions would increase considerably if further relationships between copper binding and structure of NOM were found. NMR may prove to be a useful analytical technique in this pursuit.

ii. Improve the copper titration technique or change analytical technique to enable measurements at lower copper concentrations. Most waters have copper concentrations below the detection limit found in this study and reliable copper binding predictions are not possible from this work. Several researchers have used a copper-ethylenediamine buffer to calibrate the electrode below 0.1 μ mole/l. Ma et al. (1999) calibrated their electrode down to 10^{-13} mole/l. Other researchers using this method include Benedetti et al. (1995) and Avdeef et al. (1986).

iii. Examine more river waters to detect if they are more homogeneous in copper binding properties than the single species extracts researched in this work. Related analyses on the structure of NOM may give additional insights.

iv. Investigate structural changes in NOM and effects on copper binding properties as influenced by geochemical processes occurring during transport.

v. This work has only investigated copper binding at constant pH, ionic strength and organic matter concentration. Changes in copper binding due to variations, especially in pH, might reveal important information about copper binding groups not visualized in this thesis.

REFERENCES

Aiken G.R., McKnight D.M., Wershaw R.L. and MacCarthy P., Humic Substances in Soil, Sediment and water. John Wiley and Sons, New York (1985)

Allison J.D., Brown D.S. and Novo-Gradack H., MINTEQA2 – A Geochemical Assessment Model for Environmental Systems. Center for Exposure Assessment Modeling, U.S. Envirnmental Protection Agency. (1993) Version 3.11

Amdour M.O., Doull J. and Klaassen C.D., Casarrett and Doull's Toxicology - The Basic Science of Poisons. Fourth Ed. Pergamon Press, New York (1991)

Avdeef A., Zabronsky J.and Stutting H.H., Calibration of copper ion selective electrode response to pCu 19. Analytical Chemistry (1983) 55:298-304.

Bartschat B.M., Cabaniss S.E. and Morel F.M.M., Oligoelectrolyte Model for Cation Binding by Humic Substances. Environmental Science and Technology (1992) 26:284-294.

Benedetti M.F., Milne C.J., Kinniburgh D.G., van Riemsdijk W.H. and Koopal L.K Metal ion binding to humic substances: Application of the non-ideal competitive adsorption model. Environmental Science and Technology (1995) 29:446-457.

Benedetti M.F., van Riemsdijk W.H. and Koopal L.K., Humic Substances Considered as a Heterogeneous Donnan Gel Phase. Environmental Science and Technology (1996) 30:1805-1813.

Bowles E.C., Antweiler R.C. and MacCarthy P., Acid-Base Titration and Hydrolysis of Fulvic Acid from the Suwannee River. In Humic Substances in the Suwannee River, Georgia; Interactions, Properties, and Proposed Structures. US Geological Survey, Open-File Report 87-557 (1987)

Breault R.F., Colman J.A., Aiken G.R. and Mcknight D. Copper Speciation and Binding by Organic Matter in Copper-Contaminated Streamwater. Environmental Science and Technology (1996) 30:3477-3486.

Buffle J., A Critical Comparison of Studies of Complex Formation Between Copper (2) and Fulvic Substances of Natural Waters. Analytica Chimica Acta (1980) 118:29-44.

Buffle J., Greter F.L. and Haerdi W., Measurements of Complexation Properties of Humic and Fulvic Acids in Natural Waters With Lead and Copper Ion-Selective Electrodes. Analytical Chemistry (1977) 49 (2):216-222.

Cabaniss S.E. and Shuman M.S., Copper Binding by Dissolved Organic Matter: 1. Suwannee River Fulvic Acid Equilibria. Geochimica et Cosmochimica Acta (1988) 52:185-193.

Chapra S., Surface Water Quality Modeling. MacGraw-Hill, New York (1997).

Cleven R.F.M.J., Heavy Metal/Polyacid Interaction – An Electrochemcial Study of the Binding of Cd(II), Pb(II) and Zn(II) to Polycarboxylic and Humic Acids. Ph.D. Thesis, Agricultural University, Wageningen (1984).

Ephraim J.H. and Allard B., Copper Binding by an Aquatic Fulvic Acid: Heterogeneity Considerations. Environmental International (1994) 20 (1):89-95.

Erickson R.J., Benoit D.A., Mattson V.R., Nelson H.P.Jr. and Leonard E.N., The Effects of Water Chemistry on the Toxicity of Copper to Fathead Minnows. Environmental Toxicology and Chemistry (1996) (15):181-193.

Florence T.M., Morrison G.M. and Stauber J.L. Determination of Trace Element Speciation and the Role of Speciation in Aquatic Toxicity. The Science of The Total Environment (1992) 125:1-13

Gamble D.S., Underdown A.W. and Langford C.H., Copper(II) Titration of Fulvic Acid Ligand Sites with Theoretical, Potentiometric, and Spectrophotometric Analysis. Analytical Chemistry (1980) 52:1901-1908.

Giesy P.J, Newell A. and Leversee G.J., Copper Speciation in Soft, Acid, Humic Waters: Effect On Copper Bioaccumulation by and Toxicity to Simocephalus Serrulatus. The Science of the Total Environment (1983) 28:23-36.

Herbelin A. L. and Westall J.C., FITEQL – A Computer Program for Determination of Chemical Equilibrium Constants From Experimental Data. Department of Chemistry, Oregon State University (1996) Version 3.2

Hering J.G. and Morel M.M., Humic Acid Complexation of Calcium and Copper. Environmental Science and Technology (1988) 22 (10):1234-1237.

Hogstrand C., Reid S.D. and Wood C.M., Ca^{2+} Versus Zn^{2+} Transport in the Gills of Freshwater Rainbow Trout and the Cost of Adaption to Waterborne Zn^{2+}. Journal Experimental Biology (1995) 198:337-348.

Hollis L., Muench L. and Playle R.C., Influence of Dissolved Organic Matter on Copper Binding, and Calcium Binding, by Gills of Rainbow Trout. Journal of Fish Biology (1997) 50:703-720

Hutchinson N.J. and Sprague J.B., Reduced Lethality of Al, Zn and Cu Mixtures to American Flagfish (Jordanella floridae) by Complexation with Humic Substances in Acidified Soft Waters. Environmental Toxicology and Chemistry (1987) 6:755-765

Kinniburgh D.G., Milne C.J., Benedetti M.F., Pinheiro J.P., Filiius J., Koopal L.K. and van Riemsdijk W.H., Metal Ion Binding by Humic Acid: Application of the NICA-Donnan Model. Environmental Science and Technology (1996) 30:1687-1698.

Kipton H., Powell H.K.J. and Fenton E., Size Fractionation of Humic Substances: Effect on Protonation and Metal Binding Properties. Analytica Chimica Acta (1996) 334:27-38.

Leenheer J.A., Wershaw R.L. and Reddy M.M., Strong-Acid Carboxyl Groups Structurers in Fulvic Acid From the Suwannee River, Georgia. 1. Minor Structures. Environmental Science and Technology (1995) 29:393-398.

Ma H., Kim S.D., Cha D.K. and Allen H.E., Effects of Kinetics of Complexation by Humic Acid on Toxicity of Copper to Ceriodaphnia dubia. Environmental Toxicology and Chemistry (1999) 18 (5):828-837.

Macalady D.L. and Ranville J.F., The Chemistry and Geochemistry of Natural Organic Matter (NOM). In Macalady D.L. (Ed.), Perspectives in Environmental Chemistry. Oxford University Press, New York (1998)

Malcolm R.L., Geochemistry of Stream Fulvic and Humic Substances. In Aiken G.R., McKnight D.M., Wershaw R.L. and McCarthy P. (Eds), Humic Substances in Soil, Sediment, and water. John Wiley and Sons, New York (1985)

Manning G.S., Counterion Binding in Polyelectrolye Theory. Accounts of Chemical Research (1979) 12:413-449.

Manning G.S., Limiting Laws and Counterion Condensation in Polyelectrolyte Solutions. 6. Theory of the Titration Curve. Journal of Physical Chemistry (1981) 85 (7):870-877.

Martell A.E. and Smith R.M., Critical Stability Constants. Vol 3 – Other Organic Ligands. Plenum, New York (1973)

Mathuthu A.S. and Ephraim J.H., Calcium Binding by Fulvic Acids Studied by an Ion Selective Electrode and an Ultrafiltration Method. Talanta (1993) 40(4):521-526.

McKnight D.M., Feder G.L., Thurman E.M., Wershaw R.L. and Westall J.C., Complexation of Copper by Aquatic Humic Substances From Different Environments. The Science of the Total Environment (1983) 28:65-76.

McKnight D.M. and Wershaw R.L., Complexation of Copper by Fulvic Acid From the Suwannee River; Effect of Counter-ion Concentration. In Averett R.C., Leenher J.A., McKnight D.M. and Thorn K.A. (Eds.) Humic Substances in the Suwannee River, Georgia; Interactions Properties, and Proposed Structures. U.S. Geological Survey, Reston, VA Report Number W 2373, Accesion Number 94-52312 (1994)

Midorikawa T, Tanoue E. and Sugimora Y., Determination of Complexing Ability of Natural Ligands in Seawater For Various Metal Ions Using Ion Selective Electrodes. Analytical Chemistry (1990) 62:1737-1746.

Palmer F.B., Butler C.A., Timperley M.H. and Evans C.W., Toxicity to Embryo and Adult Zebrafish of Copper Complexes with Two Malonic Acids as Models for Dissolved Organic Matter. Environmental Toxicology and Chemistry (1998) 17 (8):1538-1545

Perdue M.P. and Charles R.L., Distribution Model for Binding of Protons and Metal Ions by Humic Substances. Environmental Science and Technology (1983) 17 (11):654-660.

Playle C.P., Dixon D.G. and Burnison K., Copper and Cadmium Binding to Fish Gills: Estimates of Metal-Gill Stability Constants and Modelling of Metal Accumulation. Canadian Journal of Fisheries and Aquatic Sciences (1993) 50:2678-2687.

Saar R.A. and Weber J.H., Comparison of Spectrofluorometry and Ion-Selective Electrode Potentiometry for Determination of Complexes Between Fulvic Acid and Heavy-Metal Ions. Analytical Chemistry (1980) 52:2095-2100.

Saar R.A. and Weber J.H., Fulvic Acid: Modifier of Metal-ion Chemistry. Environmental Science and Technology (1982) 16 (9):510-517.

Sahu S. and Banerjee D.K., Complexation of Copper(II), Cadmium(II) and Lead(II) with Humic and Fulvic Acids of Yamuna River Sedimments. In Pawlowski et al. (Eds) Chemistry for the Protection of the Environment 2. Plenum Press, New York (1996)

Salmon J.T., New Zealand Native Trees. Reed Publishing, Singapore (1994)

Schwarzenbach R.P., Gschwend P.M., Imboden D.M., Environmental Organic Chemistry. John Wiley and Sons, New York (1993)

Skoog D.A. and Leary J.J., Principles of Instrumental Analysis, Fourth ed. Saunders College Publishing (1992)

Stewart K., Native Trees of New Zealand. HarperCollinsPublishers New Zealand, Hong Kong (1993)

Temminghoff E.J.M., Van der Zee S.E.A.T and de Haan F.A.M., Copper Mobility in a Copper-Contaminated Sandy Soil as Affected by pH and Soil and Dissolved Organic Matter. Environmental Science and Technology (1997) 31:1109-1115.

Thurman E.M., Organic Geochemistry of Natural Waters, Martinus Nijhoff/Dr.W. Junk Publishers, Dordrecht (1985).

