

AN ABSTRACT OF THE THESIS OF

Ariel M. Mosbrucker for the degree of Master of Science in Environmental Engineering
presented on August 26, 2016

Title: Copper Speciation in Wastewater-Impacted Surface Waters

Abstract Approved:

Jeffrey A. Nason

This study investigates the relationship between total and ionic copper in samples taken from the Tualatin River and wastewater treatment facilities (WWTFs) that discharge into the Tualatin River. Copper speciation was analyzed by determining conditional stability constants and densities of copper binding ligands on the dissolved organic matter (DOM) in each sample using a two-ligand model. Differences in copper binding between wastewater and river water were identified, changes in copper binding following the mixing of WWTF effluent and receiving waters were observed, and the ability of the Biotic Ligand Model (BLM) to predict copper speciation in each sample was determined. WWTF effluent samples were found to bind copper more strongly than river water samples through a larger conditional stability constant for the strong binding site. At low dilution ratios, copper binding characteristics in waters downstream of WWTF outfalls deviated from those of upstream waters by approaching the WWTF effluent characteristics for copper binding. The BLM adequately predicted copper speciation in waters without a high percentage of WWTF effluent, but overpredicted ionic copper concentrations in effluent and downstream waters during low-flow by as much as 2-3 orders of magnitude. The differences observed highlight the need to account for WWTF DOM in the BLM framework.

©Copyright by Ariel M. Mosbrucker

August 26, 2016

All Rights Reserved

Copper Speciation in Wastewater-Impacted Surface Waters

by

Ariel M. Mosbrucker

A THESIS

submitted to

Oregon State University

in partial fulfillment of

the requirements for the

degree of

Master of Science

Presented August 26, 2016

Commencement June 2017

Master of Science thesis of Ariel M. Mosbrucker presented on August 26, 2016.

APPROVED:

Major Professor, representing Environmental Engineering

Head of the School of Chemical, Biological, and Environmental Engineering

Dean of the Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Ariel M. Mosbrucker, Author

ACKNOWLEDGEMENTS

I would like to express my sincere appreciation to my advisor, Jeffrey A. Nason, for his support and guidance. I would also like to acknowledge Clean Water Services for funding this research, providing water samples and background analysis, and offering editorial advice. In particular, I would like to thank Leila Barker of Clean Water Services for answering countless questions and for serving as a liaison for this project. I would also like to express my gratitude to the Environmental Engineering department for providing me with Teaching Assistantship positions which made this research possible. In addition, I add my thanks to Justine Feist for her assistance with titrations.

Furthermore, I would like to thank my family for their tireless support, the entire Nason lab group for keeping it light through extracurricular activities, and my friends inside and outside of the CBEE graduate program for their support, encouragement, and positive attitudes.

TABLE OF CONTENTS

| | <u>Page</u> |
|--|-------------|
| 1. Introduction..... | 1 |
| 1.1 Overview and Motivation | 1 |
| 1.2 Problem Statement | 3 |
| 1.3 Objectives..... | 4 |
| 1.4 Approach | 5 |
| 2. Background..... | 7 |
| 2.1 Copper Toxicity..... | 7 |
| 2.2 Copper Speciation and the Role of Dissolved Organic Matter | 8 |
| 2.3 Copper-Ligand Chemistry..... | 10 |
| 2.4 Regulating Copper in Waterways..... | 12 |
| 2.5 Wastewater Organic Matter | 14 |
| 2.6 The BLM and Wastewater Organics..... | 15 |
| 2.7 CuISEs | 17 |
| 2.8 The Influence of Cu:DOM Ratio | 18 |
| 2.9 Conclusion | 18 |
| 3. Manuscript..... | 20 |
| 3.1 Abstract | 21 |
| 3.2 Introduction..... | 21 |
| 3.3 Methods | 24 |
| 3.4 Results | 29 |
| 3.5 Discussion..... | 38 |

TABLE OF CONTENTS

| | <u>Page</u> |
|--|-------------|
| 3.6 Associated Content | 42 |
| 3.7 Acknowledgements | 43 |
| 3.8 Supplementary Information:..... | 43 |
| 4. Conclusions..... | 52 |
| Bibliography | 55 |
| APPENDICES | 60 |
| Appendix A – Method Verification..... | 61 |
| Appendix B – Willamette River Dilution Study | 62 |

LIST OF FIGURES

| <u>Figure</u> | <u>Page</u> |
|--|-------------|
| 1. An example of titration data plotted on a Scatchard plot. The non-linear nature | 12 |
| 2. An example of titration data plotted on a Langmuir plot. The non-linear nature | 12 |
| 3. Results from the Forest Grove site during the high-flow period. Binding..... | 31 |
| 4. Results from the Rock Creek site during the high-flow period (left) and the low- | 32 |
| 5. Results from the Durham site during the high-flow period (left) and the low- | 33 |
| 6. Comparison of ionic versus total copper concentrations (ppb) for the high-flow | 36 |
| 7. Comparison of ionic versus total copper concentrations (ppb) for the low-flow | 37 |
| 8. An example calibration curve for the CuISE in ion mode. | 44 |
| 9. An example experimental copper titration curve. The 1:1 line represents the | 44 |
| 10. An example of a log-transformed Scatchard plot used for determination of the.... | 45 |
| 11. An across-WWTF comparison for the high-flow period of binding strengths..... | 47 |
| 12. An across-WWTF comparison for the low-flow period of binding strengths..... | 48 |
| 13. A river-length comparison of high-flow results. Binding strengths are on top | 49 |
| 14. A river-length comparison of low-flow results. Binding strengths are on top | 50 |
| 15. An example of predicted versus observed copper speciation based on a 2 and | 51 |
| 16. A comparison of a 10% and a 60% humic acid assumption in the BLM for the | 52 |

LIST OF APPENDIX FIGURES

| <u>Figure</u> | <u>Page</u> |
|--|-------------|
| A1. Determined stability constants (left) and ligand densities (right) for Suwannee | 61 |
| B1. Results from the Willamette River dilution experiments. Determined binding | 63 |

1. Introduction

1.1 Overview and Motivation

The issue of copper pollution in the world's aquatic systems is a subject that has been researched and debated for many decades. It is well established that even low levels of ionic copper (Cu^{2+}) can impose negative effects upon fish and other aquatic organisms, including, but not limited to, a reduction in their sensitivity to chemosensory cues^{1,2}. It is also understood that certain species of fish are more susceptible to these effects than others. Many salmon species that are listed as threatened or endangered in the United States, including the coho (*Oncorhynchus kisutch*) and the chinook (*Oncorhynchus tshawytscha*), are among the species that are particularly sensitive to the presence of copper, while predators of juveniles of these species, such as the cutthroat trout (*Oncorhynchus clarki*) do not seem to be as negatively impacted by the presence of copper ions^{1,2}. This makes the issue of copper contamination a very significant concern in places like the Pacific Northwest, where conservation of endangered salmon species is a major subject of interest.

Copper can enter aquatic systems in a great variety of ways. Many algacides and fungicides contain high concentrations of copper, brake pads from cars release copper onto road ways, accelerated erosion of soils creates an excess of labile copper, and a vast variety of industrial and architectural sources result in the release of copper into the environment^{3,4}. Some of this copper enters aquatic systems directly when it gets washed off of surfaces during storm events, but the rest is directed to wastewater treatment facilities (WWTFs) where it gets combined with both household sources of copper and leached copper from copper piping. Because not all copper is removed during the treatment of wastewater, most of the copper that enters a WWTF is discharged with the treated effluent. Thus, releases from WWTFs end up contributing about 8% of all of the copper that enters aquatic systems³. Discharge regulations can be placed upon these WWTFs, as they are point sources, but not upon the runoff from storm events, as that copper cannot be linked to one specific, controllable point of

entrance. Thus, it is the WWTFs that are hit with strict copper release regulations once an aquatic system exceeds its maximum copper criteria.

Not all forms of copper are similarly toxic, however. Most of the copper that enters aquatic systems forms complexes with organic and inorganic species. While some of these species are still considered toxic to some extent, the cupric ion is generally considered the most toxic species⁵. Thus, the complexation of ionic copper significantly reduces the amount of copper that exists in the most bioavailable, ionic form. Complexes that form include copper hydroxides, copper sulfates, copper carbonates, and copper complexes that form with dissolved organic matter (DOM), which is a blanket term encompassing any carbonaceous organic material less than 0.45 μm in diameter. Because of the limitless number of sources for DOM, its composition varies greatly by both origin and geographic location⁶⁻⁸. As a result of this vast variability in structure, it is nearly impossible to define a universal quantitative relationship for how copper interacts with DOM. Thus, it is difficult to determine what fraction of total copper in an aquatic system exists as toxic ions.

Ionic copper concentrations are not easy to measure directly. Because analytical techniques for determining these concentrations are time-consuming, expensive, and very sensitive to contamination, it is impractical for regulatory agencies to monitor ionic copper by direct measurements. As such, models have been developed in attempt to predict ionic copper concentrations based upon total copper concentrations, so that appropriate regulatory constraints can be put into place on a per-system basis. Older regulations use a hardness-based equation to calculate the criterion maximum concentration for copper. This equation relies upon the observed relationship that acute toxicity to copper decreases as hardness increases⁹. This model, though simple and easy to use, fails to capture many of the important variations in water quality that can cause changes in ionic copper concentrations. As such, the Biotic Ligand Model (BLM) has been developed in attempt to more accurately reflect copper speciation and bioavailability in aquatic systems. This model is a combination of the Windermere Humic

Aqueous Model (WHAM), which models binding of copper to DOM, Chemical Equilibria in Soils and Solutions (CHESS) for inorganic speciation, and a biotic ligand interaction model, which predicts metal toxicity to aquatic organisms by modelling metal accumulation at the surface of a biotic ligand, such as a fish gill¹⁰. The model takes an input of 10 different water quality parameters and predicts how much total dissolved copper can be in a system before toxicity limits for ionic copper are reached. Although the EPA does not yet require the use of the BLM for setting copper regulations, it has been the recommended method for setting criteria since the EPA's 2003 Draft Update of Ambient Water Quality Criteria for Copper¹¹.

Although the BLM is clearly an improvement over the hardness-based model, the BLM could still use improvement in its ability to account for the variability in DOM. As it stands, the BLM is only designed for use with the default parameters provided with WHAM for describing copper-DOM interactions. These default values were derived from a compilation of values found in published literature, and have been shown to work adequately for aquatic systems consisting of DOM derived from natural environmental sources, such as decaying plant material and naturally existing bacteria¹²⁻¹⁴. However, the accuracy of the BLM as it stands is less certain in aquatic systems with significant inputs of DOM from non-natural sources, such as that which is discharged from a WWTF. Research on ionic copper complexation with DOM continues in hopes of identifying scientific gaps and implementing improvements to the BLM. Thus, with future adjustments, the BLM should be able to predict ionic copper concentrations in wastewater-impacted surface waters well enough that agencies can trust its predictions, so that achievable, scientifically sound regulatory limits can be established, and so that copper-safe habitats for aquatic organisms can be ensured.

1.2 Problem Statement

Despite the fact that previous studies have identified differences between natural DOM and WWTF DOM^{7,15,16}, very little has been done to quantify what changes occur to copper-DOM complexes after two sources containing different types of DOM are mixed,

as occurs when effluent from a WWTF is discharged into a surface water. Although it is possible that mixing does not cause changes in copper binding, there are many reasons to believe that it could. These reasons include, but are not limited to, differences in both water quality parameters and DOM characteristics between effluent, receiving, and post-mixing waters.

Limited previous research has investigated copper complexation in wastewater, but the topic, especially with regards to how copper complexation downstream of a WWTF outfall is impacted, remains largely unexplored¹⁶⁻¹⁹. As cities continue to grow and more wastewater is generated, the impact of discharged wastewater on aquatic systems will continue to increase. Because nearly all surface waters are now impacted in some way by anthropogenic influences, this aspect of copper complexation needs to be addressed if the BLM is to become the de facto model for copper regulations. However, we currently lack a good understanding of how DOM in WWTF effluent complexes copper, and what the implications are for copper complexation in surface waters that are highly impacted by effluent from WWTFs.

One surface water system that receives a particularly high contribution from WWTF effluent is the Tualatin River. The Tualatin River is a rain-fed river draining a portion of the coast range in northwestern Oregon, and, as such, exhibits drastically different discharge rates during different times of the year. Thus, while the WWTF effluent makes up little (~0-5%) of the river's total discharge during the high-flow winter and spring periods, the river's discharge can consist of upwards of 30% WWTF effluent during the low-flow summer months.

1.3 Objectives

We hypothesize that adding WWTF effluent to a surface water system alters the copper-DOM complexation chemistry in that environment. Thus, the study was conducted with the following goals in mind:

- 1) To compare copper complexation by natural DOM and DOM in WWTF effluent.

- 2) To investigate how copper-DOM complexation changes after WWTF effluent has been added to a surface water system.
- 3) To investigate how copper-DOM complexation changes along the course of a river system with repeated additions of WWTF effluent.
- 4) To determine how well the BLM predicts ionic copper concentrations throughout the river.
- 5) To develop strategies for incorporating significant findings into regulatory framework based upon the BLM.

1.4 Approach

To accomplish these objectives, the first necessary task was to develop a repeatable method for measuring ionic copper concentrations at environmentally-relevant (sub-ppb) levels. This was accomplished with the use of a cupric ion-selective electrode (CuISE) to directly measure cupric ion concentrations, and the assistance of similar methods described in previously published work²⁰⁻²³. The method was verified by conducting titrations with Suwannee River Fulvic Acid (SRFA), a well-characterized organic matter isolate obtained through the International Humic Substances Society. As ligand densities and conditional stability constants determined by other researchers for SRFA are readily available in published literature, the values obtained through the methods used by this research were able to be compared and validated to those obtained through similar methods^{7,8}.

Once a method had been established, samples were obtained from three different WWTFs that discharge to the Tualatin River, as well as from the river at locations upstream and downstream of those WWTF outfalls. Samples were obtained during both a high-flow and a low-flow period so that any effects that the WWTF effluent might have had on the downstream river samples could be compared at high and low dilution ratios, and so that samples could be compared at different times of year. These samples were titrated across a wide range of total copper concentrations under chemical

conditions that were as identical as possible to facilitate direct comparisons between samples.

Titration experiments were analyzed using a 2-ligand based Langmuir-type binding model to determine ligand densities and conditional stability constants for copper-DOM complexes in each of the different samples. River water titrations were repeated three times and WWTF effluent titrations were repeated five times to ensure that the natural variability in samples was captured. Statistical tests, including t-tests assuming equal or unequal variances, where appropriate, as well as single-factor ANOVAs, were used to determine where significant differences between samples existed.

Finally, the BLM was run for a wide range of total dissolved copper concentrations using site-specific water quality parameters, under the pH and alkalinity conditions maintained during laboratory experiments, for each of the samples. The predicted concentrations of ionic copper from the BLM for each site were compared to modeled ionic copper concentrations obtained by inputting experimentally determined parameters into the speciation program Visual MINTEQ.

The remainder of this thesis is divided as follows: Chapter 2 contains additional background information on copper toxicity and complexation, as well as a review of published literature pertinent to the topic of copper complexation, particularly in wastewater and systems affected by wastewater; Chapter 3 contains a manuscript where the methods, results, discussion, and conclusions that can be drawn from this study are detailed; finally, conclusions are summarized, and future work related to this topic is suggested, in Chapter 4.