Thurman E.M. and Malcolm R.L., Structural Study of Humic Substances: New Approaches and Methods. In Cristman R.F. and Gjessing E.T. (Eds) Aquatic and Terrestrial Humic Materials. Ann Arbor Science, Ann Arbor (1983)

Town R.M. and Powell H.K.J., Ion-Selective Electrode Potentiometry Studies on the Complexation of Copper(II) by Soil-Derived Humic and Fulvic Acids. Analytica Chimica Acta. (1993) 279:221-233.

Turner D.R., Varney M.S., Whitfield M., Mantoura R.F.C. and Riley J.P., Electrochemical Studies of Copper and Lead complexation by Fulvic Acid. 1. Potentiometric Measurements and a Critical Comparison of Metal Binding Models. Geochimica et Cosmochimica Acta (1986) 50:289-297.

van den Hoop. M.A.G.T., van Leeuwen H.P., Pinheiro J.P., Mota A.M. and Simões Gonçalves M.d.L., Voltammetric Analysis of the Competition Between Calcium and Heavy Metals fo Complexation by Humic Material. Colloids and Surfaces A: Physicochemical and Engineering Aspects (1995) 95:305-313.

Welsh P.G., Skidmore J.F., Spry D.J., Dixon D.G., Hodson P.V., Hutchinson N.J. and Hickie B.E., Effect of pH and Dissolved Organic Carbon on the Toxicity of Copper to Larval Fathead Minnow (Pimephales promelas) in Natural Lake Waters of Low Alkalinity. Canadian Journal of Fisheries and Aquatic Sciences (1993) 50:1356-1362.

Westall J.C., Jones J.D., Turner G.P. and Zachara J.M., Models for Association of Metal Ions with Heterogeneous Environmental Sorbents. 1. Complexatiopn of Cu(II) by Leonardite Humic Acid as a function of pH and $NaClO_4$ Concentration. Environmental Science and Technology (1995) 29:951-959.

Westall J.C., Morel F.M.M. and Hume D.N., Chloride Interference in Cupric Ion Selective Electrode Measurements. Analytical Chemistry (1979) 51 (11):1792-1798.

Zhang Y.J., Bryan N.D., Livens F.R. and Jones M.N., Complexing of Metal Ions by Humic Substances. American Chemical Society Symposium Series (1996) 651 (Humic Fulvic Acids):194-206.

APPENDIX A. DESCRIPTION OF FITEQL

This section is not meant to serve as a complete description of FITEQL but only to briefly introduce the reader to the program. For a more rigorous discussion, refer to the manual (Herbelin and Westall, 1996).

FITEQL determines the "best-fit" of a model to experimental data. The code is primarily set up to fit titration data and the input experimental data is therefore given to the program as serial data. The model consists of a user-specified set of equilibrium equations and the best fit is found by minimizing the weighted sum of squares (WSOS) between the experimental data and the model. This is done by changing the equilibrium constant(s) and total species concentration(s) in the model. Copper binding by NOM can thus be described by the total concentration of NOM (the ligand(s)) and corresponding equilibrium constant(s) found by FITEQL.

Chemical compounds are defined by FITEQL as species built up of one or more components. As an example, the complex CuL is a species built up of the two components Cu and L. For each data point one mass balance equation and one mass action expression per equilibrium equation is used to solve the problem. The best fit of the model to the whole titration data is found by minimizing the sum of squares by a Newton-Raphson iteration. Activity coefficients are calculated using the Davies equation for each step in the titration procedure.

FITEQL needs a starting estimate of total ligand concentration and equilibrium constants. It was found that the code is able to fit a titration in only one run in the simplest possible system; one copper ion is bound to one ligand resulting in a matrix consisting of only one mass action expression and one mass balance equation. When the model consisted of two equilibrium equations it was necessary to fit total ligand concentration and then manually change the starting estimate of the total ligand concentration and let FITEQL find the appurtenant equilibrium constants. The new equilibrium constants were given to the program as starting estimates in the new run.

APPENDIX B. EXPERIMENTAL DATA

Experimental data is presented in the following order:

a) Copper titration at pH 6, 50 mmole/l KNO_3 and 10 mg C/l (Sutton Stream has an organic carbon concentration of 5.7 mg C/l). Blank titrations are given together with the NOM titrations.

i)	Bamboo extract
ii)	Cabbage Tree extract
iii)	Kauri extract
iv)	Manuka extract
v)	Red Beech extract
vi)	Red Tussock extract
vii)	Sutton Stream water
viii)	Suwannee River water
ix)	Swamp Cypress extract, first titration
x)	Swamp Cypress extract, second titration
xi)	Fit of two-site model to Manuka NOM with altered conditional formation constants (0.1 log units)
xii)	Fit of two-site model to Manuka NOM with altered conditional formation constants (0.5 log units)

b) Acid/base titrations between pH 3 and 11 at 50 mmole/l KNO_3.

i)	Bamboo extract
ii)	Cabbage Tree extract
iii)	Kauri extract, first titration
iv)	Kauri extract, second titration
v)	Manuka extract, first titration

vi)   Manuka extract, second titration

vii)   Red Beech extract

viii)   Red Tussock extract

ix)   Sutton Stream water

x)   Suwannee River water

xi)   Swamp Cypress extract, first titration

xii)   Swamp Cypress extract, second titration

xiii)   First blank titration

xiv)   Second blank titration

xv)   Third blank titration

xvi)   Fourth blank titration

xvii)   Fifth blank titration

xviii) Sixth blank titration

Copper titration of Bamboo extract at pH 6, 50 mmole/l KNO₃ and 10 mg C/l.

Cu$_{tot}$ (mole/l)	e.m.f. (mV)	pH	Cu$_{tot}$ (cont.) (mole/l)	e.m.f. (cont.) (mV)	pH (cont.)
0	-120.3	5.99	7.00E-06	-39.3	6.10
4.87E-08	-119.1	5.97	7.92E-06	-36.6	6.01
2.47E-07	-110.3	5.99	9.30E-06	-32.9	6.00
3.43E-07	-106.9	6.00	1.07E-05	-29.9	5.99
4.39E-07	-103.4	6.00	1.25E-05	-26.8	6.01
5.36E-07	-99.9	6.00	1.49E-05	-23.5	6.00
6.32E-07	-96.7	6.00	1.76E-05	-20.2	6.00
7.80E-07	-93.9	6.00	2.13E-05	-16.6	5.99
8.70E-07	-89.9	6.00	2.58E-05	-13.3	6.00
1.01E-06	-86.4	6.01	3.03E-05	-10.5	6.00
1.11E-06	-84.4	6.00	3.48E-05	-8.1	6.01
1.25E-06	-81.6	6.01	4.39E-05	-4.2	5.99
1.39E-06	-78.8	6.00	5.29E-05	-1.2	6.00
1.53E-06	-76.3	5.99	6.63E-05	2.3	5.99
1.72E-06	-73.6	6.00	8.9E-05	6.7	5.99
1.91E-06	-71.1	6.00	1.25E-04	12.0	5.98
2.15E-06	-68.2	6.00	1.70E-04	15.1	5.99
2.43E-06	-65.4	6.01	2.14E-04	17.1	5.98
2.81E-06	-62.3	6.02	3.03E-04	19.1	5.98
3.27E-06	-58.0	6.01	4.84E-04	24.2	5.98
3.74E-06	-55.0	6.02	7.07E-04	30.6	6.01
4.21E-06	-51.7	6.00	9.73E-04	36.3	6.01
4.68E-06	-49.0	6.00	1.33E-03	41.3	5.98
5.14E-06	-46.7	6.00	1.76E-04	45.5	5.99
6.07E-06	-42.6	6.00			

Blank titration in connection to Bamboo NOM titration at pH 6 and 50 mmole/l KNO₃.

Cu$_{tot}$ (mole/l)	e.m.f. (mV)	pH
0.00E+00	-88.2	5.87
4.85E-08	-82.5	5.94
2.46E-07	-68.1	6.05
7.29E-07	-53.5	5.99
5.55E-06	-26.4	5.96
5.37E-05	3.4	5.98
5.34E-04	32.9	5.98
2.48E-03	51.5	6.00

Copper titration of Cabbage Tree extract at pH 6, 50 mmole/l KNO_3 and 10 mg C/l.

Cu_{tot} (mole/l)	e.m.f. (mV)	pH	Cu_{tot} (cont.) (mole/l)	e.m.f. (cont.) (mV)	pH (cont.)
0.00E+00	-114.5	5.93	9.74E-06	-27.5	5.99
5.40E-08	-109.3	5.98	1.18E-05	-23.9	6.00
1.61E-07	-100.9	5.98	1.43E-05	-20.3	6.00
3.21E-07	-93.1	6.02	1.74E-05	-17.0	6.00
4.81E-07	-86.5	6.00	2.04E-05	-14.3	6.00
6.41E-07	-81.5	6.01	2.44E-05	-11.3	5.99
7.46E-07	-78.8	6.00	2.94E-05	-8.5	5.99
8.52E-07	-76.8	6.02	3.44E-05	-5.9	5.99
9.58E-07	-74.2	6.00	3.94E-05	-3.8	6.00
1.12E-06	-71.4	6.00	4.43E-05	-1.8	6.00
1.33E-06	-67.8	6.00	5.42E-05	1.3	5.98
1.54E-06	-65.1	6.01	6.90E-05	4.9	6.00
1.81E-06	-61.9	6.01	8.90E-05	8.6	6.00
2.07E-06	-59.0	6.00	1.09E-04	11.4	5.99
2.38E-06	-56.1	6.00	1.38E-04	14.8	6.00
2.69E-06	-53.5	6.00	1.67E-04	17.4	5.99
3.10E-06	-50.5	5.99	2.06E-04	20.2	6.00
3.62E-06	-47.6	6.01	2.54E-04	22.9	5.99
4.13E-06	-44.7	6.00	3.50E-04	27.2	5.99
4.64E-06	-42.3	5.99	4.46E-04	30.3	5.99
5.15E-06	-40.4	6.01	5.89E-04	33.9	5.98
6.18E-06	-36.7	6.00	8.31E-04	38.1	6.01
7.20E-06	-33.5	6.00	1.22E-03	43.1	5.99
8.22E-06	-30.5	6.00	1.69E-03	47.1	6.01

Blank titration in connection to Cabbage Tree NOM titration at pH 6 and 50 mmole/l KNO_3.

Cu_{tot} (mole/l)	e.m.f. (mV)	pH
0.00E+00	-99.3	6.04
4.84E-08	-84.0	6.03
2.45E-07	-67.8	6.01
7.28E-07	-53.8	6.01
5.49E-06	-26.2	5.98
5.32E-05	3.7	5.98
5.29E-04	33.6	6

Copper titration of Kauri extract at pH 6, 50 mmole/l KNO_3 and 10 mg C/l.

Cu_{tot} (mole/l)	e.m.f. (mV)	pH	Cu_{tot} (cont.) (mole/l)	e.m.f. (cont.) (mV)	pH (cont.)
0.00E+00	-94.1	6.03	2.20E-05	-9.7	6.00
1.98E-07	-73.0	5.96	2.67E-05	-7.0	6.00
3.95E-07	-64.1	5.96	3.59E-05	-3.0	5.99
5.90E-07	-58.8	5.99	4.52E-05	0.1	6.00
6.85E-07	-56.7	6.00	5.90E-05	3.7	5.99
8.80E-07	-53.3	5.99	7.76E-05	7.3	5.99
1.17E-06	-49.5	6.00	1.01E-04	10.8	5.99
1.45E-06	-46.2	6.00	1.28E-04	14.1	6.01
1.84E-06	-42.9	5.99	1.65E-04	17.5	6.01
2.31E-06	-39.8	5.99	2.10E-04	20.6	6.01
2.78E-06	-37.2	6.00	2.55E-04	23.1	5.99
3.73E-06	-32.8	6.00	3.46E-04	27.0	5.98
4.67E-06	-29.4	6.00	4.80E-04	31.0	5.99
6.08E-06	-26.0	6.00	7.53E-04	36.5	6.01
7.99E-06	-23.0	6.01	1.12E-03	41.5	6.01
1.11E-05	-19.3	6.02	1.56E-03	45.5	6.02
1.36E-05	-16.0	5.99	3.38E-03	54.9	6.01
1.74E-05	-12.7	6.00			

Blank titration in connection to Kauri NOM titration at pH 6 and 50 mmole/l KNO_3.

Cu_{tot} (mole/l)	e.m.f. (mV)	pH
0.00E+00	-106.4	5.95
4.85E-08	-86.3	6.01
2.46E-07	-65.7	6.02
7.30E-07	-52.1	5.99
5.56E-06	-25.3	6.01
5.38E-05	4.5	5.97
5.35E-04	33.9	5.98
2.48E-03	52.7	6.00

Copper titration of Manuka extract at pH 6, 50 mmole/l KNO_3 and 10 mg C/l.

Cu_{tot} (mole/l)	e.m.f. (mV)	pH	Cu_{tot} (cont.) (mole/l)	e.m.f. (cont.) (mV)	pH (cont.)
0.00E+00	-138.6	5.97	1.59E-05	-33.1	5.99
1.98E-07	-129.9	5.99	1.82E-05	-29.6	6.00
3.96E-07	-122.2	5.99	2.09E-05	-25.9	5.99
5.91E-07	-113.3	6.00	2.36E-05	-22.8	6.00
7.86E-07	-108.2	6.01	2.63E-05	-20.2	6.00
1.17E-06	-100.1	6.00	2.98E-05	-17.0	6.00
1.65E-06	-92.9	6.00	3.43E-05	-13.9	6.00
2.13E-06	-87.0	5.99	3.88E-05	-11.3	6.00
2.60E-06	-82.1	5.99	4.32E-05	-9.0	6.00
3.07E-06	-78.2	5.99	5.21E-05	-5.0	5.98
3.54E-06	-74.9	5.99	6.08E-05	-1.8	5.98
4.01E-06	-72.5	6.01	6.97E-05	0.4	6.00
4.49E-06	-69.6	6.01	8.77E-05	4.7	5.98
4.95E-06	-66.4	6.00	1.05E-04	7.9	5.99
5.42E-06	-63.8	6.00	1.28E-04	11.1	5.99
6.07E-06	-60.5	5.98	1.54E-04	14.1	5.98
6.71E-06	-57.9	5.99	1.89E-04	17.3	5.98
7.64E-06	-54.6	6.00	2.33E-04	20.3	5.99
8.56E-06	-51.2	6.00	3.20E-04	24.7	5.98
9.48E-06	-47.8	5.98	4.96E-04	30.2	6.00
1.08E-05	-43.4	5.98	7.57E-04	35.2	6.00
1.22E-05	-40.5	6.01	1.11E-03	40.7	6.01
1.41E-05	-36.3	5.99	1.54E-03	45.2	6.00

Blank titration in connection to Manuka NOM titration at pH 6 and 50 mmole/l KNO_3.

Cu_{tot} (mole/l)	e.m.f. (mV)	pH
0.00E+00	-85.0	6.06
4.87E-08	-78.7	5.98
2.46E-07	-60.6	6.03
7.31E-07	-50.5	6.00
5.57E-06	-26.1	6.01
5.38E-05	3.7	5.99
5.34E-04	31.8	5.98

Copper titration of Red Beech extract at pH 6, 50 mmole/l KNO$_3$ and 10 mg C/l.

Cu$_{tot}$ (mole/l)	e.m.f. (mV)	pH	Cu$_{tot}$ (cont.) (mole/l)	e.m.f. (cont.) (mV)	pH (cont.)
0.00E+00	-114.6	5.94	1.59E-05	-25.9	5.98
9.74E-08	-100.1	5.99	1.77E-05	-23.4	5.98
2.95E-07	-101.2	5.96	1.99E-05	-20.8	5.99
4.91E-07	-95.3	5.598	2.26E-05	-18.1	5.98
8.82E-07	-87.2	5.99	2.62E-05	-14.7	5.98
1.27E-06	-81.2	5.99	3.07E-05	-11.3	5.99
1.65E-06	-76.2	5.98	3.52E-05	-8.5	6.01
1.89E-06	-73.8	5.98	3.96E-05	-5.8	5.98
2.17E-06	-70.8	5.98	4.41E-05	-3.6	5.99
2.55E-06	-67.9	5.99	5.30E-05	0.0	5.99
3.02E-06	-64.2	5.97	6.19E-05	2.8	6.00
3.48E-06	-61.4	5.99	7.07E-05	5.3	5.99
3.95E-06	-58.3	5.98	8.41E-05	8.5	5.99
4.41E-06	-55.1	5.98	1.02E-04	11.8	5.99
4.87E-06	-53.4	5.98	1.20E-04	14.4	6.01
5.80E-06	-49.4	5.98	1.47E-04	17.2	5.98
6.72E-06	-46.1	5.98	1.91E-04	20.8	6.00
7.63E-06	-43.4	5.99	2.79E-04	26.0	6.00
8.55E-06	-40.8	6.00	4.10E-04	31.2	5.99
9.46E-06	-38.3	5.99	6.76E-04	36.6	6.01
1.08E-05	-35.1	6.00	1.03E-03	42.4	6.00
1.22E-05	-32.4	6.00	1.47E-03	46.7	6.03
1.40E-05	-28.9	5.99			

Blank titration in connection to Red Beech NOM titration at pH 6 and 50 mmole/l KNO$_3$.

Cu$_{tot}$ (mole/l)	e.m.f. (mV)	pH
0.00E+00	MD	MD
4.86E-08	-81.3	5.97
2.46E-07	-65.2	5.96
7.31E-07	-51.5	5.96
5.57E-06	-24.6	5.97
5.39E-05	5.6	5.97
5.36E-04	34.9	5.95

Copper titration of Red Tussock extract at pH 6, 50 mmole/l KNO_3 and 10 mg C/l.

Cu_{tot} (mole/l)	e.m.f. (mV)	pH	Cu_{tot} (cont.) (mole/l)	e.m.f. (cont.) (mV)	pH (cont.)
0.00E+00	-136.3	5.96	1.11E-05	-28.9	6.00
1.98E-07	-120.5	5.99	1.30E-05	-25.9	6.01
3.93E-07	-108.3	5.97	1.52E-05	-22.3	5.99
5.88E-07	-100.1	5.98	1.79E-05	-19.5	6.00
7.82E-07	-93.9	6.01	2.06E-05	-16.5	6.00
9.76E-07	-88.1	5.99	2.33E-05	-14.1	6.00
1.17E-06	-84.2	6.01	2.68E-05	-11.3	6.00
1.36E-06	-79.3	5.96	3.12E-05	-8.6	5.99
1.55E-06	-76.5	5.98	3.57E-05	-6.2	5.98
1.74E-06	-74.0	6.01	4.00E-05	-4.3	6.00
1.97E-06	-70.2	5.98	4.89E-05	-0.9	5.99
2.20E-06	-67.4	5.99	5.77E-05	1.7	6.00
2.44E-06	-65.5	5.99	7.09E-05	4.8	6.00
2.71E-06	-62.7	6.00	8.87E-05	8.3	6.01
3.08E-06	-59.0	5.99	1.06E-04	11.1	5.99
3.44E-06	-56.2	6.01	1.28E-04	14.0	5.98
3.90E-06	-53.2	5.99	1.54E-04	16.7	5.99
4.36E-06	-50.8	6.00	1.89E-04	19.5	5.99
4.82E-06	-48.2	6.00	2.32E-04	22.7	5.98
5.28E-06	-45.8	5.99	3.19E-04	26.4	5.98
5.74E-06	-43.9	5.99	4.48E-04	30.4	5.97
6.65E-06	-40.1	5.97	6.66E-04	35.2	6.01
7.56E-06	-37.0	5.99	1.01E-03	40.9	6.01
8.45E-06	-34.5	5.98	1.44E-03	45.4	6.02
9.79E-06	-31.4	5.99			

Blank titration in connection to Red Tussock NOM titration at pH 6 and 50 mmole/l KNO_3.

Cu_{tot} (mole/l)	e.m.f. (mV)	pH
0.00E+00	-104.4	6.05
4.86E-08	-83.2	5.99
2.46E-07	-68	5.98
7.31E-07	-53.9	5.97
5.57E-06	-26.6	5.98
5.37E-05	5.2	5.97
5.34E-04	33.6	5.98

Copper titration of Sutton Stream water at pH 6, 50 mmole/l KNO_3 and 10 mg C/l.

Cu_{tot} (mole/l)	e.m.f. (mV)	pH	Cu_{tot} (cont.) (mole/l)	e.m.f. (cont.) (mV)	pH (cont.)
0.00E+00	-127.0	5.96	1.03E-05	-24.8	6.00
9.68E-08	-107.6	6.01	1.22E-05	-21.9	6.01
1.93E-07	-100.0	6.02	1.45E-05	-18.8	6.00
3.38E-07	-92.2	6.01	1.72E-05	-15.9	5.99
5.33E-07	-85.0	6.02	1.99E-05	-13.5	6.01
7.28E-07	-78.4	6.00	2.35E-05	-10.8	6.00
8.23E-07	-75.8	5.98	2.71E-05	-8.5	5.99
9.64E-07	-72.4	5.98	3.16E-05	-6.1	6.01
1.10E-06	-69.4	5.99	3.61E-05	-4.0	6.00
1.30E-06	-66.3	6.00	4.50E-05	-0.4	6.00
1.49E-06	-63.1	5.98	5.39E-05	2.4	6.00
1.72E-06	-60.1	6.00	6.72E-05	5.8	6.00
2.00E-06	-56.8	6.01	8.04E-05	8.3	5.99
2.28E-06	-53.9	6.01	9.35E-05	10.3	6.10
2.61E-06	-51.1	6.00	1.20E-04	13.8	6.00
2.98E-06	-48.2	6.01	1.46E-04	16.8	5.99
3.44E-06	-45.4	6.01	1.82E-04	19.6	6.00
3.91E-06	-42.6	5.99	2.26E-04	22.3	6.00
4.37E-06	-40.4	5.99	3.13E-04	26.5	6.00
4.83E-06	-38.7	6.00	4.90E-04	32.1	5.98
5.75E-06	-35.5	6.02	6.67E-04	35.9	6.02
6.68E-06	-32.6	6.00	1.02E-03	41.5	5.98
8.06E-06	-28.9	5.99	1.45E-03	45.6	5.99
8.96E-06	-27.2	6.00			

Blank titration in connection to Sutton Stream water titration at pH 6 and 50 mmole/l KNO_3.

Cu_{tot} (mole/l)	e.m.f. (mV)	pH
0.00E+00	-101.9	6.00
4.84E-08	-81.2	6.03
2.45E-07	-66.7	6.03
7.27E-07	-53.1	5.98
5.53E-06	-25.7	5.94
5.35E-05	3.7	5.99
5.33E-04	32.9	5.96

Copper titration of Suwannee River water at pH 6, 50 mmole/l KNO₃ and 10 mg C/l.