2. Background

2.1 Copper Toxicity

While metals are naturally present in most systems at very dilute concentrations, the presence of metals has been considerably increased in many locales due to the impacts of anthropogenic activities. Many metals, such as copper, are considered trace nutrients at low levels but become harmful at more elevated concentrations³. Copper has been shown to cause adverse effects to organisms ranging from the small filter-feeding daphnia^{24,25} to large fish, such as the rainbow trout^{2,26}. Toxicity thresholds have been established for a range of these species, with 96-hour LC50s ranging from below 3 µg/L total copper for the Arctic grayling (*Thymallus arcticus*) to upwards of 6000 µg/L total copper for the American eel (*Anguilla rostrata*)²⁷. Perhaps the most important piece of information that can be gleaned from the plethora of toxicity information, however, is that different species have vastly different thresholds of tolerance to elevated copper concentrations – even within the same genus^{1,2,27}.

Copper has been observed to cause cell necrosis in several different fish species. In one study, cell necrosis in the olfactory bulb of several different *Oncorhynchus* species was observed at elevated concentrations of copper – 50 µg/L for *Oncorhynchus tshawytscha* and 200 µg/L for *Oncorhynchus mykiss* – at exposure times of 1 h². Because copper seems to affect the entire olfactory system, there is also some speculation of a reduction in imprinting and homing mechanisms in these species²⁸, which could result in difficulties in locating historic breeding sites. Mechanistically, copper has been shown to cause sub-lethal, degrading effects to fish by inhibiting the ability of sensory systems to perform their normal functions^{1,2,26,28,29}. The receptors affected include chemosensory receptors found on the olfactory bulb and mechanoreceptors found on the lateral line^{2,30}, both of which are used to help fish gather information about their surrounding environment.

In a normal situation, many non-predatory and juvenile fish exhibit behavioral responses to the presence of predators that make them less visible to those predators.

An example of this is the Schreckstoff alarm reaction system, in which the identification of chemicals given off by mechanical damage to a nearby friendly fish will inform the signal-receiving fish to exhibit predator avoidance behaviors, such as freezing, increased shelter use, and shoaling behaviors^{31,32}. The inhibition of the chemosensory receptors that are needed to detect these chemicals prevents the fish from acknowledging the existence of the alarm substances in the presence of copper. As a result, copper has been shown to reduce or eliminate such behavioral avoidance responses in some salmon species at concentrations of copper as low as 2-5 ppb^{28,29,33}. The reduction or elimination of these behavioral responses results in a lower survival rate for juvenile salmon, and therefore a reduced number of salmon surviving to adulthood.

In the Pacific Northwest region of the United States, the toxicity of copper to salmon species is of particular concern as many of these anadromous species breed in the Columbia and Snake River basins. Several of these threatened or endangered species, including the chinook, coho, and sockeye salmon^{1,2,34}, have shown particular sensitivity to elevated levels of copper, which means that copper contamination could be hindering salmon restoration efforts in the Pacific Northwest.

2.2 Copper Speciation and the Role of Dissolved Organic Matter

To protect aquatic organisms from the potential impacts of copper toxicity, a clear understanding of how copper behaves in natural systems is critical. Copper in its unbound, ionic form is the most bioavailable, and therefore toxic, form of copper²¹. When copper is bound to something else it is unavailable to bind to receptors on aquatic organisms, therefore reducing the toxic potential of the total copper in a system. This allows for much higher levels of copper in aquatic systems than would be safe if all forms of copper were similarly toxic^{22,26,27}. Other common copper complexes in aquatic systems include inorganic species, such as copper carbonates, copper hydroxides, and copper sulfates, as well as the complexes that are the focus of this study – copper that is complexed with DOM.

Water chemistry other than copper and DOM concentration are important not just for inorganic speciation, but also because some of these parameters can alter the availability of DOM to bind copper. Historically, hardness measurements have been used to predict ionic copper concentrations and toxicity based on total copper concentrations, with the idea that other divalent cations will compete with ionic copper for binding sites on organisms³⁵. However, Lu and Allen ran experiments to examine these competitive effects with respect to competition for binding sites on DOM, and noted that the extent of competition was lower than what a simple competition model would predict, and that the effect of competition was nonlinear, with a reduced response at higher Ca or Mg concentrations. They attributed this to the idea that, at low copper concentrations, ionic copper is more likely to bind to phenolic-type sites, while Ca and Mg are more likely to bind to the carboxylic-type sites, which would result in a decreased observable effect of competition³⁶. Elsewhere, there is evidence that the decreased binding of copper observed under conditions of high water hardness is due more to a decrease in solubility of fulvic acid than to direct competition for binding sites³.

Refocusing on copper-DOM complexation, it is important to understand that DOM is a blanket term for any carbonaceous material less than 0.45 μm in diameter. As such, it can be derived from any number of natural, altered, or synthetic materials, which results in a high chemical diversity of DOM in any given aquatic system. Thus, a wide array of potential copper binding sites, consisting of a wide array of functional groups, exist in any given aquatic system. Although the distribution of copper binding sites on DOM is almost certainly wide and continuous, DOM is frequently modeled as having a discrete number of sites, often two, with one class of ligands thought to represent carboxylic-type sites and the other meant to represent phenolic-type sites^{37,38}. Occasionally, a third, extra-strong class of ligands is needed to appropriately describe experimental data^{17,19}. Models that are much more sophisticated than these simple discrete models have been developed, with the most robust likely being WHAM.

WHAM includes the possibility for bidentate and tridentate sites, accounts for electrostatic interactions, and includes the possibility for a range of pKa values, among other considerations^{12,39,40}. It is the goal of this research to use the simplified, discrete model as a tool to identify differences between the copper complexing behavior of natural DOM and DOM in WWTF effluent. In addition, this research will compare the copper speciation predictions of WHAM through use of the BLM to modeled predictions based off of experimentally determined parameters that describe DOM-copper binding in each unique sample.

2.3 Copper-Ligand Chemistry

To extract the desired information from experimental results, data needs to be fitted to existing models. A Langmuir-type linearization of titration data is commonly employed in trace metal binding studies^{19,22,41}. Langmuir models assume a finite number of discrete binding sites, which results in parameters that can be compared in a scientifically meaningful way. Although many other models exist, few other non-empirical binding models exist. While empirical equations are useful for modeling purposes, values obtained from fitting data to these equations have little more use for comparison between samples than the ability to say which sample resulted in greater numerical value. On the other hand, a Langmuir-type model allows us to identify whether significant differences between samples are a result of the number or ligands or the binding strengths of the ligands present, and how the DOM in two different samples compares with respect to each of these parameters. To employ the Langmuir-type model, we follow common practice for metal-ligand binding and assume two discrete ligands. The concentrations of these ligands are denoted by $L_{1,T}$ and $L_{2,T}$, such that

$$L_{i,T} = L_i^{-1} + CuL_i^{+1} \quad i = 1, 2 \quad (1)$$

$$L_T = \sum_{i=1}^2 L_{i,T} \quad (2)$$

$$CuL_T = \sum_{i=1}^2 CuL_i^{+1} \quad (3)$$

where L_1^{-1} and L_2^{-1} , and are the concentrations of each of the unbound ligand types, and CuL_1^{+1} and CuL_2^{+1} are the concentrations of the respective ligand-copper complexes.

K_{CuL1} and K_{CuL2} represent conditional stability constants, also referred to as the binding strengths, for the CuL_1^{+1} and CuL_2^{+1} complexes, respectively, such that

$$Cu^{2+} + L_i^{-1} \leftrightarrow CuL_i^{+1}$$

$$K_{CuLi} = \frac{[CuL_i^{+1}]}{\{Cu^{2+}\}[L_i^{-1}]} \quad (4)$$

$$i = 1, 2$$

where square brackets denote concentration in mol/L and curly brackets denote activity. We can then employ the 2-ligand Langmuir-type model

$$[CuL_T] = \sum_{i=1}^2 \frac{[L_{i,T}]K_{CuLi}\{Cu^{2+}\}}{1 + K_{CuLi}\{Cu^{2+}\}} \quad (5)$$

to solve for our unknown values. The use of a two-ligand model as opposed to a one ligand model can be validated by both Scatchard and Langmuir plots. In a Scatchard plot the total concentration of bound metal divided by the ionic metal concentration ($\frac{[CuL_T]}{[Cu^{2+}]}$) is plotted against the bound metal concentration ($[CuL_T]$; Figure 1) while in a Langmuir plot the ionic metal concentration divided by the bound metal concentration ($\frac{[Cu^{2+}]}{[CuL_T]}$) is plotted against the ionic metal concentration ($[Cu^{2+}]$; Figure 2). In both cases a non-linear trend in plotted experimental data indicates the presence of at least two ligands. Stability constants and ligand densities for two distinct ligands can be estimated using the slopes of different portions of these overall nonlinear plots, but it is not possible to distinguish more than two ligands from these plots⁴¹.

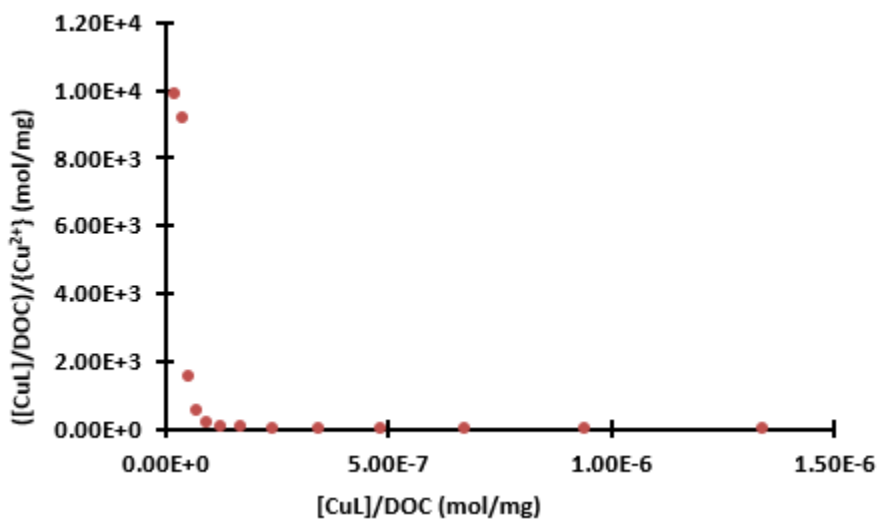


Figure 1: An example of titration data plotted on a Scatchard plot. The non-linear nature of this data reveals the presence of more than one ligand.

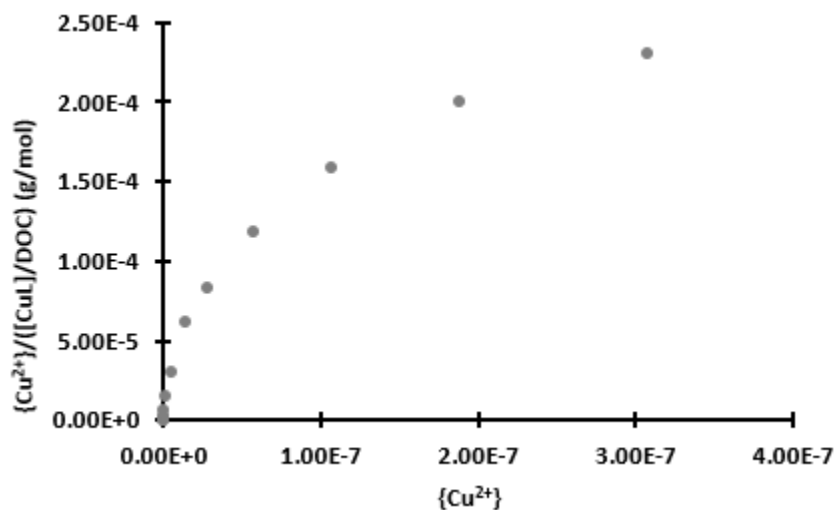


Figure 2: An example of titration data plotted on a Langmuir plot. The non-linear nature of this data reveals the presence of more than one ligand.

2.4 Regulating Copper in Waterways

The fact that copper is much more toxic in its ionic form than any of its complexed forms makes setting appropriate regulations for copper concentrations a difficult task. Because of the aforementioned variability in DOM, copper-DOM complexation has the potential to vary widely from region to region, river to river, and

even stretch to stretch of the same river system. Thus, it is not easy to predict how copper will bind to organic matter at a given location. Even if the speciation of copper can be predicted adequately through modeling, each water system still requires its own unique set of regulations to account for differences in that particular system's water quality parameters.

In many cases, this complex problem has been made feasible by the development of the BLM. To run the BLM, ten water quality parameters, many of which are already routinely analyzed by WWTFS, are needed: pH, dissolved organic carbon (DOC – a proxy for DOM), alkalinity, temperature, calcium, magnesium, sodium, potassium, sulfate, and chloride⁴². The version of the BLM being used in this study is the version available online from HydroQual, which is the version incorporating the model Chemical Equilibria in Soils and Solutions (CHESS) as well as WHAM. There is also a version of the BLM based off of MINEQL+, but that version has been determined to be the less sophisticated of the two⁵. The WHAM-based portion of the BLM assumes a 2-ligand model, with different parameter values for each of fulvic and humic acid fractions included in the program. Ligand densities for both fulvic and humic acids, as well as the stability constant of the weaker ligand for both DOM fractions, are fixed in the model, while the stability constant for the stronger ligand is calculated through an empirical equation based upon the stability constant of the weaker ligand. These values, along with the empirical equation, were determined by the creators of WHAM and based off of previously published data^{13,39}.

WHAM has been shown to predict ionic copper concentrations to within a factor of 6.3 if WHAM/Model V is used, and a factor of 3.6 if WHAM/Model VI is used in 95% of cases for natural freshwaters¹². The BLM was originally formulated with WHAM/Model V, and has not been updated to incorporate the more recent WHAM/Model VI or WHAM/Model VII versions that are now available. As copper toxicity values also have some error associated with them, these margins for error are likely acceptable for use in natural freshwaters. Thus, although the EPA has yet to require states to adopt a BLM-

based copper standard, many states are beginning to implement the use of the more sophisticated BLM-based standards in their water quality legislature.

2.5 Wastewater Organic Matter

In some aquatic systems, DOM from anthropogenic inputs makes up a significant portion of the total DOM. Because DOM is so heterogeneous, it is difficult to analyze with respect to functional groups and chemical composition, but what work has been completed on this subject does reveal some clear differences between DOM from natural and WWTF origins. Although the DOM sampled in this work was not chemically analyzed, it is informative to mention previous findings on this subject.

Ma et al. found that the DOM isolated from a WWTF effluent had almost no humic acid content, as opposed to a 13.5-28.7% humic acid content in the four natural water samples that were tested. They also found a high percentage of hydrophilic organic matter, which appeared to be made up of mostly simple aliphatic compounds⁷. The high percentage of hydrophilic organic matter in treated wastewater was supported by similar findings from Imai et al. and Pernet-coudrier et al.^{6,43}. Pernet-coudrier et al. also found a higher percent elemental composition of hydrogen, nitrogen, and sulphur, as well as a lower percentage of oxygen in WWTF effluent DOM as compared to river water. Also similar to Ma et al., Pernet-coudrier et al. found a lower aromatic content in treated wastewater as opposed to river water⁴³. These differences in chemical composition between natural and non-natural DOM support the possibility that differences in copper binding potential exist between the two differently sourced DOM categories.

In addition, strong synthetic copper chelating agents are often discharged in WWTF effluent along with DOM. The likely influence of these extra-strong ligands has been noted by many researchers, with the most commonly suggested being ethylenediaminetetraacetic acid (EDTA) and nitrilotriacetate acid (NTA)^{18,44-46}. Although these compounds are often only present in surface waters at nanomolar concentrations, because they form such strong complexes with copper they can serve as the dominant

binding ligands when copper concentrations are very low⁴⁴. Possible influences from other aminopolycarboxylates, sulfide, organophosphonates, and hydroxy carboxylates has also been suggested^{17,18,47}.

2.6 The BLM and Wastewater Organics

Although WHAM, and therefore the BLM, appears to adequately represent copper speciation in natural systems, it is unclear whether the BLM adequately accounts for systems that contain large amounts non-natural DOM, such as that in a WWTF effluent. As detailed previously, DOM coming from a WWTF is often quite chemically different from the organic matter that exists naturally in aquatic systems. This is likely due to the fact that DOM in WWTF effluent is often mainly derived from residual biological material that remains after the water is put through biological treatment processes, though it may also contain some recalcitrant material of anthropogenic origins. The ability of the BLM to model copper speciation in waters with a heavy anthropogenic influence is not well tested.

Previous research has indicated that DOM originating from wastewater effluent may bind copper and other metals more strongly than DOM derived from more natural origins. Sedlak et al. found that only 5-50% of copper in wastewater effluent was explained by the moderately strong complexes typical of humic substances and activated sludge biopolymers, while 5-60% of copper in the effluent was bound to a very strong second class of ligands with stability constants similar to those of synthetic chelating agents¹⁶. Sarathy and Allen looked at a variety of natural DOMs in addition to DOM from the effluent of a WWTF, and found that a 3 site model fit all of their samples best. In that study, the wastewater effluent had a 10-15 times greater concentration of the weakest ligand and a stronger stability constant for the strongest site. As a result, WHAM did a poor job of predicting ionic copper concentrations in the effluent, resulting in overprediction of ionic copper at low total copper concentrations and underprediction of ionic copper at high total copper concentrations¹⁷. Baken et al. found that waters with a large anthropogenic influence had a larger metal affinity than those

waters of more natural origin. This resulted in WHAM VI underestimating metal binding in waters with a strong anthropogenic influence by a factor of 3, on average, and by as great as a factor of 9¹⁸. On the other hand, Pernet-coudrier et al. found that treated wastewater didn't differ significantly from SRFA in its protective effect against copper toxicity to *Daphnia magna*⁴³.

To the author's knowledge, only one study has looked into how the addition of wastewater to a waterway affects copper speciation in receiving waters downstream of a WWTF outfall. Matar et al. investigated how copper binding compared amongst wastewater effluent and river water upstream and downstream of that WWTF's discharge site. They found that upstream and downstream waters contained two copper binding sites, while the wastewater effluent contained a third, very high affinity ligand, which accounted for more than 98% of copper binding in the wastewater effluent. They also found an increase in the molar concentration of both binding sites in the downstream water during the low-flow period as opposed to the upstream water or the downstream water during high-flow. The authors attributed this as being likely due to the added WWTF discharge in the downstream water¹⁹.

Thus, the limited previous research that has been conducted on this subject would lead one to believe that DOM from a WWTF does not bind copper the same way that DOM from natural origins does. Because the EPA has yet to set universal recommendation or requirements on how to implement the BLM⁴⁸, it is crucial to understand how sampling site selection could affect the accuracy and results of the model in aquatic systems that have the potential for large spatial variations, such as those which receive discharges from WWTFs. Although it seems evident that the BLM would need modification to be useful for copper speciation modeling within a WWTF discharge pipe, more research is required before the need for modifications to appropriately model waters downstream of WWTF outfalls can be appropriately assessed. It is the goal of this research to provide information that will shrink that gap in knowledge.

2.7 CuISEs

Few techniques are available for accurately measuring ionic copper concentrations at environmentally relevant levels. Among the available techniques are voltammetric titrations, competitive ligand exchange—solid-phase extraction (CLE-SPE), and CuISEs^{16,21,22,45,49}. For this work, the CuISE method was selected because it utilizes direct measurement of copper ions rather than relying on competitive exchange, thus reducing the potential for accumulation of error in measurements. The use of CuISEs also allows for measurement in whole-water samples, therefore facilitating the obtainment of experimentally determined parameters under conditions as similar as possible to the naturally observed conditions from which the water samples were obtained. This ability is important as much of the research on copper-DOM complexation has been performed by isolating DOM from water samples through harsh extraction processes, and then re-dissolving it in water that is free of other constituents^{7,43}. Although there is some evidence that these harsh processes do not seriously alter the characteristics of the DOM¹⁴, studying its ability to complex copper without having to subject it to these processes eliminates any potential confounding effects that could occur as a result of these processes.

Most CuISEs, including the one employed in this study (Orion 9629BNWP) are designed for use with much higher concentrations of copper than are present at environmentally relevant levels. According to the manual provided with the CuISE used in this study, the response of the CuISE begins to lose its linearity at copper concentrations below approximately pCu 6 (63.5 ppb), which is above the concentration that one would expect in natural waters, WWTF effluent, stormwater runoff, or other common discharges. Thus, direct calibration methods were determined to be insufficient for the purposes of this study. As such, a method that uses a metal chelating agent to ensure the accuracy of copper standards that bracket the concentrations observed and employed in this study was utilized. This method, which uses the chelating agent ethylenediamine (EN), has been used by many other researchers performing

similar work, and has been shown to result in a linear response of the CuISE to concentrations as low as pCu 19, which is well below the lowest measurement observed in this study of natural waters^{17,20,21,23}.

2.8 The Influence of Cu:DOM Ratio

It is also important to note that some studies have shown that the conditional stability constant and ligand density values determined from experimental data are not only dependent upon chemical conditions (pH, ionic strength, etc.), but also upon the Cu:DOM ratio. Craven et al. showed that as the Cu:DOM ratio increased, the conditional binding constants for the copper-DOM complexation reactions decreased over several orders of magnitude, meaning that the less copper that is present the more likely it is to be bound to DOM. This observation held true for results from each of voltammetry, CLE-SPE, and CuISEs⁴⁵. Thus, this ratio is important to keep in mind when attempting to directly compare values determined from different experimental samples, as this study does.

2.9 Conclusion

Protecting aquatic organisms from copper toxicity could play an important role in the recovery of several threatened and endangered species. Much effort has been put into understanding and developing proper models to determine appropriate regulations for copper concentrations. The speciation of copper in aquatic systems is fairly well predicted by the BLM in water bodies containing only natural DOM, but the model as it stands lacks the ability to account for the differences in chemical character observed between natural and non-natural DOM. Thus, the BLM may need to undergo modification before it can be used in systems with a large percentage of non-natural DOM. A few studies have looked into how copper binding between WWTF DOM compares to that of natural DOM, but even fewer have examined how copper binding changes once those two sources are mixed. Thus, more information on the effects that these mixing processes have on copper complexation is needed before appropriate and

scientifically accurate BLM-based regulations can be implemented in highly wastewater-impacted surface waters.

3. Manuscript

Copper Speciation in Wastewater-Impacted Surface Waters

Ariel Mosbrucker and Jeffrey A. Nason

Targeted for Submission to *Environmental Science & Technology*

3.1 Abstract

This study investigates the relationship between total and ionic copper in samples taken from the Tualatin River and wastewater treatment facilities (WWTFs) that discharge into the Tualatin River. Copper speciation was analyzed by determining conditional stability constants and densities of copper binding ligands on the dissolved organic matter (DOM) in each sample using a two-ligand model. We analyzed how copper binding in wastewater differs from that in river water, observed how binding changes once WWTF effluent is mixed with receiving waters, and determined how well the Biotic Ligand Model (BLM) predicts copper speciation in each sample. WWTF effluent samples were found to bind copper more strongly than river water samples through a larger conditional stability constant for the strong binding site. At low dilution ratios, binding characteristics in waters downstream of WWTF outfalls deviated from those of upstream waters by approaching the binding characteristics of the WWTF effluent. The BLM adequately predicted copper speciation in waters without a high percentage of WWTF effluent, but overpredicted ionic copper concentrations in effluent and downstream waters during low-flow by as much as 2-3 orders of magnitude. The differences observed highlight the need to account for WWTF DOM in the BLM framework.

3.2 Introduction

Copper can be harmful to aquatic organisms when present in its unbound, ionic form. Copper ions have been shown to diminish or eliminate the ability of chemosensory receptors in aquatic organisms to function properly^{1,2,26}. Many of the Pacific Northwest's threatened and endangered salmon species are particularly sensitive to impairment by copper, with some species showing sensitivity to copper at ion concentrations as low as 2-5 ppb, with no evidence of acclimation^{1,26,28,29,33}. Car brake pads, algacides, fungicides, leaching from copper pipes, and copper released as a result of soil erosion are just a few of the contributing sources to elevated levels of copper in the environment^{3,4}. As a result of the nature of many of these sources, a large portion

of the copper that enters a waterway enters through runoff, a non-point source discharge. Because it is difficult to regulate non-point source contributions, wastewater treatment facilities (WWTFs), one of the few large, point-source contributors of copper to aquatic systems, are likely to be subjected to total maximum daily load regulations (TMDLs) and effluent concentration limits if a system becomes listed as water quality impaired with respect to copper.

Copper that is bound to dissolved organic matter (DOM), or that is contained within inorganic complexes (excluding the first hydrolysis product), is much less bioavailable than ionic copper or CuOH^+ , making it much less of a concern from a toxicity standpoint^{22,26,27,39}. Due to the fact that DOM refers to any carbonaceous material less than 0.45 μm in diameter that exists in waterways, not all DOM has the same ability to form complexes with copper^{8,25,50}. This heterogeneity in the ability of DOM to bind copper makes it difficult to set regulations that both protect aquatic organisms, yet are fair and achievable for those facilities that must discharge wastewater.

The Biotic Ligand Model (BLM) was created for the purpose of determining appropriate instantaneous water quality criteria using site-specific water quality parameters. The BLM incorporates Chemical Equilibria in Soils and Solutions (CHESS), which is an inorganic speciation model, the Windermere Humic Aqueous Model (WHAM), which models binding of copper to DOM, and a biotic ligand interaction model, which predicts metal toxicity to aquatic organisms by modelling metal accumulation at the surface of a biotic ligand, such as a fish gill¹⁰. Repeated analysis of this model has shown that it does an adequate job of predicting copper speciation, and therefore providing appropriate toxicity limits, in most natural systems^{7,12,17,36,46}. However, limited past research has hinted that this model may not accurately predict the speciation of copper in wastewater or systems influenced strongly by wastewater discharges¹⁶⁻¹⁹.

With respect to wastewater, previous research has indicated that DOM originating from wastewater effluent may bind copper and other metals more strongly than DOM derived from natural origins. Sedlak et al. found that only 5-50% of copper binding in wastewater effluent was explained by the moderately strong complexes typical of humic substances and activated sludge biopolymers, with the rest being explained by strong ligands with stability constants similar to those of synthetic chelating agents¹⁶. Sarathy and Allen found that the wastewater effluent had a 10-15 times greater concentration of the weakest ligand and a stronger stability constant for the strongest site¹⁷. Baken et al. found that waters with a large anthropogenic influence had a larger metal affinity than waters of more natural origin, resulting in WHAM VI underestimating metal binding in waters with a strong anthropogenic influence by a factor of 3, on average, and by as great as a factor of 9¹⁸. In addition, Matar et al. found that waters upstream and downstream of a WWTF outfall contained two copper binding sites, while the wastewater effluent itself contained a third, very high affinity ligand, which accounted for more than 98% of copper binding in the wastewater effluent¹⁹. These findings call into question the suitability of using WHAM's default parameters for modeling copper speciation in WWTF effluent, and therefore point to the need for further research as to the BLM's accuracy at sites where wastewater makes up a large portion of total river discharge before the BLM is implemented as a regulatory model in such circumstances.

The Tualatin River, a rain-fed river draining a portion of the coast range in northwestern Oregon, has been listed as water quality impaired with respect to copper⁵¹. This river is a tributary of the Willamette River, which, in turn, is a major tributary of the Columbia River – making it part of a vital river system for many species of salmon. As such, elevated levels of copper in this particular waterway are a critical topic of concern. Oregon's current copper standards, which resulted in the listing of the Tualatin River as copper impaired, are based upon an outdated hardness-based model⁵². Oregon's copper standards are up for review, and, although a BLM-based model is not

yet explicitly required by the EPA, the possibility of switching to a standard based upon the BLM is imminent.

During the summer months, however, approximately 30 percent of the total discharge of the Tualatin River can be made up of wastewater effluent, which is a circumstance under which the BLM has yet to be tested. The work presented here assesses how binding of copper by DOM from WWTF effluent compares to that of DOM of natural origins, evaluates how contributions from wastewater alter copper binding in a flowing surface water downstream of several WWTF outfalls, and tests the accuracy of the BLM under these circumstances. In addition, possible approaches to assist in the implementation of the BLM as a regulatory tool under circumstances of high WWTF contribution to aquatic systems are presented.

3.3 Methods

3.3.1 Sample Collection and Analysis

Samples were collected at two different times of year – one during a high-flow period and one during a low-flow period – to capture the effects of WWTF effluent on copper binding at different dilution ratios. High-flow sampling was conducted on March 1 and 2, 2016, and low-flow sampling was conducted on June 13 and 14, 2016. Both sampling events were conducted using EPA-approved methods for BLM samples. For the high-flow period, water samples were obtained from the final effluent of three WWTFs that discharge into the Tualatin River, two of which provide advanced treatment processes (AWWTF, advanced processes include chemical clarification, filtration, and phosphorous removal): Forest Grove WWTF, Rock Creek AWWTF, and Durham AWWTF. Samples were also obtained from the Tualatin River upstream and downstream of each discharge site, with the downstream sample taken far enough downstream as to be beyond the mixing zone. Forest Grove WWTF is the most upstream of these three WWTFs, followed by Rock Creek AWWTF, making Durham AWWTF the most downstream of the three WWTFs. Because Forest Grove WWTF is not permitted to

discharge directly to the river when the river's flow is below 250 cfs (generally April-November), only samples from the upstream, downstream, and effluent sites associated with Rock Creek AWWTF and Durham AWWTF were analyzed for the low-flow period. After collection, samples were immediately filtered on-site through a 0.45 μm filter by use of a peristaltic pump. Samples were placed in acid-washed polyethylene containers and transported on ice to Oregon State University, whereupon samples were stored at 4°C for the duration of the study. The sample from downstream of Rock Creek was taken a few miles further downstream during the low-flow period than during the high-flow period, as the sampling location from the high-flow period was retrospectively deemed unsafe for sampling. No significant inputs to the river exist between these two locations.

Background concentrations of BLM parameters in all water samples were determined using EPA-approved analytical methods. Chloride and sulfate concentrations were determined via ion chromatography; calcium, hardness, potassium, magnesium, sodium, and both total and dissolved copper were determined via ICP-MS. Temperature and pH measurements were taken in the field.

3.3.2 Copper Titrations

Samples were analyzed for copper binding ability by titrating the collected samples with a standard solution of $\text{Cu}(\text{NO}_3)_2$ (Orion 942906 stock). Ionic copper concentrations were determined via cupric ion-selective electrode (CuISE, Orion 9629BNWP) using a method similar to those used in many previous studies^{17,20-23}. The CuISE was stored dry overnight and prior to each use the sensing surface was polished and the CuISE was subsequently soaked in a 10^{-8} M $\text{Cu}(\text{NO}_3)_2$, 0.025 M H_2SO_4 , and DDI water for 10 minutes each. All titrations were performed in acid-washed glassware (see Supporting Information [SI] for details).