Cu$_{tot}$ (mole/l)	e.m.f. (mV)	pH	Cu$_{tot}$ (cont.) (mole/l)	e.m.f. (cont.) (mV)	pH (cont.)
0.00E+00	-108.2	5.94	1.93E-05	-17.9	5.99
1.97E-07	-97.1	5.95	2.39E-05	-14.3	5.99
2.93E-07	-93.9	5.98	3.32E-05	-9.0	5.99
4.88E-07	-87.4	5.97	4.69E-05	-3.1	5.98
9.66E-07	-76.7	6.00	6.56E-05	1.8	5.99
1.44E-06	-69.5	6.00	1.03E-04	8.5	6.00
1.92E-06	-63.5	5.99	1.49E-04	13.9	5.98
2.87E-06	-54.9	5.98	1.94E-04	17.8	5.98
3.81E-06	-49.4	6.00	2.85E-04	23.0	5.99
4.75E-06	-45.0	6.00	4.21E-04	28.2	5.99
6.16E-06	-39.9	6.01	6.49E-04	34.2	6.00
8.07E-06	-34.2	6.00	1.01E-03	40.1	6.00
1.09E-05	-28.3	6.00	1.46E-03	44.3	6.00
1.47E-05	-22.5	5.98	3.29E-03	54.3	6.00

Blank titration in connection to Suwannee River water titration at pH 6 and 50 mmole/l KNO₃.

Cu$_{tot}$ (mole/l)	e.m.f. (mV)	pH
0.00E+00	-119.7	5.93
4.86E-09	-118	5.94
5.34E-08	-99.5	5.98
5.38E-07	-62.8	5.98
5.38E-06	-27.9	6.00
5.37E-05	2.1	5.98
5.36E-04	32.1	5.99
2.49E-03	51.6	5.99

First copper titration of Swamp Cypress extract at pH 6, 50 mmole/l KNO_3 and 10 mg C/l.

Cu_{tot} (mole/l)	e.m.f. (mV)	pH	Cu_{tot} (cont.) (mole/l)	e.m.f. (cont.) (mV)	pH (cont.)
0.00E+00	-115.2	5.97	7.97E-06	-26.4	5.99
1.99E-07	-94.7	5.98	9.84E-06	-23.1	6.01
2.95E-07	-87.8	5.99	1.17E-05	-20.4	6.00
3.92E-07	-82.3	5.99	1.40E-05	-17.6	6.00
4.39E-07	-80.0	5.99	1.68E-05	-14.8	5.99
5.35E-07	-75.9	5.99	2.04E-05	-12.0	5.99
6.30E-07	-72.9	5.99	2.49E-05	-8.9	6.00
7.25E-07	-70.1	5.99	2.95E-05	-6.3	6.00
8.20E-07	-67.7	5.99	3.40E-05	-4.2	6.00
9.61E-07	-64.3	5.99	4.30E-05	-0.8	5.99
1.10E-06	-61.9	6.01	5.20E-05	1.9	5.99
1.29E-06	-58.9	6.01	6.10E-05	4.3	5.99
1.53E-06	-55.6	6.01	7.43E-05	7.3	5.99
1.76E-06	-52.8	6.01	9.26E-05	10.1	6.00
2.04E-06	-49.8	6.01	1.20E-04	13.6	5.99
2.42E-06	-46.6	5.98	1.55E-04	16.3	5.99
2.88E-06	-43.8	6.00	2.00E-04	18.2	5.99
3.35E-06	-41.1	6.01	2.88E-04	21.2	5.97
3.81E-06	-38.8	6.00	4.68E-04	27.3	6.01
4.74E-06	-34.9	5.99	6.91E-04	33.6	6.01
5.67E-06	-31.9	6.00	1.05E-03	40.2	6.00
6.59E-06	-29.5	5.99	1.48E-03	45.2	5.99

Blank titration in connection to first Swamp Cypress NOM titration at pH 6 and 50 mmole/l KNO_3.

Cu_{tot} (mole/l)	e.m.f. (mV)	pH
0.00E+00	-84.4	5.99
4.87E-08	-78.5	5.99
2.47E-07	-65.7	5.98
7.31E-07	-52.6	5.98
5.57E-06	-26.1	6.01
5.39E-05	5.4	5.98
5.35E-04	33.4	5.98

Second copper titration of Swamp Cypress extract at pH 6, 50 mmole/l KNO_3 and 10 mg C/l.

Cu_{tot} (mole/l)	e.m.f. (mV)	pH	Cu_{tot} (cont.) (mole/l)	e.m.f. (cont.) (mV)	pH (cont.)
0.00E+00	-99.8	5.97	7.98E-06	-26.0	6.00
1.98E-07	-86.0	5.97	9.86E-06	-22.5	5.99
2.95E-07	-80.7	5.98	1.17E-05	-19.9	6.00
3.90E-07	-76.7	5.97	1.40E-05	-17.0	5.98
4.38E-07	-75.4	5.98	1.68E-05	-14.3	6.00
5.33E-07	-73.0	6.01	2.04E-05	-11.3	5.99
6.29E-07	-70.5	6.00	2.50E-05	-8.3	5.99
7.24E-07	-68.2	5.99	3.40E-05	-3.8	6.00
8.18E-07	-66.5	5.97	3.85E-05	-2.0	6.00
9.58E-07	-64.0	6.00	4.76E-05	0.9	5.99
1.10E-06	-61.4	6.00	5.65E-05	3.3	6.01
1.29E-06	-58.2	5.99	6.55E-05	5.5	5.99
1.53E-06	-55.1	6.01	7.89E-05	8.2	5.99
1.77E-06	-52.3	5.99	9.71E-05	11.1	5.99
2.05E-06	-49.5	6.00	1.24E-04	14.5	5.98
2.42E-06	-46.4	6.00	1.60E-04	17.4	5.99
2.89E-06	-43.3	6.00	1.99E-04	19.5	5.97
3.36E-06	-40.7	6.01	2.88E-04	21.3	5.99
3.82E-06	-38.1	5.99	4.68E-04	27.9	6.01
4.75E-06	-34.7	6.00	6.90E-04	34.4	6.00
5.68E-06	-31.7	6.00	1.05E-03	40.6	6.00
6.60E-06	-29.0	5.98	1.48E-03	45.3	6.00

Blank titration in connection to second Swamp Cypress NOM titration at pH 6 and 50 mmole/l KNO_3.

Cu_{tot} (mole/l)	e.m.f. (mV)	pH
0.00E+00	-87.3	5.99
4.88E-08	-79.0	5.99
2.47E-07	-66.2	5.99
7.33E-07	-52.2	5.96
5.59E-06	-26.3	6.00
5.40E-05	3.9	5.97
5.38E-04	33.8	5.98

Output from FITEQL modeling of Manuka copper titration using two-site model with altered conditional formation constants kept constant at 4.94 and 6.67.

Cu_{free} (mole/l)	$logL_{1,tot}$ (mole/l)	$L_{2,tot}$ (mole/l)	$LogK_2$	$logK_1$
-6.969	-4.668	-5.235	-6.694	-5.533
-6.886	-4.669	-5.265	-6.612	-5.48
-6.785	-4.671	-5.306	-6.513	-5.42
-6.675	-4.672	-5.357	-6.404	-5.361
-6.585	-4.674	-5.404	-6.316	-5.318
-6.471	-4.677	-5.471	-6.205	-5.27
-6.381	-4.68	-5.528	-6.117	-5.238
-6.266	-4.684	-5.607	-6.008	-5.202
-6.149	-4.691	-5.694	-5.896	-5.172
-6.031	-4.698	-5.787	-5.787	-5.148
-5.879	-4.712	-5.915	-5.648	-5.123
-5.779	-4.723	-6.003	-5.559	-5.11
-5.633	-4.745	-6.134	-5.435	-5.096
-5.523	-4.766	-6.236	-5.345	-5.088
-5.402	-4.794	-6.35	-5.252	-5.081
-5.273	-4.831	-6.473	-5.161	-5.075
-5.166	-4.868	-6.576	-5.091	-5.071
-5.076	-4.904	-6.664	-5.037	-5.069
-4.966	-4.954	-6.772	-4.977	-5.066
-4.858	-5.01	-6.877	-4.925	-5.065
-4.768	-5.061	-6.966	-4.887	-5.063
-4.689	-5.111	-7.045	-4.857	-5.063
-4.55	-5.205	-7.182	-4.812	-5.061
-4.44	-5.286	-7.292	-4.783	-5.061
-4.364	-5.345	-7.368	-4.766	-5.06
-4.215	-5.467	-7.516	-4.739	-5.06
-4.104	-5.562	-7.626	-4.723	-5.059

Output from FITEQL modeling of Manuka copper titration using two-site model with altered conditional formation constants kept constant at 4.54 and 6.27.

Cu_{free} (mole/l)	$logL_{1,tot}$ (mole/l)	$L_{2,tot}$ (mole/l)	$LogK_2$	$logK_1$
-6.969	-4.669	-4.954	-7.095	-5.652
-6.886	-4.669	-4.969	-7.012	-5.584
-6.785	-4.670	-4.991	-6.912	-5.505
-6.675	-4.671	-5.019	-6.802	-5.423
-6.585	-4.671	-5.047	-6.713	-5.360
-6.471	-4.672	-5.087	-6.600	-5.287
-6.381	-4.674	-5.124	-6.511	-5.234
-6.266	-4.676	-5.178	-6.399	-5.173
-6.149	-4.678	-5.241	-6.284	-5.119
-6.031	-4.681	-5.312	-6.169	-5.072
-5.879	-4.687	-5.415	-6.023	-5.022
-5.779	-4.692	-5.488	-5.928	-4.996
-5.633	-4.701	-5.602	-5.792	-4.965
-5.523	-4.711	-5.694	-5.690	-4.946
-5.402	-4.724	-5.799	-5.582	-4.930
-5.273	-4.741	-5.914	-5.472	-4.916
-5.166	-4.760	-6.012	-5.383	-4.907
-5.076	-4.779	-6.096	-5.312	-4.902
-4.966	-4.807	-6.201	-5.229	-4.896
-4.858	-4.839	-6.304	-5.154	-4.891
-4.768	-4.870	-6.391	-5.096	-4.888
-4.689	-4.902	-6.468	-5.047	-4.886
-4.550	-4.965	-6.603	-4.972	-4.883
-4.440	-5.023	-6.712	-4.920	-4.881
-4.364	-5.067	-6.787	-4.888	-4.880
-4.215	-5.163	-6.935	-4.835	-4.878
-4.104	-5.241	-7.044	-4.802	-4.878

Base titration of Bamboo NOM at 50 mmole/l KNO_3 at 29 mg C/l. Data for acid titration of Bamboo is missing. NaOH concentration equals 0.104 mole/l.