The CuISE was calibrated in ion mode each day using an Accumet AR50 multimeter. The calibration solution consisted of $\text{Cu}(\text{NO}_3)_2$, ethylenediamine (EN), and NaNO_3 , such that the standards bracketed the range of expected cupric ion activity

values in the subsequent experiment as calculated by Visual MINTEQ 3.0⁵³. Equilibrium constants were taken to be the default values in the Visual MINTEQ database: $\log K = 10.5$ for $\text{Cu}^{2+} + \text{EN} \leftrightarrow \text{Cu}(\text{EN})$ and $\log K = 19.58$ for $\text{Cu}^{2+} + 2\text{EN} \leftrightarrow \text{Cu}(\text{EN})_2$. The solution was purged with water-saturated ultrapure N_2 gas (grade 5.0) for 30 minutes prior to recording the first measurement, and constant N_2 bubbling was maintained throughout all calibrations and titrations. Stabilization criteria for the CuISE were ± 0.1 mV/min, and with a minimum time of 5 minutes between recordings. Additional standard values (5 total) were reached by acidifying the original standard solution using ultrapure HNO_3 . After calibration and between titrations the CuISE was subsequently soaked in 0.025M H_2SO_4 and DDI water for 10 minutes. This calibration method produced a slope of -26.2 ± 0.70 and a y-intercept of 139.2 ± 5.07 (mean \pm SD, $n=47$). Although this slope was lower than the theoretical Nernstian response of 29.6 mV/pCu, it was consistent.

Experimental samples were also purged with water-saturated, ultrapure N_2 gas for 30 minutes prior to recording the initial reading, and were kept carbonate-free by constant bubbling throughout each titration. Titrations were performed at pH 6.00 ± 0.05 , with pH adjustments made using dilute, ultrapure HNO_3 and dilute, trace-grade NaOH. Ionic strength was adjusted to ~ 25 mM with NaNO_3 (Orion 940011 stock) and data points were recorded using a stabilization criteria of ± 0.1 mV/min and a minimum of 5 minutes for equilibration.

All titrations were performed in the dark to eliminate any possible interference from light. Measured ionic copper activities were converted to concentrations using a Davies activity coefficient. Effluent samples from the low-flow period were diluted 50% with DDI water prior to titration analysis due to their high complexation capacity and correspondingly slow equilibration times at full strength. River water samples were titrated in triplicate, and effluent samples in quintuplicate.

3.3.3 Determination of the “Experimental Window”

Experimental data points at both the upper and lower ends of each titration were eliminated to account for CuISE functionality and to ensure that the ratio of Cu to

DOC fell within a consistent range. In nearly all titrations, the measured $p[\text{Cu}^{2+}]$ remained essentially unchanged for the first several additions, indicating that the electrode was not sensitive enough to detect changes in $p[\text{Cu}^{2+}]$ at such low concentrations. When viewed on a log-transformed Scatchard plot, the first 1-4 points on each titration were found to deviate from expected trends (SI, Figure 8). On this basis, it was determined that the CuISE had a lower detection limit which differed slightly from day to day. Each daily limit was determined by plotting the data on a log-transformed Scatchard plot and excluding any points whose y-value was smaller than that of the following data point (see SI). In addition, any points whose mV reading were below that of the lowest calibration standard were not included in analysis. This resulted in a lower Cu to DOC ratio limit of between 1.1×10^{-3} and 3.4×10^{-3} on a mass basis.

An upper bound on experimental data was instituted to ensure that titration data were analyzed within a consistent range of Cu to DOC ratios, as it has been shown that the Cu to DOC ratio influences the determined values of the conditional stability constants and ligand densities⁴⁵. As such, the titration that ended at the lowest Cu to DOC ratio was identified and all points in other titrations that fell above this ratio were excluded from analysis. This upper bound was determined to be a Cu to DOC ratio of 0.11 on a mass basis, which resulted in an upper Cu to DOC ratio of between 7.0×10^{-2} and 1.1×10^{-1} on a mass basis. This upper range falls within the Cu:DOC ratio that was determined by Ahmed et al. to be accurately predicted by existing speciation models¹⁴.

3.3.4 Chemistry

Precedent has determined that most DOM can be modeled as having two discrete sites for copper binding, also known as ligands. These sites are generally thought to depict a carboxylic-type site (L_1) and a phenolic-type site (L_2), with the phenolic-type site having a stronger affinity for copper than the carboxylic-type site³⁸. It should be emphasized that the chemical makeup of these sites was not analyzed, and as such these site names are simply generalized labels for groupings of sites likely present

on this heterogeneous material. The concentrations of these ligands are denoted by $L_{1,T}$ and $L_{2,T}$, such that

$$L_{i,T} = L_i^{-1} + CuL_i^{+1} \quad i = 1, 2 \quad (1)$$

$$L_T = \sum_{i=1}^2 L_{i,T} \quad (2)$$

$$CuL_T = \sum_{i=1}^2 CuL_i^{+1} \quad (3)$$

where L_1^{-1} and L_2^{-1} , and are the concentrations of each of the unbound ligand types, and CuL_1^{+1} and CuL_2^{+1} are the concentrations of the respective ligand-copper complexes.

K_{CuL1} and K_{CuL2} represent conditional stability constants, also referred to as binding strengths, for the CuL_1^{+1} and CuL_2^{+1} complexes, respectively, such that

$$Cu^{2+} + L_i^{-1} \leftrightarrow CuL_i^{+1}$$

$$K_{CuLi} = \frac{[CuL_i^{+1}]}{\{Cu^{2+}\}[L_i^{-1}]} \quad (4)$$

$$i = 1, 2$$

where square brackets denote concentration in mol/L and curly brackets denote activity here and elsewhere.

3.3.5 Parameter determination

Titration data was analyzed using a 2-ligand Langmuir-type model

$$[CuL_T] = \sum_{i=1}^2 \frac{[L_{i,T}]K_{CuLi}\{Cu^{2+}\}}{1 + K_{CuLi}\{Cu^{2+}\}} \quad (5)$$

with parameters fitted using a sum of squared error (SSE) approach on log-transformed data via EXCEL's solver function.

A 2-ligand model was chosen for several reasons. As mentioned above, previous research has indicated that a 2-ligand model is often most appropriate in modeling Cu-DOM complexation. This is supported by the fact that the SSE of the two-ligand model was improved over that of a one ligand model by as much as 2 orders of magnitude. In

addition, plotting experimental data on Langmuir and Scatchard plots results in a non-linear trend, which suggests the presence of more than one ligand⁴¹. In addition, the fact that WHAM uses a 2-ligand model allowed for more direct comparison of experimental results with existing models.

Although a 3-ligand fit was possible in some cases, it was not possible for all samples. This, coupled with the fact that modeled results for both the 2- and 3-ligand approach did not differ greatly, resulted in the decision to describe all samples with a 2-ligand model (see SI).

3.3.6 Modeling

The method used to model copper speciation via experimentally determined parameters was the same as that presented by Craven et al⁴⁵. The speciation program Visual MINTEQ was used to model copper binding in each sample by creating a component for both the weak and strong ligand and changing their concentration and stability constants according to the experimentally determined parameters for each sample. The measured BLM-required parameters were input into the speciation program, with the laboratory conditions of pH 6.00, 25.0°C and no carbonate being input to assure that the conditions under which the conditional stability constants were measured were met. The default value of 10% humic acid was assumed for all BLM runs, as the actual percent humic acid in each sample was unknown. Including the presence of the ionic strength adjuster did not significantly alter the results of the models, so the additional sodium present in solution after ionic strength adjustment was not included in modeling. Predicted ionic copper concentrations determined by Visual MINTEQ were then compared to analogous predictions calculated using the Biotic Ligand Model, which was also run using the laboratory conditions.

3.4 Results

3.4.1 Comparing Effluent and River Water

Figure 3 illustrates the results from the Forest Grove site, which was the most upstream site, and was only analyzed during the high-flow period. A significant difference was found between the effluent and river water samples with respect to the binding strength of the stronger ligand, with that ligand-copper complex being stronger in the effluent. All other characteristics were similar between all three samples taken at this site.

Figure 4 illustrates the results from samples taken from the Rock Creek site during both the high- and low-flow periods. Rock Creek AWWTF discharges to the Tualatin River between Forest Grove WWTF and Durham AWWTF. Similar to the Forest Grove site, the only significant difference identified between effluent and river waters was in the binding strength of the stronger ligand, with that ligand-copper complex being stronger in the effluent sample. For the low-flow period, the binding strength of both ligands were significantly different between the effluent and both river water samples, with both ligand-copper complexes being stronger in the effluent.

Figure 5 illustrates the results from samples taken from the Durham site for both the high- and low-flow periods. Durham AWWTF discharges at the furthest downstream location of the three WWTFs. Similar to both of the other sites, the binding strength of the stronger ligand was significantly different from the river water samples for the high-flow period, with that ligand-copper complex being stronger in the effluent than in the river water. Here, the density of the weaker ligand was also significantly different between effluent and river water samples, with there being a higher density of the weaker ligand on the effluent DOM. For the low-flow period, the only significant difference that was consistent between the effluent and both river water samples was the density of the stronger ligand, with that ligand existing at a more dense concentration on the effluent DOM than the river water DOM.

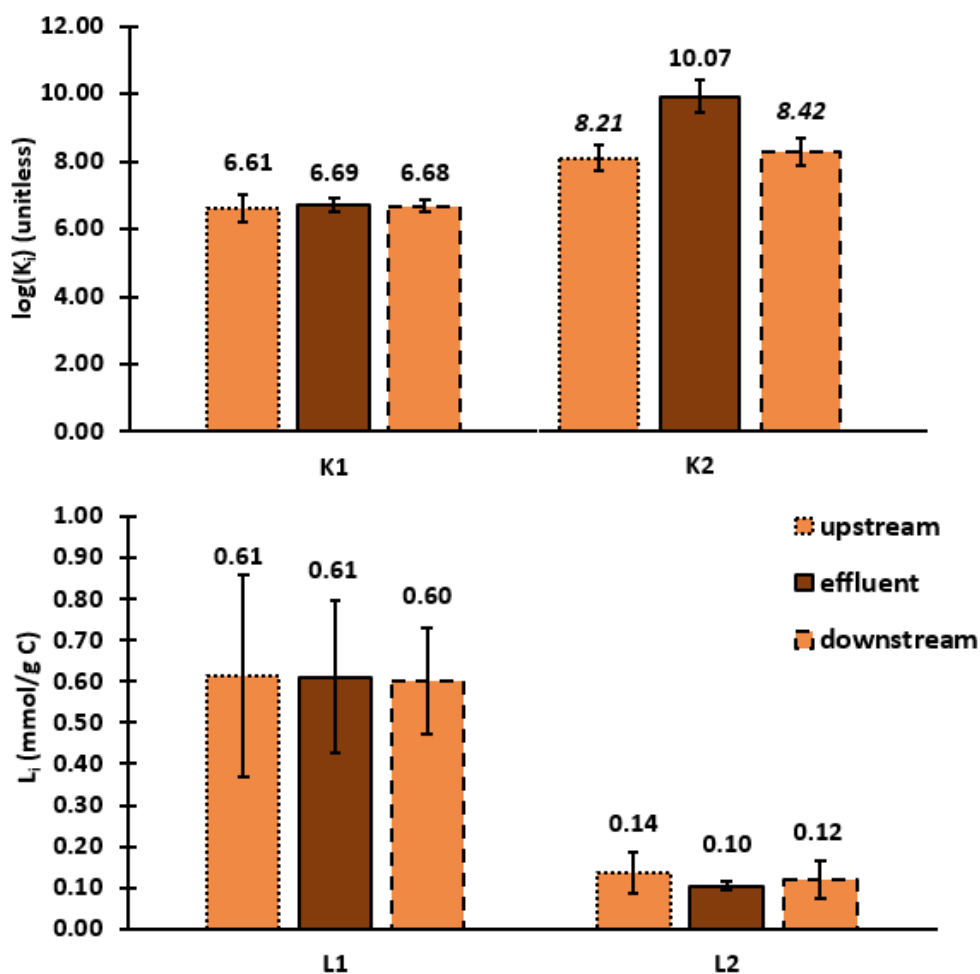


Figure 3: Results from the Forest Grove site during the high-flow period. Binding strengths are found on top and ligand densities on bottom. Data labels represent the mean of repeated titrations and error bars represent 95% confidence intervals. Italic data labels represent a significant difference from the associated effluent parameter, and underlined data labels indicate a downstream parameter that is significantly different from its associated upstream sample (two-tailed t-tests, $\alpha=0.05$).

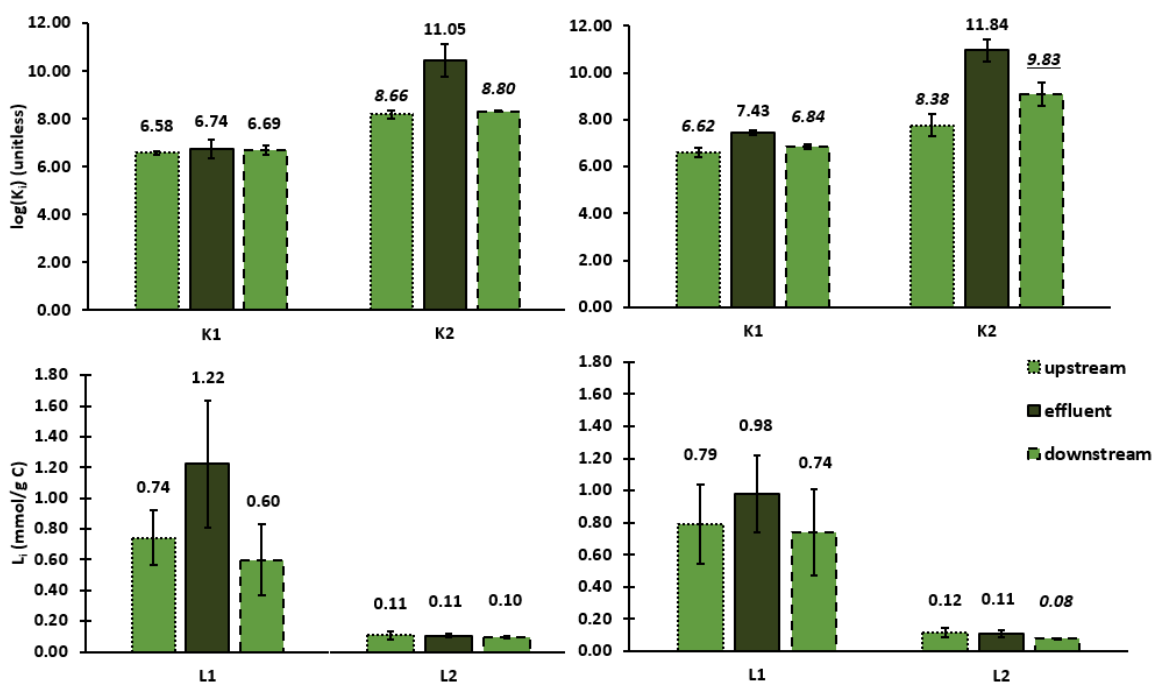


Figure 4: Results from the Rock Creek site during the high-flow period (left) and the low-flow period (right). Binding strengths are found on top and ligand densities on bottom. Data labels represent the mean of repeated titrations and error bars represent 95% confidence intervals. Italic data labels represent a significant difference from the associated effluent parameter, and underlined data labels indicate a downstream parameter that is significantly different from its associated upstream sample (two-tailed t-tests, $\alpha=0.05$).

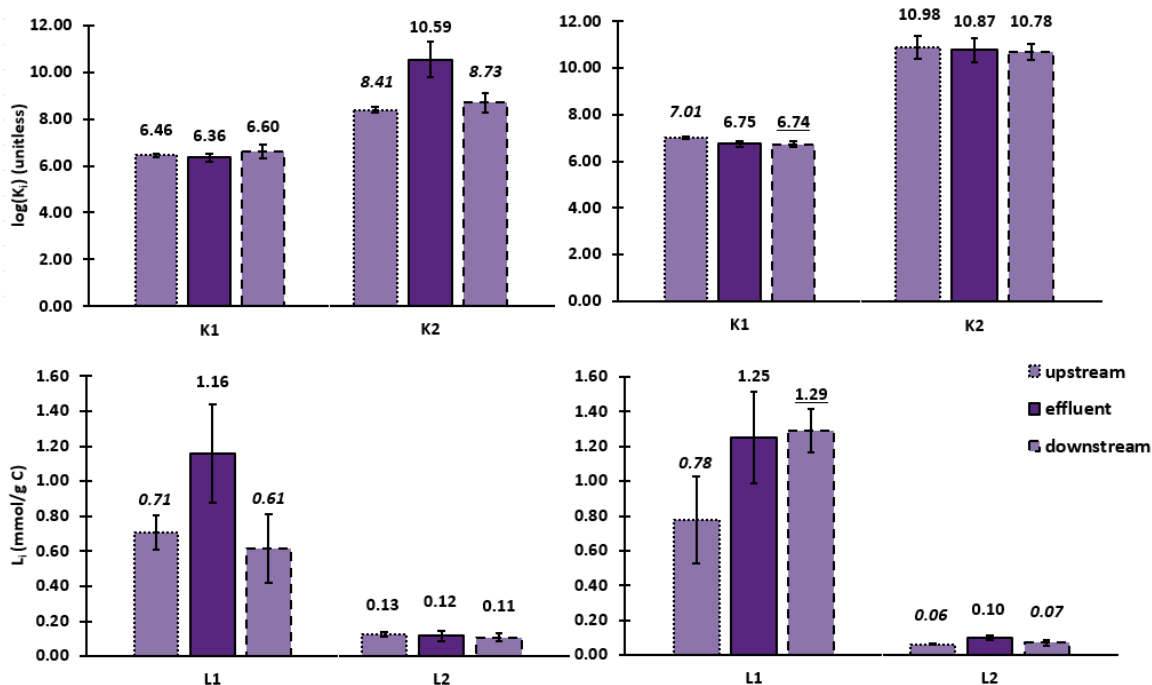


Figure 5: Results from the Durham site during the high-flow period (left) and the low-flow period (right). Binding strengths are found on top and ligand densities on bottom. Data labels represent the mean of repeated titrations and error bars represent 95% confidence intervals. Italic data labels represent a significant difference from the associated effluent parameter, and underlined data labels indicate a downstream parameter that is significantly different from its associated upstream sample (two-tailed t-tests, $\alpha=0.05$).

3.4.2 Comparing Upstream and Downstream Samples

In comparing upstream and downstream samples, no significant differences were found between any of the upstream and downstream sample pairs for the high-flow period (Figures 3, 4, 5). Indeed, a one-way ANOVA analysis performed on all six of the river water samples revealed that no significant differences existed along the length of the river with respect to copper binding.

For the low-flow period, a significant difference was identified between the upstream and downstream Rock Creek samples with respect to the binding strength of the stronger ligand. Significant differences were also identified between the upstream and downstream Durham samples with respect to both the binding strength and the density of the weaker ligand. These significant differences revealed a trend in which the

parameter values for the downstream samples became more similar to those of the respective effluent values. One-way ANOVA tests revealed significant differences with respect to all four of the copper binding parameters among the four river samples taken during the low-flow period: the binding strength of weaker site: ($\log(K_1)$: $F(3,8)=4.07$, $p=0.02$), the density of the weaker site: (L_1 : $F(3, 8)=4.07$, $p=0.03$), the binding strength of the stronger site ($\log(K_2)$: $F(3, 8)=4.07$, $p<0.01$), and the density of stronger site (L_2 : $F(3, 8)=4.07$, $p<0.01$, see SI).

3.4.3 High/Low Flow Comparison

No significant differences were identified at any of the four parameters between the most upstream sites during the two sampling periods (upstream of Forest Grove for the high-flow period, and upstream of Rock Creek for the low-flow period), or between the upstream of Rock Creek samples for the high and low flow periods.

The binding strength of the stronger site was significantly different for all of the other river water samples: downstream of Rock Creek ($\log(K_2)$: $t(4)=2.78$, $p=0.02$), upstream of Durham ($\log(K_2)$: $t(4)=2.78$, $p<0.01$), and downstream of Durham ($\log(K_2)$: $t(4)=2.78$, $p<0.01$). In addition, the density of the weaker ligand was significantly different between the two periods for the downstream of Rock Creek sample (L_1 : $t(4)=2.78$, $p=0.02$) and the downstream of Durham sample (L_1 : $t(4)=2.78$, $p=0.01$), while both the binding strength of the weaker ligand ($\log(K_1)$: $t(4)=2.78$, $p<0.01$) and the density of the stronger ligand (L_2 : $t(4)=2.78$, $p<0.01$) were also significantly different for the upstream of Durham site.

With respect to the effluent samples, the binding strength of the weaker site was the only significant difference identified between high and low flow periods for both Rock Creek AWWTF ($\log(K_1)$: $t(8)=2.31$, $p=0.01$) and Durham AWWTF ($\log(K_1)$: $t(8)=2.31$, $p=0.01$).

3.4.4 Modeling

As seen in Figure 6, both upstream and downstream river water samples taken during the high-flow period were predicted within a factor of 1-2.41 for values within the range of total dissolved copper measured in samples obtained for this study (0.737-3.97 $\mu\text{g/L}$, Table SI1). The upstream and downstream of Forest Grove samples, as well as the upstream samples from both Rock Creek and Durham were consistently underpredicted by the BLM with respect to ionic copper concentration within the modeled range, though generally by only a factor of 1-2. The downstream samples from both Rock Creek and Durham were slightly overpredicted with respect to ionic copper concentration at low total dissolved copper concentrations and underpredicted at higher total dissolved copper concentrations, with maximum overprediction being by a factor of 1.23, and the crossover occurring at approximately 5 ppb for both sites.

Ionic copper concentrations in effluent samples were consistently overpredicted at low total dissolved copper concentrations, and consistently underpredicted at high total dissolved copper concentrations. The error in prediction between the two models reached a maximum of 2.46 orders of magnitude, which occurred in the Rock Creek effluent sample. The transition from overprediction to underprediction occurred at approximately 30, 50, and 70 ppb for each of the Forest Grove, Rock Creek, and Durham effluent samples, respectively.

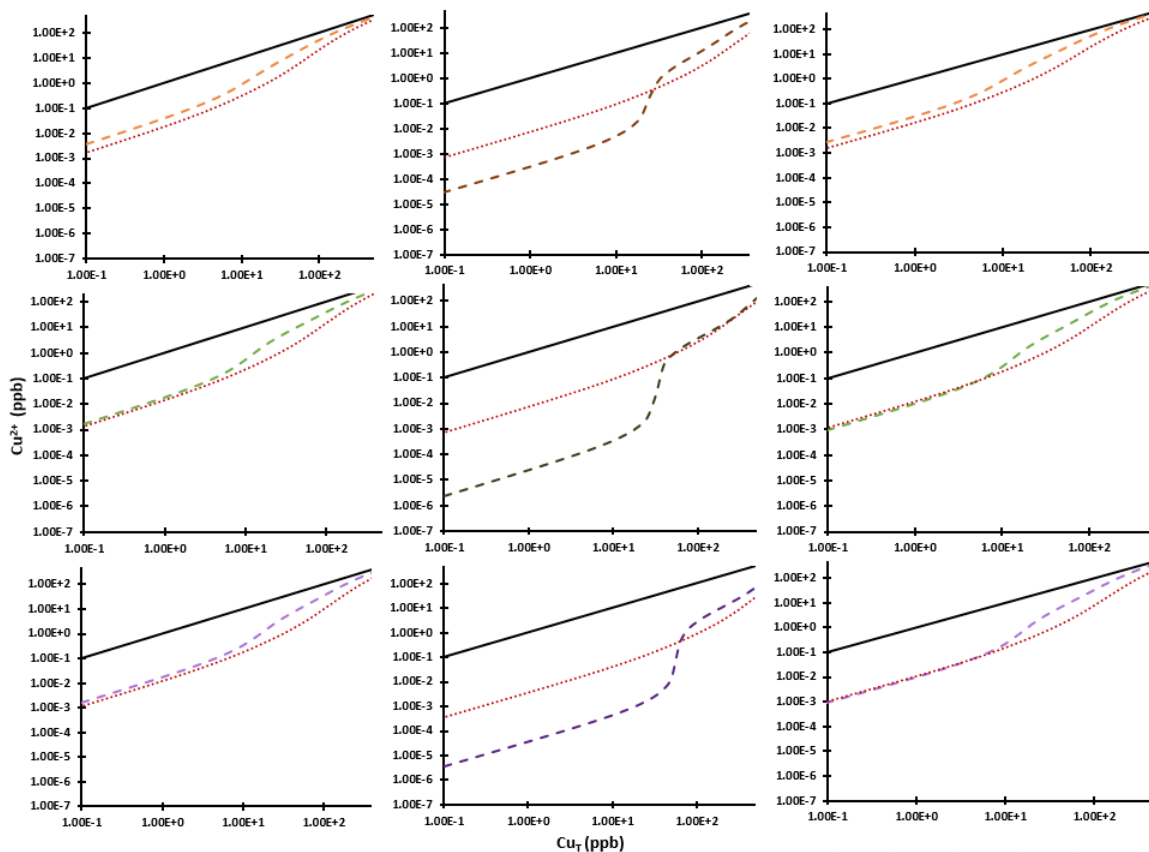


Figure 6: Comparison of ionic versus total copper concentrations (ppb) for the high-flow period at each of the Forest Grove (top), Rock Creek (middle) and Durham (bottom) sites. Model results from upstream samples are found on the left, effluent samples are in the middle, and downstream samples are on the right. Solid lines represent when $Cu_T = Cu^{2+}$, dashed lines represent values modeled using Visual MINTEQ and the experimentally determined 2-ligand parameters, and dotted lines represent predicted BLM speciation based upon site-specific water quality parameters.

Figure 7 details the modeling results from the low-flow period. As can be seen from this figure, the ionic copper concentrations in the sample taken upstream of Rock Creek were predicted with an error of a factor of 1.02-1.19. This sample was underpredicted for the entire range of copper modeled. The ionic copper concentration for the upstream of Durham sample was consistently overpredicted throughout the entire modeled range, with a maximum overprediction of 2.49 orders of magnitude occurring at the first modeled point, and a minimum overprediction of a factor of 1.19

at the final modeled point. The ionic copper concentrations in both the downstream samples were overpredicted at low total dissolved copper concentrations and overpredicted at higher total dissolved copper concentrations. The maximum overprediction at the downstream of Rock Creek site was by 1.07 orders of magnitude, and by 1.98 orders of magnitude at the downstream of Durham site. The maximum underpredictions were by a factor of 2.55 and 1.76, respectively. The Visual MINTEQ modeled values and the BLM predicted values intersected at total dissolved copper concentrations of approximately 15 and 18 ppb for these respective sites.

Ionic copper concentrations in effluent samples were consistently overpredicted below approximately 50 ppb in both samples. The maximum overprediction occurred for the Rock Creek effluent sample, and was by 3.33 orders of magnitude. The Visual MINTEQ model and the BLM predictions intersected at approximately 500 and 75 ppb for the Rock Creek and Durham effluent samples, respectively.

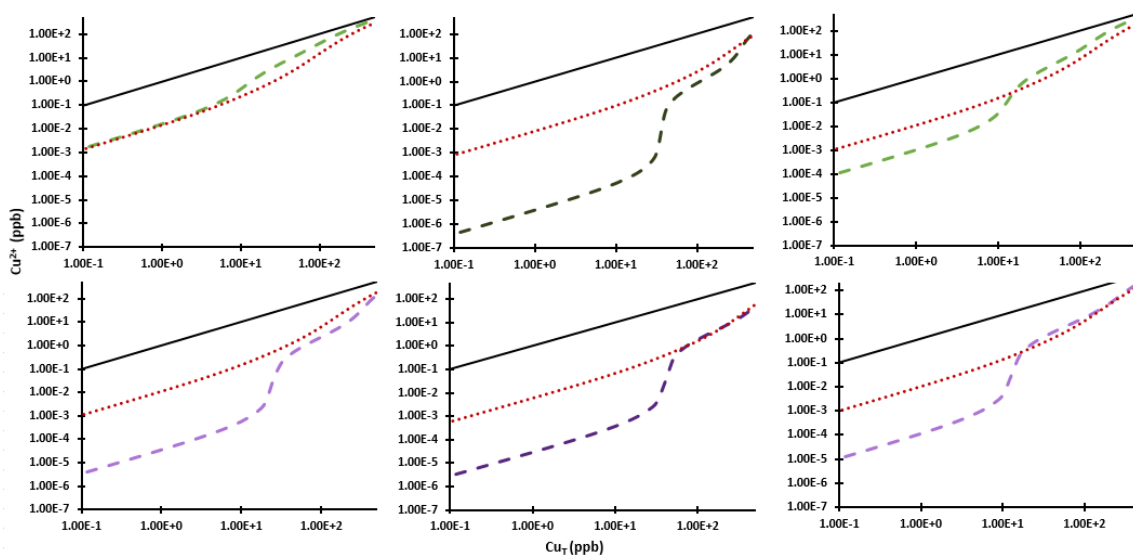


Figure 7: Comparison of ionic versus total copper concentrations (ppb) for the low-flow period at each of the Rock Creek (top) and Durham (bottom) sites. Modeled results from upstream samples are on the left, effluent samples are in the middle, and downstream samples are on the right. Solid lines represent when $Cu_T = Cu^{2+}$, dashed lines represent values modeled using Visual MINTEQ and the experimentally-determined 2-ligand parameters, and dotted lines represent predicted BLM speciation based upon site-specific water quality parameters.

3.5 Discussion

3.5.1 Sample Comparisons

Results clearly show that there are differences between copper binding in effluent and river waters. The most consistent of these differences was in the binding strength of the stronger ligand, with that ligand-copper complex being stronger at each of the sampled sites during both time periods, save for the high-flow Durham samples (Figures 3-5). This one exception is likely due to the fact that the low-flow upstream of Durham sample was already quite similar to the Durham effluent prior to effluent addition, which is likely due to the fact that Durham is the furthest downstream WWTF on the Tualatin River. Thus, this sample already has a fair amount of WWTF influence prior to the addition of Durham AWWTF effluent. These findings are consistent with findings from Sedlak et al., Sarathy and Allen, Baken et al., and Matar et al.¹⁶⁻¹⁹. In particular, our findings in relation to the mechanism for this strong binding agree with the findings of Sedlak et al. and Baken et al. in that strong binding results from ligands with a greater binding affinity for copper^{16,18}. Sarathy and Allen and Matar et al., in contrast, found that the cause was due to an increased concentration of copper binding ligands^{17,19}.

Although some differences arose elsewhere, the fact that the stronger ligand forms a ligand-copper complex that is several orders of magnitude stronger than that of the complex formed with the weaker ligand means that the weaker ligand will only play a very small role in the binding of copper in systems that are not subject to exceedingly high levels of copper. Thus, the small differences in the densities and binding strengths of the weaker ligand are likely to be unimportant at low copper concentrations, such as those seen in most natural systems, receiving waters, and WWTF effluents. Also, the fact that the stronger site was ubiquitously stronger in the effluent samples as

compared to the river water samples shows that most of the copper discharged with WWTF effluent will be entering the river as a strongly-bound complex.