pH	NaOH (cont.) (ml)	pH	NaOH (cont.) (ml)	pH	NaOH (cont.) (ml)	pH	NaOH (cont.) (ml)
2.99	0.000	6.77	0.965	8.72	1.357	9.46	1.969
3.05	0.080	6.82	0.972	8.75	1.375	9.47	1.987
3.12	0.160	6.87	0.979	8.78	1.393	9.49	2.005
3.20	0.240	6.93	0.986	8.81	1.411	9.51	2.023
3.31	0.320	6.98	0.993	8.84	1.429	9.52	2.041
3.44	0.400	7.03	1.000	8.87	1.447	9.61	2.121
3.61	0.480	7.09	1.007	8.89	1.465	9.69	2.201
3.86	0.560	7.14	1.014	8.92	1.483	9.77	2.281
4.21	0.640	7.20	1.021	8.94	1.501	9.85	2.361
4.30	0.658	7.25	1.028	8.97	1.519	9.94	2.441
4.40	0.676	7.31	1.035	8.99	1.537	10.01	2.521
4.50	0.694	7.36	1.042	9.02	1.555	10.10	2.601
4.60	0.712	7.42	1.049	9.04	1.573	10.20	2.681
4.71	0.730	7.48	1.056	9.06	1.591	10.31	2.761
4.82	0.748	7.54	1.063	9.08	1.609	10.41	2.841
4.94	0.766	7.59	1.070	9.10	1.627	10.52	2.921
5.07	0.784	7.65	1.077	9.12	1.645	10.61	3.001
5.22	0.802	7.70	1.084	9.14	1.663	10.70	3.081
5.38	0.820	7.75	1.091	9.16	1.681	10.78	3.161
5.55	0.838	7.80	1.098	9.19	1.699	10.85	3.241
5.75	0.856	7.85	1.105	9.21	1.717	10.91	3.321
5.96	0.874	7.97	1.123	9.22	1.735	10.97	3.401
6.03	0.881	8.07	1.141	9.24	1.753	11.02	3.481
6.10	0.888	8.16	1.159	9.26	1.771	11.06	3.561
6.17	0.895	8.24	1.177	9.28	1.789	11.11	3.641
6.24	0.902	8.31	1.195	9.30	1.807	11.14	3.721
6.31	0.909	8.37	1.213	9.32	1.825		
6.37	0.916	8.42	1.231	9.33	1.843		
6.43	0.923	8.47	1.249	9.35	1.861		
6.49	0.930	8.52	1.267	9.37	1.879		
6.54	0.937	8.57	1.285	9.39	1.897		
6.60	0.944	8.61	1.303	9.40	1.915		
6.66	0.951	8.65	1.321	9.42	1.933		
6.71	0.958	8.68	1.339	9.44	1.951		

Base titration of Cabbage Tree NOM at 50 mmole/l KNO_3 at 33 mg C/l. NaOH concentration equals 0.104 mole/l.

pH	NaOH (ml)	pH (cont)	NaOH (cont.) (ml)
2.95	0.000	9.48	1.053
3.00	0.080	9.67	1.071
3.07	0.160	10.14	1.151
3.15	0.240	10.39	1.231
3.25	0.320	10.57	1.311
3.37	0.400	10.70	1.391
3.53	0.480	10.80	1.471
3.75	0.560	10.88	1.551
4.04	0.640	10.95	1.631
4.11	0.658	11.01	1.711
4.19	0.676	11.06	1.791
4.26	0.694	11.11	1.871
4.34	0.712		
4.42	0.730		
4.49	0.748		
4.57	0.766		
4.65	0.784		
4.74	0.802		
4.82	0.820		
4.91	0.838		
5.00	0.856		
5.11	0.874		
5.22	0.892		
5.36	0.910		
5.51	0.928		
5.74	0.946		
6.10	0.964		
6.34	0.971		
6.61	0.978		
6.99	0.985		
7.48	0.992		
8.04	0.999		
8.84	1.017		
9.22	1.035		

Acid titration of Cabbage Tree NOM at 50 mmole/l KNO_3 at 33 mg C/l. HCl concentration equals 0.0924 mole/l.

pH	HCl (ml)	pH (cont.)	HCl (cont.) (ml)
11.07	0.000	4.85	1.166
11.03	0.080	4.77	1.184
10.98	0.160	4.70	1.202
10.92	0.240	4.63	1.220
10.85	0.320	4.56	1.238
10.78	0.400	4.49	1.256
10.69	0.480	4.42	1.274
10.58	0.560	4.36	1.292
10.43	0.640	4.29	1.310
10.24	0.720	4.23	1.328
9.94	0.800	4.16	1.346
9.84	0.818	3.89	1.426
9.73	0.836	3.66	1.506
9.61	0.854	3.49	1.586
9.47	0.872	3.36	1.666
9.29	0.890	3.25	1.746
9.09	0.908	3.16	1.826
8.81	0.926	3.09	1.906
8.51	0.944	3.03	1.986
7.84	0.962	2.97	2.066
7.45	0.969	2.92	2.146
7.10	0.976	2.88	2.226
6.81	0.983		
6.57	0.990		
6.36	0.997		
6.19	1.004		
5.86	1.022		
5.63	1.040		
5.46	1.058		
5.33	1.076		
5.21	1.094		
5.11	1.112		
5.02	1.130		
4.93	1.148		

First base titration of Kauri NOM at 50 mmole/l KNO_3 at 33 mg C/l. NaOH concentration equals 0.104 mole/l.

pH	NaOH (ml)	pH (cont.)	NaOH (cont.) (ml)
2.90	0.000	5.34	0.988
2.94	0.070	5.42	1.000
3.00	0.140	5.52	1.012
3.06	0.210	5.65	1.024
3.13	0.280	5.80	1.036
3.22	0.350	5.88	1.041
3.32	0.420	5.97	1.046
3.45	0.490	6.07	1.051
3.62	0.560	6.20	1.056
3.84	0.630	6.35	1.061
4.11	0.700	6.55	1.066
4.16	0.712	6.79	1.071
4.20	0.724	7.09	1.076
4.25	0.736	7.49	1.081
4.29	0.748	8.06	1.086
4.34	0.760	8.97	1.098
4.39	0.772	9.34	1.110
4.43	0.784	9.56	1.122
4.48	0.796	10.14	1.192
4.52	0.808	10.40	1.262
4.57	0.820	10.56	1.332
4.61	0.832	10.68	1.402
4.66	0.844	10.78	1.472
4.71	0.856	10.85	1.542
4.75	0.868	10.92	1.612
4.80	0.880	10.97	1.682
4.85	0.892	11.02	1.752
4.90	0.904	11.06	1.822
4.95	0.916	11.09	1.892
5.01	0.928	11.13	1.962
5.07	0.940		
5.13	0.952		
5.19	0.964		
5.26	0.976		

First acid titration of Kauri NOM at 50 mmole/l KNO_3 at 46 mg C/l. HCl concentration equals 0.0924 mole/l.

pH	HCl (ml)	pH (cont.)	HCl (cont.) (ml)	pH (cont.)	HCl (cont.) (ml)
11.11	0.000	5.26	1.086	3.58	1.584
11.07	0.080	5.20	1.098	3.52	1.614
11.03	0.160	5.15	1.110	3.46	1.644
10.98	0.240	5.09	1.122	3.41	1.674
10.92	0.320	5.04	1.134	3.37	1.704
10.86	0.400	4.99	1.146	3.32	1.734
10.79	0.480	4.95	1.158	3.28	1.764
10.71	0.560	4.90	1.170	3.25	1.794
10.60	0.640	4.86	1.182	3.22	1.824
10.46	0.720	4.81	1.194	3.18	1.854
10.24	0.800	4.77	1.206	3.16	1.884
9.84	0.880	4.73	1.218	3.13	1.914
9.74	0.892	4.69	1.230	3.10	1.944
9.61	0.904	4.65	1.242	3.08	1.974
9.45	0.916	4.61	1.254	3.06	2.004
9.22	0.928	4.56	1.266	3.03	2.034
8.84	0.940	4.52	1.278	3.01	2.064
7.90	0.952	4.48	1.290	3.00	2.094
7.47	0.957	4.44	1.302	2.98	2.124
7.15	0.962	4.40	1.314	2.96	2.154
6.90	0.967	4.36	1.326	2.94	2.184
6.69	0.972	4.32	1.338	2.92	2.214
6.51	0.977	4.27	1.350	2.91	2.244
6.36	0.982	4.23	1.362	2.89	2.274
6.23	0.987	4.19	1.374		
6.12	0.992	4.15	1.386		
6.03	0.997	4.11	1.398		
5.95	1.002	4.07	1.410		
5.79	1.014	4.03	1.422		
5.66	1.026	3.99	1.434		
5.56	1.038	3.89	1.464		
5.47	1.050	3.80	1.494		
5.40	1.062	3.72	1.524		
5.33	1.074	3.65	1.554		

Second base titration of Kauri NOM at 50 mmole/l KNO_3 at 33 mg C/l. NaOH concentration equals 0.108 mole/l.

pH	NaOH (ml)	pH (cont.)	NaOH (cont.) (ml)
2.89	0.000	8.22	1.126
2.94	0.080	9.20	1.144
3.00	0.160	9.55	1.162
3.06	0.240	10.18	1.242
3.14	0.320	10.45	1.322
3.24	0.400	10.62	1.402
3.36	0.480	10.74	1.482
3.52	0.560	10.83	1.562
3.73	0.640	10.91	1.642
4.01	0.720	10.98	1.722
4.08	0.738	11.03	1.802
4.15	0.756	11.08	1.882
4.22	0.774	11.13	1.962
4.29	0.792		
4.36	0.810		
4.43	0.828		
4.49	0.846		
4.56	0.864		
4.63	0.882		
4.70	0.900		
4.77	0.918		
4.84	0.936		
4.91	0.954		
5.00	0.972		
5.08	0.990		
5.18	1.008		
5.28	1.026		
5.41	1.044		
5.57	1.062		
5.78	1.080		
6.14	1.098		
6.37	1.105		
6.71	1.112		
7.26	1.119		

Second acid titration of Kauri NOM at 50 mmole/l KNO$_3$ at 46 mg C/l. HCl concentration equals 0.0993 mole/l.

pH	HCl (ml)	pH (cont.)	HCl (cont.) (ml)
11.11	0.000	4.67	1.188
11.07	0.080	4.61	1.206
11.02	0.160	4.54	1.224
10.96	0.240	4.48	1.242
10.90	0.320	4.43	1.260
10.84	0.400	4.36	1.278
10.76	0.480	4.31	1.296
10.66	0.560	4.25	1.314
10.52	0.640	4.18	1.332
10.33	0.720	3.91	1.412
10.02	0.800	3.68	1.492
9.92	0.818	3.50	1.572
9.79	0.836	3.37	1.652
9.64	0.854	3.26	1.732
9.43	0.872	3.17	1.812
9.13	0.890	3.10	1.892
8.53	0.908	3.04	1.972
7.02	0.926	2.98	2.052
6.71	0.933	2.93	2.132
6.49	0.940	2.89	2.212
6.31	0.947		
6.16	0.954		
5.85	0.972		
5.64	0.990		
5.48	1.008		
5.36	1.026		
5.25	1.044		
5.16	1.062		
5.07	1.080		
5.00	1.098		
4.93	1.116		
4.86	1.134		
4.79	1.152		
4.73	1.170		

First base titration of Manuka NOM at 50 mmole/l KNO_3 at 55 mg C/l. NaOH concentration equals 0.104 mole/l.

pH	NaOH (ml)	pH (cont.)	NaOH (cont.) (ml)
2.91	0.000	9.52	1.038
2.96	0.080	10.07	1.118
3.02	0.160	10.37	1.198
3.08	0.240	10.56	1.278
3.16	0.320	10.69	1.358
3.26	0.400	10.80	1.438
3.38	0.480	10.89	1.518
3.54	0.560	10.96	1.598
3.76	0.640	11.02	1.678
4.14	0.720	11.08	1.758
4.25	0.738	11.12	1.838
4.40	0.756		
4.57	0.774		
4.77	0.792		
5.01	0.810		
5.28	0.828		
5.61	0.846		
6.02	0.864		
6.19	0.871		
6.35	0.878		
6.50	0.885		
6.65	0.892		
6.80	0.899		
6.95	0.906		
7.11	0.913		
7.25	0.920		
7.40	0.927		
7.57	0.934		
7.75	0.941		
7.94	0.948		
8.44	0.966		
8.83	0.984		
9.12	1.002		
9.34	1.020		