The lack of difference in binding strengths and ligand densities between upstream and downstream samples for the high-flow collection period can be attributed to the high dilution factors related to WWTF effluent at the time of sampling. Effluent from Forest Grove WWTF, Rock Creek AWWTF, and Durham AWWTF made up only 1, 4, and 2%, respectively, of the total river discharge downstream of each respective site at the time of collection. Thus, because the dilution of the wastewater effluent entering the river at each location was so great, it is understandable that no significant differences were revealed (Figures 3-5). This is consistent with the results from highly diluted wastewater presented by Matar et al.¹⁹.

During the low-flow period, there were clear differences between the upstream and downstream samples at both of the sites. During this period the Rock Creek and Durham effluents made up 23% and 8%, respectively, of the total downstream river discharge at each of the respective sites. The binding strength of the stronger ligand clearly increased down the length of the river until it reached and thereafter maintained its highest value at the upstream of Durham site. This increase in copper binding is consistent with findings by Matar et al., although, as previously noted, the observed mechanism for the increased binding seen in these studies differs¹⁹.

It is unclear why there was such a large difference between the downstream of Rock Creek and the upstream of Durham samples as there are no other significant inputs to the river between these two sampling locations. However, these differences can likely be attributed to both spatial differences and the fact that these samples were taken on consecutive days, rather than both on the same day. Also, some stratification of the river leading to incomplete mixing at the downstream Rock Creek site cannot be ruled out. Due to the fact that this increase in the binding strength of the strong ligand complex was not seen during the high-flow sampling event, its presence during the low-flow period provides evidence for of the increased influence of wastewater during the

low-flow sampling period. Thus, it would seem that as dilution decreases, receiving waters downstream of discharge sites begin to take on the copper binding characteristics of WWTF effluent.

3.5.2 Modeling

The BLM does a good job of predicting copper speciation in river waters without a large, undiluted contribution of wastewater (Figures 6, 7). Although the concentration of ionic copper in these samples is slightly underpredicted in most cases, the error between speciation modeled with Visual MINTEQ and that predicted by the BLM is no more than a factor of 2.5. Because copper toxicity values, as well as the determined parameters used for modeling in this study, have some error associated with them, these margins for error in speciation are reasonable. Thus, all of the river water samples from the high-flow period, as well as the upstream of Rock Creek sample for the low-flow period, are reasonably well predicted by the BLM at relevant total dissolved copper concentrations. Thus, it appears that the BLM is capable of predicting copper speciation in both natural waters and waters that are only slightly impacted by wastewater discharge. The story is very different for the effluent samples and the highly wastewater-impacted samples, however.

In all cases, ionic copper concentrations in effluent samples were grossly overpredicted by the BLM at environmentally relevant total dissolved copper concentrations (Figures 6, 7). It is clear that the sample-specific water quality parameters that the BLM requires are not sufficient to account for differences in the copper-binding abilities of effluent water as opposed to river water. The more downstream samples from the low-flow period – downstream of Rock Creek, upstream of Durham, and downstream of Durham – resulted in errors similar in order of magnitude to those seen in the effluent modeling results.

The BLM's relatively accurate modeling of natural waters as well as its over-prediction of ionic copper in WWTF effluent observed in this research is consistent with modeling performed in other published research with WHAM – the DOM-copper

complexation model that is incorporated into the BLM. Both Sarathy and Allen and Baken et al. found that WHAM overpredicts the amount of ionic copper that will be in waters at low total copper concentrations in waters with a strong anthropogenic influence^{17,18}.

It is important to note that although it is possible that BLM predictions could be improved by the determination of actual percent humic acid values for each site instead of simply employing a 10 percent humic acid assumption, changing that humic acid assumption to 60 percent, which is the maximum value that the BLM is calibrated for, still results in errors that are on the scale of orders of magnitude. Thus, it appears that the BLM as it stands currently is unable to accurately model waters with large inputs of DOM from WWTF effluent. As a result, the BLM is in need of modification before it can be applied as a regulatory tool at such sites as these.

3.5.3 Strategies for BLM Improvement

Several strategies are possible to improve this BLM's ability to predict copper speciation in highly wastewater-impacted waters. First, because all three WWTF effluents sampled had DOM with similar copper binding characteristics for both time periods, a possible solution would be to add a percent wastewater parameter to the BLM. This parameter might work much like the existing percent humic acid parameter in that the user could input a known dilution ratio, resulting in the model changing its assumptions to include stronger binding constants typical of these highly wastewater-impacted sites.

Because this would require modification of the entire model, another, less invasive solution might be to use the percent humic acid function as a tool for percent wastewater. In doing this, the WHAM database would need to be modified so that the fulvic acid parameters are reflective of natural DOM, and the humic acid parameters are reflective of effluent DOM. Then, one might be able to input percent wastewater instead of percent humic acid, and as a result be able to obtain speciation predictions that better reflect copper binding characteristics at these downstream sites.

Another solution is to allow for user modification of ligand parameters based upon experimental results such as those presented in this study. The trouble with that, however, is that most public works facilities that are in charge of WWTF effluent quality do not have these values, or the means necessary to obtain them. Also, without these parameters a mass-balance approach for copper binding downstream of a WWTF outfall would not be useful, as it is clear from the Visual MINTEQ modeling and BLM prediction comparisons presented in this study that incorporating accurate site-specific water quality parameters, but not accounting for changes in copper binding ability between natural and non-natural DOM, is not sufficient to accurately model copper speciation in highly wastewater-impacted surface waters.

Finally, because the BLM still uses WHAM/Model V, it is possible that the BLM could be improved by updating the version of WHAM used to WHAM/Model VI, which has been shown to better model copper binding to DOM¹², or the even more recently released WHAM/Model VII.

Overall, this study illustrates the importance of considering wastewater discharge when determining appropriate water quality regulations with respect to copper, and demonstrates a need for modification to the BLM before it can be implemented as a regulatory tool in locations where WWTF effluent contributes largely to a river's total discharge.

3.6 Associated Content

Supporting information associated with this work is available. This supporting information contains information on labware and cleaning procedures, graphical examples of a calibration, a titration curve, and a log-transformed Scatchard plot for experimental window determination. Also available are a table of BLM parameters for each sample, graphs comparing results across WWTFs and down the length of the river, as well as a graphical example of both the difference in fit between a 2- and a 3-ligand model and a 10% versus 60% humic acid assumption.

3.7 Acknowledgements

The authors would like to sincerely thank Clean Water Services for funding this research, as well as for providing water samples, conducting background testing, and providing editorial assistance.

3.8 Supplementary Information:

3.8.1 Labware and Cleaning Procedures

Where possible, plastic containers were used to avoid adsorption of copper ions to container walls. Glassware was used where plastic was unavailable. All glass and plasticware was washed and rinsed thoroughly with Alconox soap and water. Labware was then placed in a 10% HNO₃ bath for a minimum of 4 hours, rinsed at least three times with distilled, deionized water (DDI, 18.2 MΩ resistance), and placed in a DDI water bath for a minimum of 30 min. After removal from the DDI water bath, labware was again rinsed at least three times with DDI water and then left to dry upside down on a drying rack. Once dry, labware was covered with parafilm or capped and stored in a closed cabinet until use.

3.8.2 Method Support

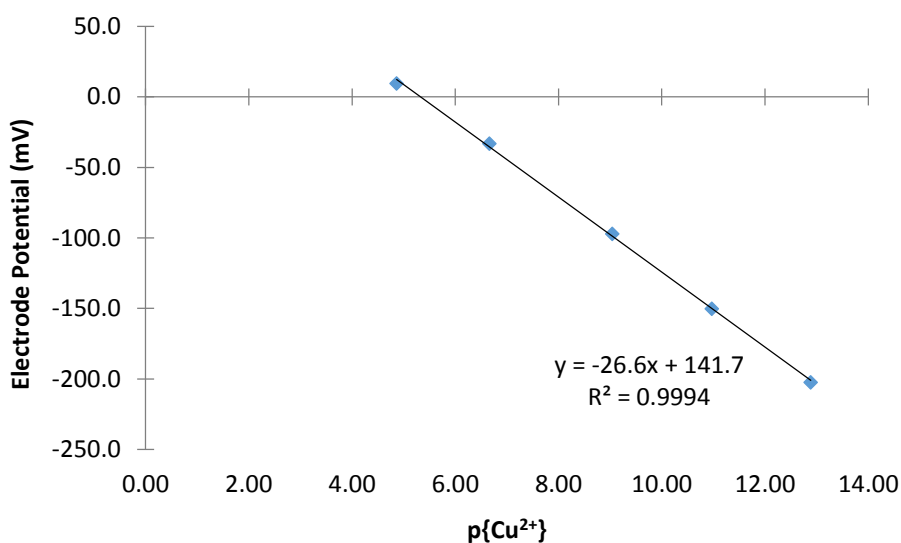


Figure 8: An example calibration curve for the CuISE in ion mode.

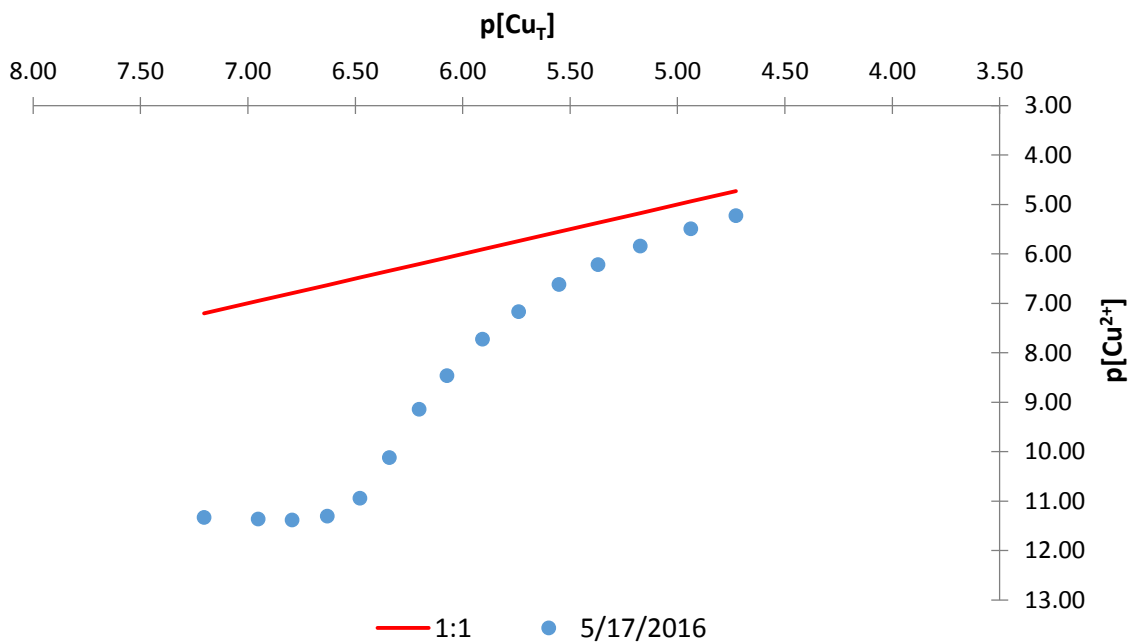


Figure 9: An example experimental copper titration curve. The 1:1 line represents the theoretical value of p{Cu²⁺} if all of the copper in the sample was in its ionic form.

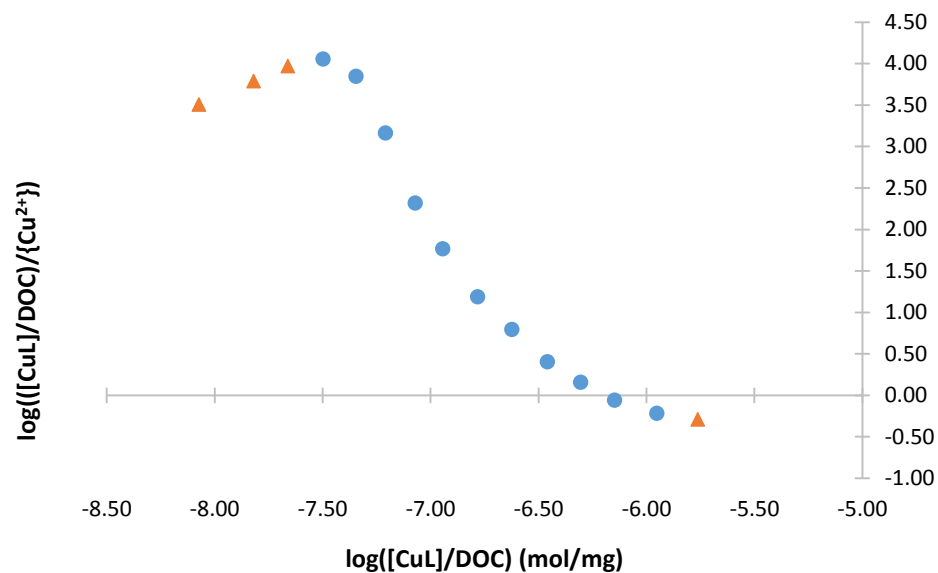


Figure 10: An example of a log-transformed Scatchard plot used for determination of the lower truncation point. Points included in analysis are shown as circles, and those truncated from both the lower and upper end of the titration are shown as triangles.

3.8.3 Water Quality Information

Table 1: Water quality parameters for sampled water titrated in this study.

| Sample Date | Sample ID | Alkalinity (mg/L) | Cl (mg/L) | SO4 (mg/L) | Ca (mg/L) | K (mg/L) | Mg (mg/L) | Na (mg/L) | Dissolved Cu (µg/L) | DOC (mg/L) | Temperature (°C) | pH |
|-------------|-------------------------|-------------------|-----------|------------|-----------|----------|-----------|-----------|---------------------|------------|------------------|------|
| 3/1/2016 | Forest Grove upstream | 29.0 | 3.32 | 2.93 | 7.07 | 0.572 | 2.54 | 4.28 | 0.792 | 1.16 | 8.0 | 7.01 |
| 3/1/2016 | Forest Grove effluent | 78.8 | 15.4 | 45.2 | 19.4 | 10.9 | 5.90 | 29.2 | 2.79 | 3.80 | 13.4 | 6.80 |
| 3/1/2016 | Forest Grove downstream | 29.7 | 3.45 | 2.91 | 7.22 | 0.574 | 2.53 | 4.37 | 0.737 | 1.16 | 8.1 | 7.13 |
| 3/1/2016 | Rock Creek upstream | 33.2 | 4.19 | 4.10 | 8.54 | 0.948 | 3.00 | 4.96 | 0.764 | 1.52 | 8.8 | 7.04 |
| 3/1/2016 | Rock Creek effluent | 123 | 35.5 | 123 | 21.6 | 12.5 | 6.74 | 80.2 | 2.73 | 4.67 | 16.5 | 7.16 |
| 3/1/2016 | Rock Creek downstream | 40.9 | 5.84 | 9.11 | 10.3 | 1.48 | 3.51 | 8.05 | 0.953 | 1.88 | 9.4 | 7.09 |
| 3/2/2016 | Durham upstream | 40.3 | 5.88 | 9.19 | 9.96 | 1.41 | 3.43 | 8.24 | 0.930 | 1.92 | 9.6 | 7.01 |
| 3/2/2016 | Durham effluent | 88.4 | 37.7 | 29.6 | 15.8 | 8.03 | 4.93 | 39.2 | 3.97 | 7.51 | 14.5 | 7.04 |
| 3/2/2016 | Durham downstream | 40.3 | 6.31 | 8.88 | 10.0 | 1.54 | 3.46 | 8.90 | 0.997 | 2.09 | 9.7 | 7.04 |
| 6/1/2016 | Rock Creek upstream | 40.2 | 3.95 | 2.69 | 9.30 | 0.899 | 3.26 | 5.16 | 1.03 | 1.5 | 15.3 | 7.4 |
| 6/1/2016 | Rock Creek effluent | 107 | 50.5 | 228 | 57.0 | 20.4 | 7.60 | 122 | 2.92 | 5.12 | 21.9 | 7.11 |
| 6/1/2016 | Rock Creek downstream | 62.8 | 12.6 | 36.9 | 19.0 | 4.13 | 4.95 | 24.1 | 1.33 | 2.55 | 17.4 | 7.3 |
| 6/2/2016 | Durham upstream | 64.2 | 3.66 | 56.4 | 21.2 | 5.74 | 5.75 | 36.7 | 1.87 | 2.7 | 19.5 | 7.1 |
| 6/2/2016 | Durham effluent | 88.7 | 53.1 | 82.4 | 49.8 | 13.7 | 4.94 | 58.9 | 2.63 | 6.37 | 20.0 | 7.03 |
| 6/2/2016 | Durham downstream | 66.0 | 3.96 | 54.9 | 22.7 | 5.83 | 5.90 | 36.1 | 1.79 | 2.9 | 19.4 | 7.2 |

3.8.4 WWTF and River-Length Comparisons

It is interesting to compare results across WWTF effluent samples, as well as down the length of the entire river. Very few differences were seen between WWTF effluents with respect to copper binding for either time period (Figures 11, 12). For the high-flow period, the density of weaker ligand was significantly different between treatment plants (one-way ANOVA, L_1 : $F(2, 12)=3.89$, $p=0.04$), and for the low-flow period the binding strength of the weaker ligand was significantly different (two-tailed unpaired t-test, $\log(K_1)$: $t(8)=2.31$, $p<0.01$). As for river-length comparisons, as detailed in the body of this research, no significant differences were found during the high-flow period using one-way ANOVA tests, but significant differences were identified at all four parameters during the low-flow period (Figures 13, 14).