First acid titration of Manuka NOM at 50 mmole/l KNO_3 at 55 mg C/l. HCl concentration equals 0.0924 mole/l.

pH	HCl (ml)	pH (cont.)	HCl (cont.) (ml)
11.08	0.000	5.77	1.078
11.04	0.080	5.47	1.096
10.99	0.160	5.21	1.114
10.93	0.240	4.98	1.132
10.86	0.320	4.79	1.150
10.78	0.400	4.61	1.168
10.69	0.480	4.46	1.186
10.57	0.560	4.32	1.204
10.42	0.640	4.21	1.222
10.21	0.720	4.11	1.240
9.88	0.800	3.77	1.320
9.78	0.818	3.56	1.400
9.67	0.836	3.41	1.480
9.54	0.854	3.29	1.560
9.38	0.872	3.20	1.640
9.20	0.890	3.12	1.720
8.97	0.908	3.05	1.800
8.70	0.926	2.99	1.880
8.37	0.944	2.94	1.960
7.98	0.962	2.89	2.040
7.83	0.969		
7.68	0.976		
7.53	0.983		
7.38	0.990		
7.25	0.997		
7.12	1.004		
6.99	1.011		
6.86	1.018		
6.74	1.025		
6.61	1.032		
6.49	1.039		
6.36	1.046		
6.23	1.053		
6.10	1.060		

Second base titration of Manuka NOM at 50 mmole/l KNO_3 at 55 mg C/l. NaOH concentration equals 0.108 mole/l.

pH	NaOH (ml)	pH (cont.)	NaOH (cont.) (ml)
2.87	0.000	10.38	1.246
2.92	0.080	10.57	1.326
2.97	0.160	10.71	1.406
3.03	0.240	10.81	1.486
3.10	0.320	10.90	1.566
3.18	0.400	10.97	1.646
3.28	0.480	11.03	1.726
3.41	0.560	11.09	1.806
3.58	0.640	11.13	1.886
3.82	0.720		
4.25	0.800		
4.39	0.818		
4.56	0.836		
4.77	0.854		
5.01	0.872		
5.30	0.890		
5.67	0.908		
6.07	0.926		
6.23	0.933		
6.39	0.940		
6.59	0.947		
6.71	0.954		
6.83	0.961		
7.05	0.968		
7.22	0.975		
7.41	0.982		
7.60	0.989		
7.82	0.996		
8.37	1.014		
8.80	1.032		
9.12	1.050		
9.36	1.068		
9.54	1.086		
10.09	1.166		

Second acid titration of Manuka NOM at 50 mmole/l KNO_3 at 55 mg C/l. HCl concentration equals 0.0993 mole/l.

pH	HCl (ml)	pH (cont.)	HCl (cont.) (ml)
11.09	0.000	6.27	1.027
11.05	0.080	6.15	1.034
10.99	0.160	5.83	1.052
10.93	0.240	5.53	1.070
10.86	0.320	5.27	1.088
10.77	0.400	5.04	1.106
10.67	0.480	4.84	1.124
10.55	0.560	4.66	1.142
10.38	0.640	4.51	1.160
10.14	0.720	4.37	1.178
10.07	0.738	4.25	1.196
9.99	0.756	4.15	1.214
9.90	0.774	3.80	1.294
9.80	0.792	3.59	1.374
9.69	0.810	3.44	1.454
9.56	0.828	3.32	1.534
9.41	0.846	3.22	1.614
9.23	0.864	3.15	1.694
9.01	0.882	3.08	1.774
8.76	0.900	3.02	1.854
8.41	0.918	2.97	1.934
7.97	0.936	2.92	2.014
7.82	0.943	2.88	2.091
7.65	0.950		
7.49	0.957		
7.35	0.964		
7.21	0.971		
7.07	0.978		
6.88	0.985		
6.82	0.992		
6.70	0.999		
6.62	1.006		
6.49	1.013		
6.38	1.020		

Base titration of Red Beech NOM at 50 mmole/l KNO$_3$ at 70 mg C/l. NaOH concentration equals 0.104 mole/l.

pH	NaOH (ml)	pH (cont.)	NaOH (cont.) (ml)
2.97	0.000	9.16	0.987
3.03	0.080	9.29	1.005
3.10	0.160	9.41	1.023
3.18	0.240	9.51	1.041
3.29	0.320	9.60	1.059
3.42	0.400	9.95	1.139
3.59	0.480	10.21	1.219
3.85	0.560	10.41	1.299
4.23	0.640	10.57	1.379
4.33	0.658	10.69	1.459
4.44	0.676	10.79	1.539
4.56	0.694	10.87	1.619
4.68	0.712	10.94	1.699
4.82	0.730	11.00	1.779
4.97	0.748	11.05	1.859
5.13	0.766	11.09	1.939
5.33	0.784	11.14	2.019
5.58	0.802		
5.91	0.820		
6.06	0.827		
6.23	0.834		
6.40	0.841		
6.58	0.848		
6.76	0.855		
6.94	0.862		
7.11	0.869		
7.30	0.876		
7.49	0.883		
7.68	0.890		
7.87	0.897		
8.29	0.915		
8.60	0.933		
8.84	0.951		
9.01	0.969		

Acid titration of Red Beech NOM at 50 mmole/l KNO_3 at 70 mg C/l. HCl concentration equals 0.0924 mole/l.

pH	HCl (ml)	pH (cont.)	HCl (cont.) (ml)
11.08	0.000	7.34	1.239
11.04	0.080	7.19	1.246
10.99	0.160	7.05	1.253
10.94	0.240	6.91	1.260
10.88	0.320	6.77	1.267
10.82	0.400	6.63	1.274
10.74	0.480	6.50	1.281
10.65	0.560	6.36	1.288
10.55	0.640	6.23	1.295
10.42	0.720	6.11	1.302
10.27	0.800	5.80	1.320
10.07	0.880	5.55	1.338
10.01	0.898	5.35	1.356
9.96	0.916	5.17	1.374
9.90	0.934	5.02	1.392
9.84	0.952	4.89	1.410
9.78	0.970	4.77	1.428
9.71	0.988	4.65	1.446
9.64	1.006	4.55	1.464
9.56	1.024	4.44	1.482
9.49	1.042	4.35	1.500
9.40	1.060	4.26	1.518
9.31	1.078	4.17	1.536
9.21	1.096	3.84	1.616
9.09	1.114	3.61	1.696
8.96	1.132	3.44	1.776
8.81	1.150	3.31	1.856
8.62	1.168	3.21	1.936
8.39	1.186	3.13	2.016
8.09	1.204	3.06	2.096
7.96	1.211	3.00	2.176
7.81	1.218	2.94	2.256
7.66	1.225	2.89	2.336
7.50	1.232		

Base titration of Red Tussock NOM at 50 mmole/l KNO_3 at 60 mg C/l. NaOH concentration equals 0.104 mole/l.

pH	NaOH (ml)	pH (cont.)	NaOH (cont.) (ml)
2.89	0.000	8.85	1.071
2.93	0.080	8.91	1.089
2.99	0.160	8.96	1.107
3.06	0.240	9.01	1.125
3.14	0.320	9.06	1.143
3.24	0.400	9.11	1.161
3.36	0.480	9.15	1.179
3.52	0.560	9.19	1.197
3.74	0.640	9.23	1.215
4.11	0.720	9.26	1.233
4.22	0.738	9.30	1.251
4.36	0.756	9.33	1.269
4.50	0.774	9.37	1.287
4.68	0.792	9.40	1.305
4.90	0.810	9.43	1.323
5.17	0.828	9.47	1.341
5.53	0.846	9.50	1.359
6.02	0.864	9.63	1.439
6.24	0.871	9.76	1.519
6.47	0.878	9.90	1.599
6.71	0.885	10.03	1.679
6.95	0.892	10.16	1.759
7.18	0.899	10.30	1.839
7.40	0.906	10.43	1.919
7.59	0.913	10.55	1.999
7.75	0.920	10.66	2.079
7.89	0.927	10.76	2.159
8.13	0.945	10.84	2.239
8.30	0.963	10.91	2.319
8.43	0.981	10.97	2.399
8.54	0.999	11.03	2.479
8.64	1.017	11.01	2.559
8.72	1.035	11.01	2.639
8.79	1.053	11.01	2.719

Acid titration of Red Tussock NOM at 50 mmole/l KNO₃ at 60 mg C/l. HCl
concentration equals 0.0924 mole/l.

pH	HCl (ml)	pH (cont.)	HCl (cont.) (ml)	pH (cont.)	HCl (cont.) (ml)
11.00	0.000	9.44	1.232	7.12	1.734
10.95	0.080	9.41	1.250	6.93	1.741
10.89	0.160	9.38	1.268	6.73	1.748
10.83	0.240	9.35	1.286	6.53	1.755
10.75	0.320	9.32	1.304	6.33	1.762
10.67	0.400	9.29	1.322	6.14	1.769
10.57	0.480	9.26	1.340	5.70	1.787
10.47	0.560	9.22	1.358	5.35	1.805
10.35	0.640	9.19	1.376	5.08	1.823
10.23	0.720	9.15	1.394	4.85	1.841
10.10	0.800	9.11	1.412	4.67	1.859
10.08	0.818	9.08	1.430	4.50	1.877
10.05	0.836	9.04	1.448	4.36	1.895
10.02	0.854	9.00	1.466	4.24	1.913
9.99	0.872	8.95	1.484	4.13	1.931
9.96	0.890	8.91	1.502	3.79	2.011
9.94	0.908	8.86	1.520	3.56	2.091
9.91	0.926	8.81	1.538	3.41	2.171
9.88	0.944	8.75	1.556	3.29	2.251
9.85	0.962	8.69	1.574	3.19	2.331
9.83	0.980	8.63	1.592	3.12	2.411
9.80	0.998	8.55	1.610	3.05	2.491
9.77	1.016	8.47	1.628	3.01	2.571
9.75	1.034	8.36	1.646	3.01	2.651
9.72	1.052	8.24	1.664		
9.70	1.070	8.18	1.671		
9.67	1.088	8.12	1.678		
9.64	1.106	8.05	1.685		
9.61	1.124	7.97	1.692		
9.59	1.142	7.87	1.699		
9.56	1.160	7.76	1.706		
9.53	1.178	7.63	1.713		
9.50	1.196	7.48	1.720		
9.47	1.214	7.31	1.727		

Base titration of Sutton Stream water at 50 mmole/l KNO_3 at 5.7 mg C/l. NaOH concentration equals 0.104 mole/l.

pH	NaOH (ml)
2.95	0.000
3.00	0.080
3.07	0.160
3.16	0.240
3.27	0.320
3.42	0.400
3.65	0.480
4.10	0.560
4.23	0.572
4.40	0.584
4.65	0.596
5.17	0.608
6.31	0.620
6.68	0.625
7.02	0.630
7.36	0.635
7.80	0.640
8.28	0.645
8.91	0.657
9.24	0.669
9.45	0.681
9.61	0.693
10.17	0.773
10.43	0.853
10.60	0.933
10.71	1.013
10.81	1.093
10.88	1.173
10.95	1.253
11.00	1.333
11.05	1.413
11.09	1.493
MD	1.573

Acid titration of Sutton Stream water at 50 mmole/l KNO_3 at 5.7 mg C/l. HCl concentration equals 0.0924 mole/l.

pH	HCl (ml)	pH (cont.)	HCl (cont.) (ml)
11.12	0.000	3.59	1.250
11.09	0.080	3.51	1.280
11.05	0.160	3.45	1.310
11.00	0.240	3.39	1.340
10.95	0.320	3.34	1.370
10.90	0.400	3.29	1.400
10.83	0.480	3.25	1.430
10.76	0.560	3.21	1.460
10.66	0.640	3.18	1.490
10.55	0.720	3.15	1.520
10.40	0.800	3.12	1.550
10.18	0.880	3.09	1.580
9.80	0.960	3.06	1.610
9.71	0.972	3.04	1.640
9.60	0.984	3.02	1.670
9.47	0.996	2.99	1.700
9.31	1.008	2.97	1.730
9.10	1.020	2.95	1.760
8.77	1.032	2.93	1.790
8.08	1.044	2.91	1.820
7.21	1.056	2.90	1.850
6.92	1.061	2.89	1.880
6.63	1.066		
6.32	1.071		
5.91	1.076		
5.07	1.088		
4.66	1.100		
4.43	1.112		
4.26	1.124		
4.13	1.136		
4.04	1.148		
3.95	1.160		
3.80	1.190		
3.68	1.220		

Base titration of Suwannee River water at 50 mmole/l KNO_3 at 49 mg C/l. NaOH concentration equals 0.104 mole/l.

pH	NaOH (ml)	pH (cont.)	NaOH (cont.) (ml)
2.92	0.000	10.89	1.421
2.96	0.080	10.96	1.501
3.03	0.160	11.02	1.581
3.11	0.240	11.07	1.661
3.19	0.320	11.12	1.741
3.29	0.400	11.16	1.821
3.43	0.480	MD	1.901
3.61	0.560		
3.91	0.640		
4.51	0.720		
4.73	0.738		
5.00	0.756		
5.36	0.774		
5.79	0.792		
5.96	0.799		
6.15	0.806		
6.35	0.813		
6.54	0.820		
6.72	0.827		
6.90	0.834		
7.08	0.841		
7.25	0.848		
7.42	0.855		
7.61	0.862		
7.81	0.869		
8.42	0.887		
8.93	0.905		
9.25	0.923		
9.49	0.941		
10.09	1.021		
10.39	1.101		
10.57	1.181		
10.71	1.261		
10.81	1.341		

Acid titration of Suwannee River water at 50 mmole/l KNO_3 at 49 mg C/l. HCl concentration equals 0.0924 mole/l.

pH	HCl (ml)	pH (cont.)	HCl (cont.) (ml)
11.17	0.000	5.37	1.224
11.14	0.080	5.08	1.242
11.10	0.160	4.85	1.260
11.05	0.240	4.64	1.278
11.00	0.320	4.47	1.296
10.95	0.400	4.33	1.314
10.89	0.480	4.21	1.332
10.81	0.560	4.11	1.350
10.73	0.640	3.77	1.430
10.62	0.720	3.56	1.510
10.49	0.800	3.40	1.590
10.30	0.880	3.29	1.670
10.00	0.960	3.19	1.750
9.91	0.978	3.12	1.830
9.80	0.996	3.05	1.910
9.67	1.014	2.99	1.990
9.52	1.032	2.94	2.070
9.32	1.050	2.89	2.150
9.05	1.068		
8.65	1.086		
8.11	1.104		
7.90	1.111		
7.72	1.118		
7.54	1.125		
7.38	1.132		
7.22	1.139		
7.06	1.146		
6.89	1.153		
6.73	1.160		
6.57	1.167		
6.42	1.174		
6.26	1.181		
6.10	1.188		
5.69	1.206		

First acid titration of Swamp Cypress NOM at 50 mmole/l KNO_3 at 42 mg C/l. HCl concentration equals 0.0924 mole/l. Data for base titration of Swamp Cypress is missing.

pH	HCl (ml)	pH (cont.)	HCl (cont.) (ml)	pH (cont.)	HCl (cont.) (ml)	pH (cont.)	HCl (cont.) (ml)
10.88	0.000	9.45	1.046	8.25	1.636	3.30	2.243
10.82	0.080	9.42	1.064	8.21	1.643	3.21	2.323
10.74	0.160	9.39	1.082	8.16	1.650	3.13	2.403
10.66	0.240	9.37	1.100	8.11	1.657	3.06	2.483
10.56	0.320	9.34	1.118	8.06	1.664	3.01	2.563
10.45	0.400	9.32	1.136	7.99	1.671	3.01	2.643
10.33	0.480	9.29	1.154	7.93	1.678	3.01	2.723
10.20	0.560	9.27	1.172	7.85	1.685	3.01	2.803
10.19	0.578	9.24	1.190	7.77	1.692		
10.16	0.596	9.22	1.208	7.67	1.699		
10.14	0.614	9.19	1.226	7.57	1.706		
10.11	0.632	9.16	1.244	7.47	1.713		
10.08	0.650	9.14	1.262	7.36	1.720		
10.05	0.668	9.11	1.280	7.25	1.727		
10.01	0.686	9.08	1.298	7.14	1.734		
9.98	0.704	9.06	1.316	7.04	1.741		
9.95	0.722	9.03	1.334	6.93	1.748		
9.92	0.740	9.00	1.352	6.83	1.755		
9.89	0.758	8.97	1.370	6.73	1.762		
9.86	0.776	8.94	1.388	6.62	1.769		
9.83	0.794	8.91	1.406	6.51	1.776		
9.80	0.812	8.88	1.424	6.40	1.783		
9.77	0.830	8.84	1.442	6.28	1.790		
9.75	0.848	8.81	1.460	6.14	1.797		
9.72	0.866	8.77	1.478	5.73	1.815		
9.69	0.884	8.73	1.496	5.30	1.833		
9.66	0.902	8.68	1.514	4.97	1.851		
9.63	0.920	8.64	1.532	4.71	1.869		
9.61	0.938	8.59	1.550	4.50	1.887		
9.58	0.956	8.53	1.568	4.34	1.905		
9.55	0.974	8.47	1.586	4.20	1.923		
9.53	0.992	8.40	1.604	3.81	2.003		
9.50	1.010	8.31	1.622	3.58	2.083		
9.47	1.028	8.28	1.629	3.42	2.163		

Second, and only valid, base titration of Swamp Cypress NOM at 50 mmole/l KNO_3 at 42 mg C/l. NaOH concentration equals 0.108 mole/l.

pH	NaOH (ml)	pH (cont.)	NaOH (cont.) (ml)
2.89	0.000	8.81	1.056
2.94	0.080	8.86	1.074
3.00	0.160	8.91	1.092
3.06	0.240	8.95	1.110
3.15	0.320	9.00	1.128
3.25	0.400	9.04	1.146
3.37	0.480	9.08	1.164
3.54	0.560	9.11	1.182
3.80	0.640	9.15	1.200
4.32	0.720	9.18	1.218
4.52	0.738	9.22	1.236
4.78	0.756	9.25	1.254
5.16	0.774	9.28	1.272
5.71	0.792	9.31	1.290
6.25	0.810	9.35	1.308
6.48	0.817	9.37	1.326
6.56	0.824	9.40	1.344
6.69	0.831	9.43	1.362
6.82	0.838	9.46	1.380
6.89	0.845	9.49	1.398
7.09	0.852	9.52	1.416
7.23	0.859	9.65	1.496
7.37	0.866	9.78	1.576
7.50	0.873	9.91	1.656
7.63	0.880	10.06	1.736
7.75	0.887	10.21	1.816
7.86	0.894	10.36	1.896
8.08	0.912	10.50	1.976
8.23	0.930	10.62	2.056
8.36	0.948	10.72	2.136
8.46	0.966	10.81	2.216
8.55	0.984	10.87	2.296
8.62	1.002	10.93	2.376
8.69	1.020	10.98	2.456
8.75	1.038		

Second acid titration of Swamp Cypress NOM at 50 mmole/l KNO_3 at 42 mg C/l. HCl concentration equals 0.0993 mole/l.

pH	HCl (ml)	pH (cont.)	HCl (cont.) (ml)	pH (cont.)	HCl (cont.) (ml)
10.93	0.000	9.40	1.108	7.95	1.665
10.87	0.080	9.37	1.126	7.87	1.672
10.80	0.160	9.34	1.144	7.79	1.679
10.72	0.240	9.31	1.162	7.70	1.686
10.62	0.320	9.28	1.180	7.60	1.693
10.52	0.400	9.25	1.198	7.50	1.700
10.40	0.480	9.23	1.216	7.39	1.707
10.29	0.560	9.20	1.234	7.28	1.714
10.16	0.640	9.16	1.252	7.17	1.721
10.13	0.658	9.14	1.270	7.07	1.728
10.11	0.676	9.11	1.288	6.96	1.735
10.08	0.694	9.08	1.306	6.86	1.742
10.05	0.712	9.05	1.324	6.77	1.749
10.01	0.730	9.02	1.342	6.67	1.756
9.98	0.748	8.98	1.360	6.57	1.763
9.95	0.766	8.95	1.378	6.47	1.770
9.92	0.784	8.91	1.396	6.36	1.777
9.89	0.802	8.88	1.414	6.24	1.784
9.86	0.820	8.84	1.432	6.12	1.791
9.83	0.838	8.81	1.450	5.72	1.809
9.80	0.856	8.76	1.468	5.27	1.827
9.77	0.874	8.73	1.486	4.92	1.845
9.74	0.892	8.68	1.504	4.66	1.863
9.71	0.910	8.63	1.522	4.45	1.881
9.68	0.928	8.57	1.540	4.29	1.899
9.65	0.946	8.52	1.558	4.16	1.917
9.62	0.964	8.45	1.576	3.77	1.997
9.59	0.982	8.38	1.594	3.54	2.077
9.56	1.000	8.30	1.612	3.39	2.157
9.53	1.018	8.20	1.630	3.27	2.237
9.51	1.036	8.16	1.637	3.18	2.317
9.48	1.054	8.11	1.644	3.11	2.397
9.45	1.072	8.07	1.651	3.04	2.477
9.42	1.090	8.01	1.658		

Blank titration number 1 performed at 50 mmole/l KNO_3. Concentration of NaOH and HCl was 0.104 and 0.0924 mole/l, respectively.