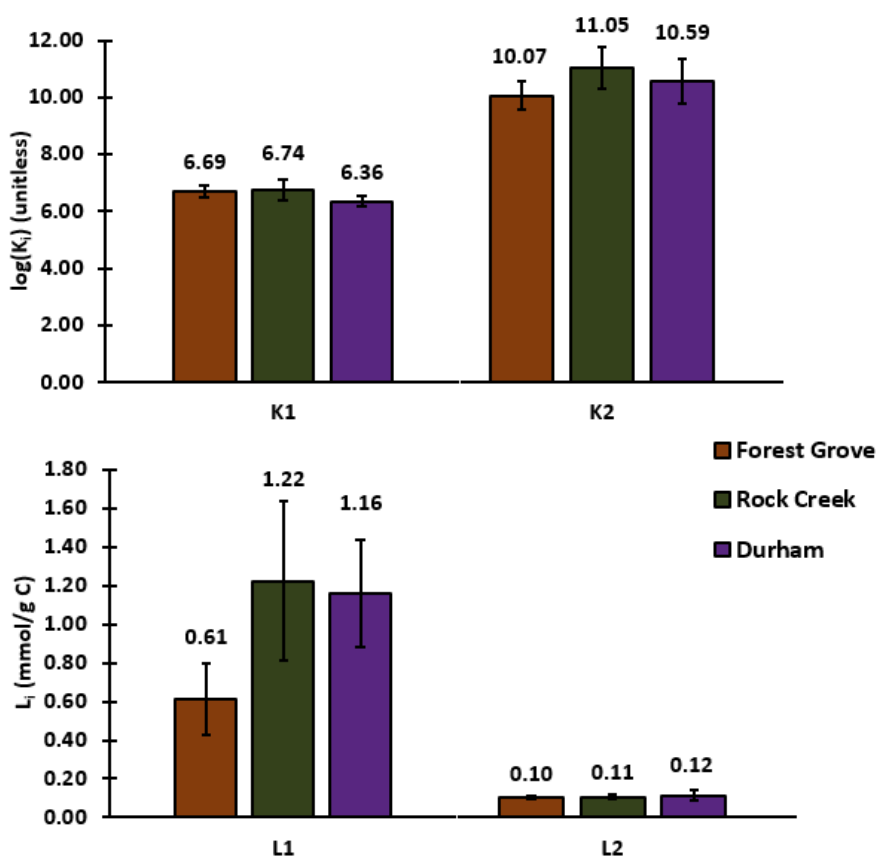


Figure 11: An across-WWTF comparison for the high-flow period of binding strengths (top) and ligand densities (bottom) of each of the two types of copper-ligand complexes. Data labels represent the mean of repeated titrations (n=5), and error bars represent 95% confidence intervals.

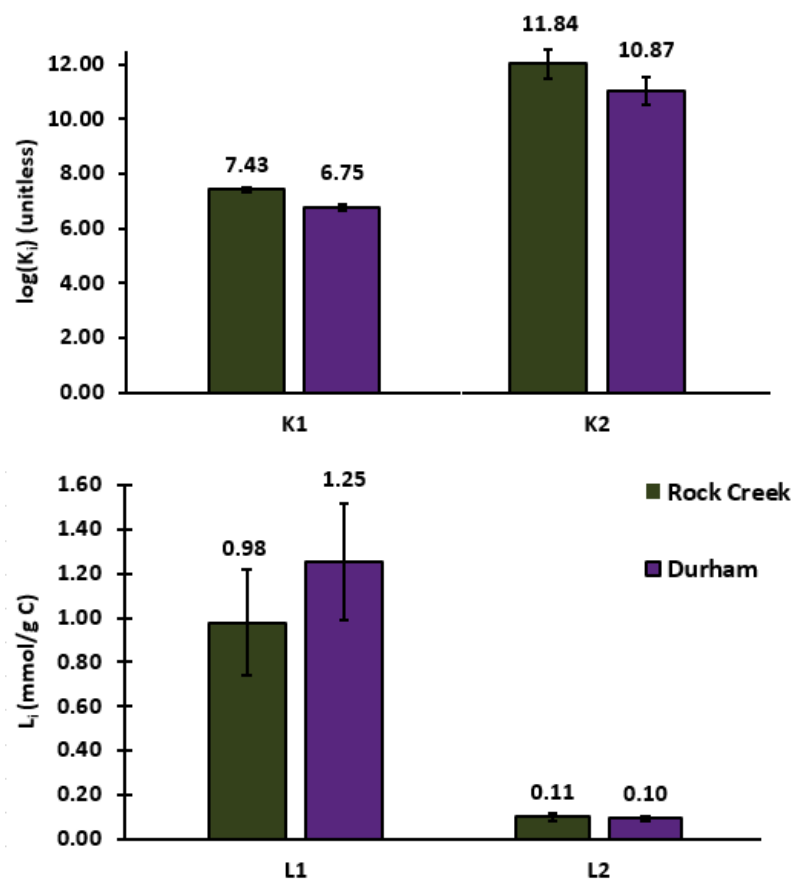


Figure 12: An across-WWTF comparison for the low-flow period of binding strengths (top) and ligand densities (bottom) of each of the two types of copper-ligand complexes. Data labels represent the mean of repeated titrations ($n=5$), and error bars represent 95% confidence intervals.

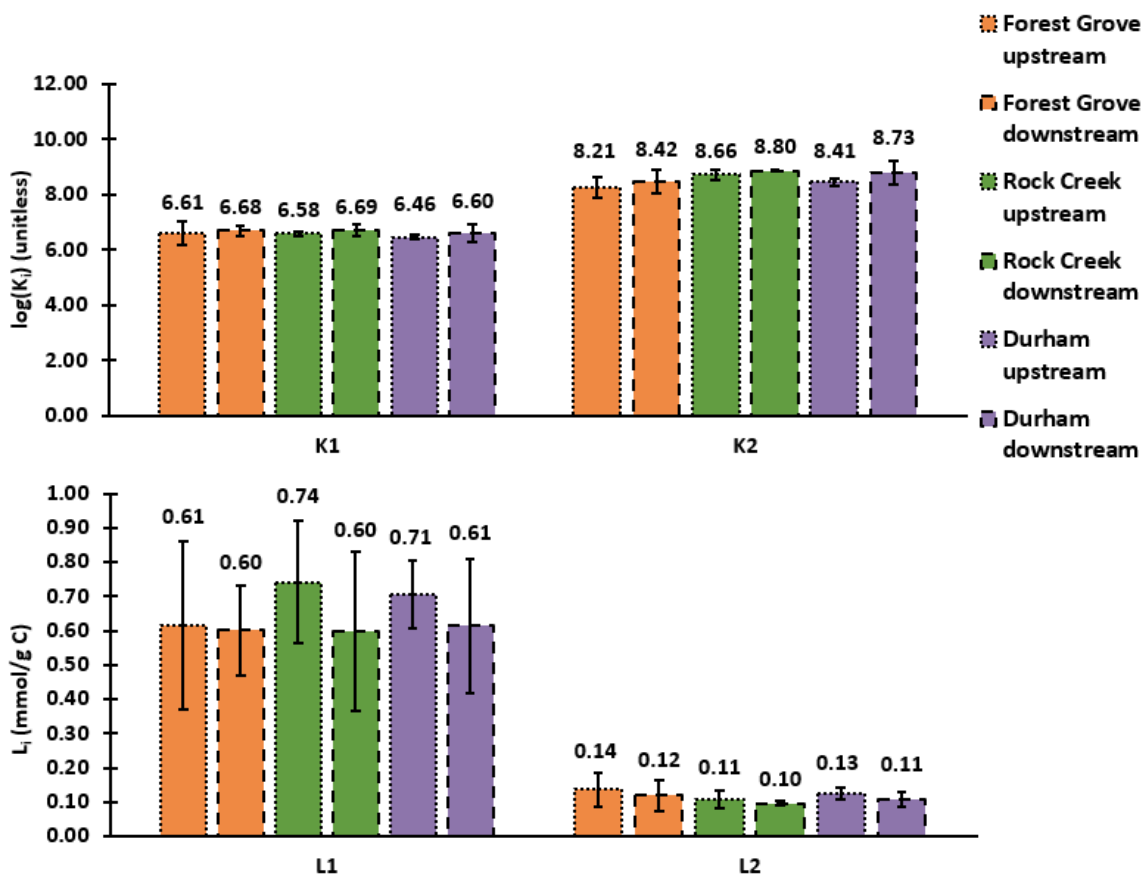


Figure 13: A river-length comparison of high-flow results. Binding strengths are on top and ligand densities are on the bottom. Results are presented in order of relative wastewater influence, with pure river water (upstream of Forest Grove) being the leftmost bar, and downstream of Durham being the rightmost bar. Data labels represent the mean of repeated titrations (n=3), and error bars represent 95% confidence intervals.

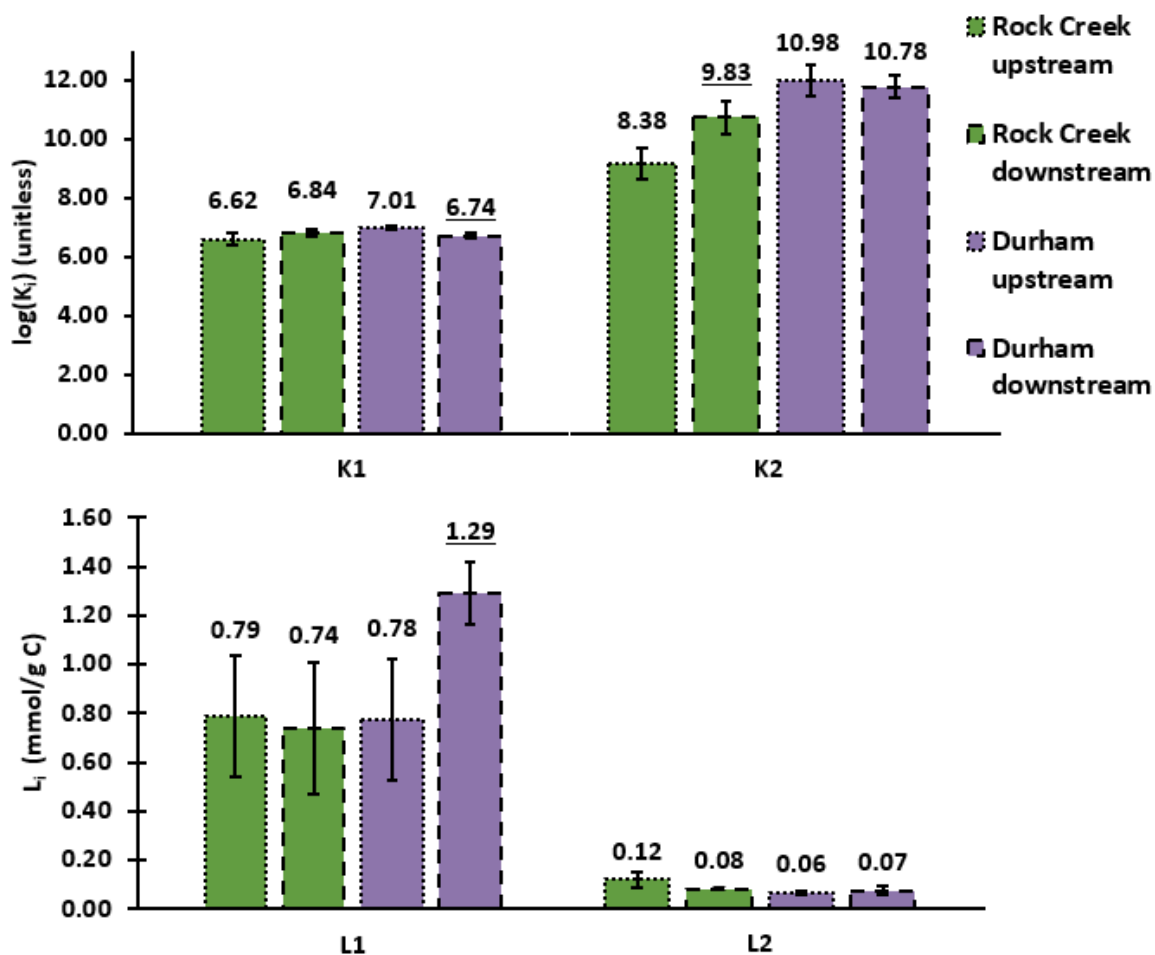


Figure 14: A river-length comparison of low-flow results. Binding strengths are on top and ligand densities are on the bottom. Results are presented in order of relative wastewater influence, with pure river water (upstream of Rock Creek) being the leftmost bar, and downstream of Durham being the rightmost bar. Data labels represent the mean of repeated titrations ($n=3$), and error bars represent 95% confidence intervals.

3.8.5 Modeling with Three Ligands

Although a two-ligand model was chosen for interpreting the results of this study, a three-ligand fit was also possible at all of the sites save for the upstream and downstream Forest Grove samples during the high-flow period, and the upstream of Rock Creek sample taken during the low-flow period. In all other cases, the 3-ligand model did at least somewhat improve the sum of squared errors for the EXCEL solver-based fitting method. However, after modeling each site in Visual MINTEQ with both a two and three ligand model, it was determined that the three ligand model did not improve the fit significantly enough to warrant the use of the additional set of fitting parameters. By use of a two-ligand model instead, the same model was able to be used for all samples, and the trends observed were unchanged by this decision (Figure 15).