Base Titration		Acid Titration			
pH	NaOH (ml)	pH		HCl (ml)	
2.89	0.000	11.00	3.25	0.000	0.984
2.91	0.050	10.97	3.18	0.050	1.034
2.94	0.100	10.92	3.13	0.100	1.084
2.98	0.150	10.88	3.08	0.150	1.134
3.02	0.200	10.83	3.04	0.200	1.184
3.07	0.250	10.78	3.00	0.250	1.234
3.12	0.300	10.71	2.96	0.300	1.284
3.17	0.350	10.64	2.93	0.350	1.334
3.24	0.400	10.55	2.88	0.400	1.384
3.32	0.450	10.44		0.450	
3.41	0.500	10.30		0.500	
3.53	0.550	10.09		0.550	
3.69	0.600	9.72		0.600	
3.96	0.650	9.66		0.605	
4.71	0.700	9.59		0.610	
4.93	0.705	9.51		0.615	
5.33	0.710	9.41		0.620	
6.35	0.715	9.30		0.625	
7.11	0.718	9.15		0.630	
8.06	0.721	8.93		0.635	
8.78	0.726	8.59		0.640	
9.10	0.731	7.64		0.645	
9.30	0.736	6.99		0.648	
9.44	0.741	6.33		0.651	
9.55	0.746	5.70		0.654	
10.10	0.796	5.12		0.659	
10.35	0.846	4.84		0.664	
10.50	0.896	4.66		0.669	
10.61	0.946	4.53		0.674	
10.70	0.996	4.43		0.679	
10.77	1.046	4.34		0.684	
10.84	1.096	3.89		0.734	
10.89	1.146	3.67		0.784	
10.94	1.196	3.52		0.834	
10.98	1.246	3.41		0.884	
11.01	1.296	3.32		0.934	

Blank titration number 2 performed at 50 mmole/l KNO$_3$. Concentration of NaOH and HCl was 0.104 and 0.0924 mole/l, respectively.

Base Titration				Acid Titration			
pH		NaOH (ml)		pH		HCl (ml)	
2.89	10.48	0.000	0.884	11.09	6.37	0.000	0.837
2.92	10.59	0.050	0.934	11.06	6.19	0.050	0.840
2.96	10.68	0.100	0.984	11.03	5.98	0.100	0.843
3.00	10.75	0.150	1.034	11.00	5.54	0.150	0.848
3.04	10.82	0.200	1.084	10.96	5.15	0.200	0.853
3.09	10.87	0.250	1.134	10.92	4.88	0.250	0.858
3.14	10.92	0.300	1.184	10.88	4.71	0.300	0.863
3.21	10.96	0.350	1.234	10.83	4.57	0.350	0.868
3.28	11.00	0.400	1.284	10.78	4.47	0.400	0.873
3.37	11.04	0.450	1.334	10.71	4.39	0.450	0.878
3.48	11.07	0.500	1.384	10.64	4.32	0.500	0.883
3.62	11.10	0.550	1.434	10.56	4.25	0.550	0.888
3.84		0.600		10.46	4.20	0.600	0.893
4.27		0.650		10.33	4.15	0.650	0.898
4.35		0.655		10.14	4.11	0.700	0.903
4.45		0.660		9.83	4.07	0.750	0.908
4.57		0.665		9.79	3.79	0.755	0.958
4.73		0.670		9.73	3.62	0.760	1.008
5.08		0.675		9.68	3.50	0.765	1.058
5.59		0.680		9.62	3.41	0.770	1.108
6.12		0.685		9.55	3.33	0.775	1.158
6.35		0.688		9.47	3.26	0.780	1.208
6.54		0.691		9.37	3.21	0.785	1.258
6.71		0.694		9.26	3.16	0.790	1.308
6.91		0.697		9.11	3.11	0.795	1.358
7.11		0.700		8.91	3.07	0.800	1.408
7.30		0.703		8.58	3.03	0.805	1.458
7.53		0.706		8.05	3.00	0.810	1.508
7.84		0.709		7.74	2.97	0.813	1.558
8.58		0.714		7.51	2.94	0.816	1.608
9.01		0.719		7.32	2.91	0.819	1.658
9.24		0.724		7.16		0.822	
9.41		0.729		7.01		0.825	
9.53		0.734		6.83		0.828	
10.08		0.784		6.68		0.831	
10.32		0.834		6.53		0.834	

Blank titration number 3 performed at 50 mmole/l KNO_3. Concentration of NaOH and HCl was 0.104 and 0.0924 mole/l, respectively.

Base Titration				Acid Titration			
pH		NaOH (ml)		pH		HCl (ml)	
2.93	10.78	0.000	0.943	11.01	3.46	0.000	0.851
2.96	10.84	0.050	0.993	10.98	3.36	0.050	0.901
3.00	10.89	0.100	1.043	10.94	3.28	0.100	0.951
3.04	10.94	0.150	1.093	10.90	3.22	0.150	1.001
3.09	10.98	0.200	1.143	10.85	3.16	0.200	1.051
3.15	11.02	0.250	1.193	10.79	3.11	0.250	1.101
3.21		0.300		10.73	3.06	0.300	1.151
3.29		0.350		10.66	3.02	0.350	1.201
3.38		0.400		10.57	2.98	0.400	1.251
3.50		0.450		10.46	2.95	0.450	1.301
3.66		0.500		10.32	2.92	0.500	1.351
3.91		0.550		10.11	2.89	0.550	1.401
3.95		0.555		9.72		0.600	
3.99		0.560		9.66		0.605	
4.02		0.565		9.58		0.610	
4.07		0.570		9.49		0.615	
4.11		0.575		9.37		0.620	
4.17		0.580		9.23		0.625	
4.23		0.585		9.04		0.630	
4.30		0.590		8.72		0.635	
4.40		0.595		7.73		0.640	
4.51		0.600		6.60		0.643	
4.67		0.605		5.80		0.646	
4.91		0.610		5.15		0.651	
5.40		0.615		4.87		0.656	
7.50		0.620		4.68		0.661	
8.43		0.623		4.54		0.666	
8.95		0.628		4.43		0.671	
9.22		0.633		4.35		0.676	
9.39		0.638		4.27		0.681	
9.52		0.643		4.21		0.686	
10.10		0.693		4.16		0.691	
10.34		0.743		4.11		0.696	
10.50		0.793		4.06		0.701	
10.61		0.843		3.76		0.751	
10.70		0.893		3.59		0.801	

Blank titration number 4 performed at 50 mmole/l KNO_3. Concentration of NaOH and HCl was 0.104 and 0.0924 mole/l, respectively.

Base Titration				Acid Titration			
pH		NaOH (ml)		pH		HCl (ml)	
2.86	10.84	0.000	1.076	11.01	4.72	0.000	0.669
2.89	10.89	0.050	1.126	10.98	4.57	0.050	0.674
2.93	10.94	0.100	1.176	10.94	4.46	0.100	0.679
2.96	10.99	0.150	1.226	10.90	4.37	0.150	0.684
3.00	11.03	0.200	1.276	10.84	4.29	0.200	0.689
3.05		0.250		10.79	4.23	0.250	0.694
3.10		0.300		10.73	4.18	0.300	0.699
3.16		0.350		10.65	4.13	0.350	0.704
3.23		0.400		10.56	4.08	0.400	0.709
3.31		0.450		10.45	3.78	0.450	0.759
3.41		0.500		10.31	3.61	0.500	0.809
3.54		0.550		10.10	3.48	0.550	0.859
3.72		0.600		10.07	3.38	0.555	0.909
4.03		0.650		10.04	3.30	0.560	0.959
4.08		0.655		10.00	3.24	0.565	1.009
4.14		0.660		9.97	3.18	0.570	1.059
4.20		0.665		9.93	3.13	0.575	1.109
4.27		0.670		9.90	3.09	0.580	1.159
4.36		0.675		9.85	3.05	0.585	1.209
4.46		0.680		9.81	3.01	0.590	1.259
4.61		0.685		9.76	2.98	0.595	1.309
4.82		0.690		9.71	2.94	0.600	1.359
5.21		0.695		9.65	2.92	0.605	1.409
6.22		0.700		9.58		0.610	
7.65		0.703		9.50		0.615	
8.50		0.706		9.42		0.620	
8.98		0.711		9.31		0.625	
9.23		0.716		9.17		0.630	
9.42		0.721		8.98		0.635	
9.54		0.726		8.68		0.640	
10.09		0.776		7.95		0.645	
10.34		0.826		7.11		0.648	
10.49		0.876		6.50		0.651	
10.61		0.926		5.97		0.654	
10.70		0.976		5.28		0.659	
10.77		1.026		4.93		0.664	

Blank titration number 5 performed at 50 mmole/l KNO₃. Concentration of NaOH and HCl was 0.108 and 0.0993 mole/l, respectively.

Base Titration		Acid Titration			
pH	NaOH (ml)	pH		HCl (ml)	
2.87	0.000	10.99	4.37	0.000	0.663
2.90	0.050	10.96	4.30	0.050	0.668
2.94	0.100	10.92	4.24	0.100	0.673
2.98	0.150	10.87	4.19	0.150	0.678
3.02	0.200	10.81	4.14	0.200	0.683
3.07	0.250	10.75	4.09	0.250	0.688
3.13	0.300	10.68	3.78	0.300	0.738
3.19	0.350	10.59	3.61	0.350	0.788
3.26	0.400	10.48	3.48	0.400	0.838
3.34	0.450	10.35	3.38	0.450	0.888
3.45	0.500	10.15	3.30	0.500	0.938
3.59	0.550	9.83	3.23	0.550	0.988
3.80	0.600	9.79	3.18	0.555	1.038
4.19	0.650	9.74	3.12	0.560	1.088
8.99	0.700	9.68	3.08	0.565	1.138
9.97	0.750	9.62	3.04	0.570	1.188
10.26	0.800	9.55	3.00	0.575	1.238
10.44	0.850	9.48	2.97	0.580	1.288
10.57	0.900	9.39	2.93	0.585	1.338
10.66	0.950	9.29	2.90	0.590	1.388
10.74	1.000	9.16		0.595	
10.81	1.050	9.00		0.600	
10.87	1.100	8.77		0.605	
10.92	1.150	8.38		0.610	
10.96	1.200	7.86		0.615	
		7.68		0.618	
		7.54		0.621	
		7.38		0.624	
		7.16		0.627	
		6.64		0.630	
		5.79		0.633	
		5.14		0.638	
		4.87		0.643	
		4.69		0.648	
		4.56		0.653	
		4.46		0.658	

Blank titration number 6 performed at 50 mmole/l KNO_3. Concentration of NaOH and HCl was 0.108 and 0.0993 mole/l, respectively.

Base Titration		Acid Titration			
pH	NaOH (ml)	pH		HCl (ml)	
2.90	0.000	11.00	8.72	0.000	0.610
2.93	0.050	11.00	7.32	0.005	0.620
2.97	0.100	11.00	4.23	0.010	0.670
3.01	0.150	11.00	4.13	0.015	0.680
3.05	0.200	10.99	4.05	0.020	0.690
3.10	0.250	10.99	3.76	0.025	0.740
3.15	0.300	10.98	3.59	0.030	0.790
3.21	0.350	10.98	3.47	0.035	0.840
3.28	0.400	10.97	3.38	0.040	0.890
3.37	0.450	10.97	3.30	0.045	0.940
3.47	0.500	10.96	3.23	0.050	0.990
3.61	0.550	10.96	3.18	0.055	1.040
3.82	0.600	10.95	3.13	0.060	1.090
4.21	0.650	10.95	3.08	0.065	1.140
9.11	0.700	10.94	3.04	0.070	1.190
9.99	0.750	10.94	3.01	0.075	1.240
10.28	0.800	10.93	2.97	0.080	1.290
10.45	0.850	10.93	2.94	0.085	1.340
10.58	0.900	10.92	2.91	0.090	1.390
10.68	0.950	10.92		0.095	
10.76	1.000	10.91		0.100	
10.82	1.050	10.91		0.105	
10.88	1.100	10.90		0.110	
10.93	1.150	10.85		0.160	
10.98	1.200	10.79		0.210	
		10.73		0.260	
		10.66		0.310	
		10.57		0.360	
		10.46		0.410	
		10.31		0.460	
		10.10		0.510	
		9.76		0.560	
		9.65		0.570	
		9.51		0.580	
		9.35		0.590	
		9.12		0.600	