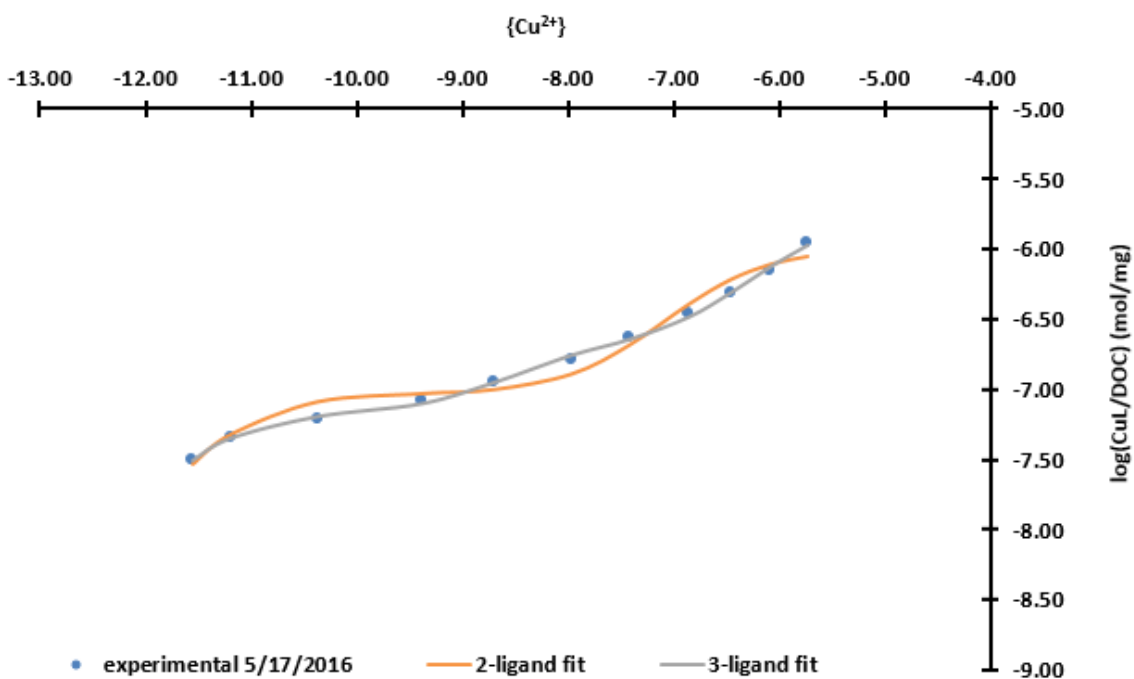


Figure 15: An example of predicted versus observed copper speciation based on a 2 and 3-ligand model.

3.8.6 Percent Humic Acid Assumptions

Due to the low concentrations of DOC observed in some of the samples in this study, percent humic and fulvic acids were unable to be determined. As such, the default BLM value of 10% humic acid was used in modeling. The BLM, however, is calibrated for humic acid values between 10 and 60%. Using an assumption of 60% humic acid results in some improvement of error (approximately by a factor of 5) between the VisualMINTEQ and BLM models for effluent and highly-effluent impacted samples. With that being said, this alternate assumption still results in both a large error and little improvement in the BLM's ability to model the shape of the VisualMINTEQ curve. In addition, past research has shown that WWTF effluent generally has less humic acid than natural waters and is perhaps made up of less than 10% humic acid^{6,7,15,17}. As such, assuming 10% humic acid was determined to be the most scientifically sound assumption (Figure 16).

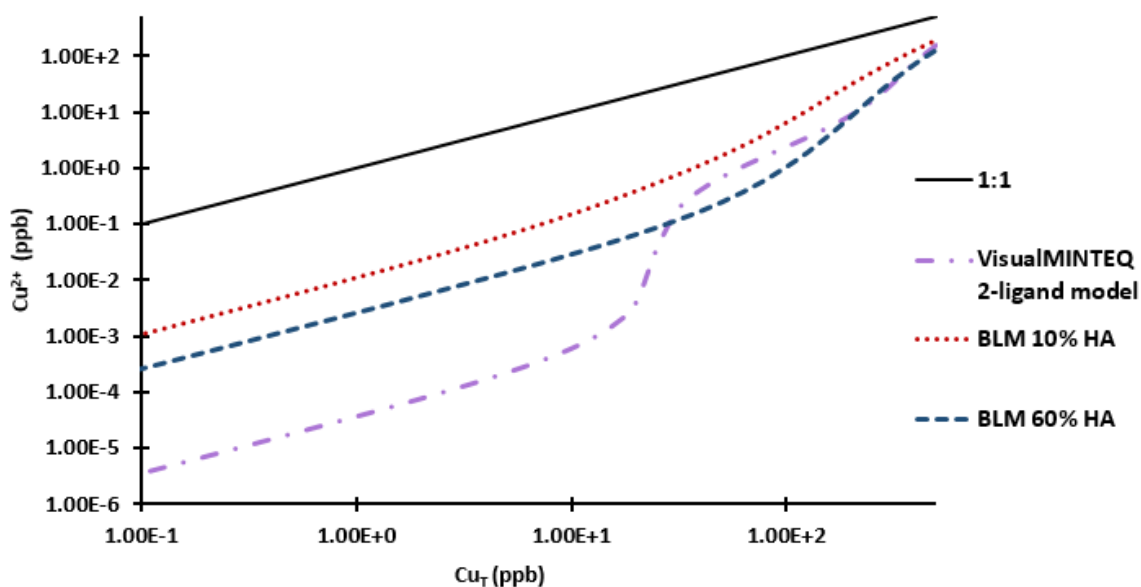


Figure 16: A comparison of a 10% and a 60% humic acid assumption in the BLM for the Durham upstream sample during the low-flow period. Although the 60% assumption improves the fit slightly over the 10% assumption, there is still a large error at environmentally relevant concentrations, and the shape of the curves from the VisualMINTEQ and BLM models are still quite different.

4. Conclusions

This study investigated the binding of copper to DOM in WWTF effluent and natural river water, the influence of WWTF effluent on copper speciation downstream of a WWTF outfall, and the ability of a commonly-employed regulatory tool to provide scientifically accurate copper speciation predictions in each of the aforementioned circumstances. Results from laboratory experiments make a strong case that copper is bound more strongly to DOM in WWTF effluent than it is to DOM in natural river waters. Results also indicate that WWTF effluent can have a strong impact on in-river copper speciation downstream of a WWTF outfall during periods of low dilution. Finally, modeled results indicate that the BLM is capable of sufficiently predicting copper speciation in river waters not impacted by WWTF effluent, as well as those impacted by WWTF effluent during periods of high dilution. However, the BLM is not currently capable of providing acceptable copper speciation predictions in WWTF effluent, or in aquatic systems where WWTF effluent makes up a large percentage of total river discharge.

Suggestions for future work related to this study include the following:

- Determining actual percent humic and fulvic acid values in each of the sampled waters so that this parameter does not have to be given an assumed value when running the BLM. This was not possible for this study as many of the water samples obtained contained DOC concentrations in the 1-2 mg/L range, which would make conducting humic and fulvic acid extraction procedures very difficult. The Nason lab group is currently constructing a DOM concentration system based upon a similar system developed by the University of Texas at Austin to increase the concentration of DOM seen in these sampled waters so that determination of these values becomes possible⁵⁴.
- Analyzing these water samples for the presence of synthetic chelating agents, such as EDTA and NTA.

- Analyzing water samples from additional time periods to help determine at what dilution ratio the BLM becomes significantly less accurate.
- Analyzing water samples from additional aquatic systems to ensure that trends seen here are observed elsewhere as well.
- Implementing BLM improvement strategies and testing their effectiveness in these and other wastewater-impacted surface waters.
- Examining the kinetics of these copper-binding relationships upon the mixing of natural water and WWTF effluent.

All of these suggestions for future work would serve to inform BLM-based regulatory decisions so as to more accurately reflect copper speciation in anthropogenically-altered aquatic systems

Bibliography

1. McIntyre, J. K., Baldwin, D., Beauchamp, D. & Scholz, N. Low-level copper exposures increase visibility and vulnerability of juvenile coho salmon to cutthroat trout predators. *Ecol. Appl.* **22**, 1460–1471 (2012).
2. Hansen, J. A., Rose, J. D., Jenkins, R. A., Gerow, K. G. & Bergman, H. L. Chinook Salmon (*Oncorhynchus tshawytscha*) and Rainbow Trout (*Oncorhynchus mykiss*) Exposed to Copper: Neurophysiological and Histological Effects on the Olfactory System. *Environ. Toxicol. Chem.* **18**, 1999 (1999).
3. U.S. Agency for Toxic Substances and Disease Registry (ASTDR). *Toxicological Profile for Copper*. (2004).
4. California Department of Pesticide Regulation (CDPR). *Copper Sources in Urban Runoff and Shoreline Activities*. (2004).
5. Paquin, P. R. *et al.* The biotic ligand model : a historical overview. *Comp. Biochem. Physiol. Part C* **133**, 3–35 (2002).
6. Imai, A., Fukushima, T., Matsushige, K., Kim, Y.-H. & Choi, K. Characterization of dissolved organic matter in effluents from wastewater treatment plants. *Water Res.* **36**, 859–870 (2002).
7. Ma, H., Allen, H. E. & Yin, Y. Characterization of isolated fractions of dissolved organic matter from natural waters and a wastewater effluent. *Water Res.* **35**, 985–996 (2001).
8. McKnight, D. M., Feder, G. L., Thurman, E. M. & Wershaw, R. L. Complexation of Copper by Aquatic Humic Substances From Different Environments. *Sci. Total Environ.* **28**, 65–76 (1983).
9. United States Environmental Protection Agency. *Quality Criteria for Water 1986*. (1986).
10. Di Toro, D. M. *et al.* Biotic Ligand Model of the Acute Toxicity of Metals. 1. Technical Basis. *Environ. Toxicol. Chem.* **20**, 2383–2396 (2001).
11. United States Environmental Protection Agency. *2003 Draft Update of Ambient Water Quality Criteria For Copper*. (2003).

12. Bryan, S. E., Tipping, E. & Hamilton-Taylor, J. Comparison of measured and modelled copper binding by natural organic matter in freshwaters. *Comp. Biochem. Physiol. Part C* **133**, 37–49 (2002).
13. Tipping, E. Modelling ion binding by humic acids. *Colloids Surfaces A Physicochemical Eng. Asp.* **73**, 117–131 (1993).
14. Ahmed, I. A. M. *et al.* Testing Copper-Speciation Predictions in Freshwaters over a Wide Range of Metal – Organic Matter Ratios. *Environ. Sci. Technol.* **47**, 1487–1495 (2013).
15. Pernet-coudrier, B. *et al.* Dissolved organic matter from treated effluent of a major wastewater treatment plant: characterization and influence on copper toxicity. *Chemosphere* **73**, 593–9 (2008).
16. Sedlak, D. L., Phinney, J. T. & Bedsworth, W. W. Strongly Complexed Cu and Ni in Wastewater Effluents and Surface Runoff. *Environ. Sci. Technol.* **31**, 3010–3016 (1997).
17. Sarathy, V. & Allen, H. E. Copper complexation by dissolved organic matter from surface water and wastewater effluent. *Ecotoxicol. Environ. Saf.* **61**, 337–44 (2005).
18. Baken, S., Degryse, F., Verheyen, L., Merckx, R. & Smolders, E. Metal Complexation Properties of Freshwater Dissolved Organic Matter Are Explained by Its Aromaticity and by Anthropogenic Ligands. *Environ. Sci. Technol.* **45**, 2584–2590 (2011).
19. Matar, Z. *et al.* Influence of organic matter from urban effluents on trace metal speciation and bioavailability in river under strong urban pressure. *Environ. Sci. Pollut. Res.* **22**, 19461–19472 (2015).
20. Avdeef, A., Zabronsky, J. & Stuting, H. H. Calibration of copper ion selective electrode response to pCu. *Anal. Chem.* **55**, 298–304 (1983).
21. Brooks, M. L., McKnight, D. M. & Clements, W. H. Photochemical Control of Copper Complexation by Dissolved Organic Matter in Rocky Mountain Streams, Colorado. *Limnol. Oceanogr.* **52**, 766–779 (2007).
22. Ma, H., Kim, S. D., Cha, D. K. & Allen, H. E. Effect of Kinetics of Complexation by Humic Acid on Toxicity of Copper to *Ceriodaphnia dubia*. *Environ. Toxicol. Chem.* **18**, 828–837 (1999).

23. Christl, I., Milne, C. J., Kinniburgh, D. G. & Kretzschmar, R. Relating Ion Binding by Fulvic and Humic Acids to Chemical Composition and Molecular Size. 2. Metal Binding. *Environ. Sci. Technol.* **35**, 2512–2517 (2001).
24. Constantino, C., Gardner, M., Comber, S. D. W., Scrimshaw, M. D. & Ellor, B. The impact of tertiary wastewater treatment on copper and zinc complexation. *Environ. Technol.* **3330**, 1–9 (2015).
25. De Schampelaere, K. A., Vasconcelos, F. M., Tack, F. M., Allen, H. E. & Janssen, C. R. Effect of Dissolved Organic Matter Source on Acute Copper Toxicity to *Daphnia magna*. *Environ. Toxicol. Chem.* **23**, 1248–1255 (2004).
26. McGeer, J. C., Szebedinszky, C., McDonald, D. G. & Wood, C. M. The role of dissolved organic carbon in moderating the bioavailability and toxicity of Cu to rainbow trout during chronic waterborne exposure. *Comp. Biochem. Physiol. Part C Toxicol. Pharmacol.* **133**, 147–160 (2002).
27. United States Environmental Protection Agency. *Aquatic life ambient freshwater quality criteria - copper*. (2007).
28. Baldwin, D. H., Sandahl, J. F., Labenia, J. S. & Scholz, N. L. Sublethal effects of copper on coho salmon: Impacts on nonoverlapping receptor pathways in the peripheral olfactory nervous system. *Environ. Toxicol. Chem.* **22**, 2266–2274 (2003).
29. Sandahl, J. F., Baldwin, D. H., Jenkins, J. J. & Scholz, N. L. Odor-evoked field potentials as indicators of sublethal neurotoxicity in juvenile coho salmon (*Oncorhynchus kisutch*) exposed to copper, chlorpyrifos, or esfenvalerate. *Can. J. Fish. Aquat. Sci.* **61**, 404–413 (2004).
30. Linbo, T. L., Stehr, C. M., Incardona, J. P. & Scholz, N. L. Dissolved copper triggers cell death in the peripheral mechanosensory system of larval fish. *Environ. Toxicol. Chem.* **25**, 597–603 (2006).
31. Mathis, A. & Smith, R. J. F. Intraspecific and Cross-Superorder Responses to Chemical Alarm Signals by Brook Stickleback. *Ecology* **74**, 2395–2404 (1993).
32. Brown, G. E., Chivers, D. P. & Smith, R. J. F. Fathead minnows avoid conspecific and heterospecific alarm pheromones in the faeces of northern pike. *J. Fish Biol.* **47**, 387–393 (1995).

33. Sandahl, J. F., Baldwin, D. H. & Jenkins, J. J. A Sensory System at the Interface between Urban Stormwater Runoff and Salmon Survival. *Environ. Sci. Technol.* **41**, 2998–3004 (2007).
34. Donaldson, E. M. & Dye, H. M. Corticosteroid Concentrations in Sockeye Salmon (*Oncorhynchus nerka*) Exposed to Low Concentrations of Copper. *J. Fish. Res. Board Canada* **32**, 533–539 (1975).
35. Playle, R. C., Gensemer, R. W. & Dixon, D. G. Copper Accumulation on Gills of Fathead Minnows: Influence of Water Hardness, Complexation and pH of the Gill Micro-Environment. *Environ. Tox* **11**, 381–391 (1992).
36. Lu, Y. & Allen, H. E. Characterization of copper complexation with natural dissolved organic matter (DOM)—link to acidic moieties of DOM and competition by Ca and Mg. *Water Res.* **36**, 5083–5101 (2002).
37. Croue, J.-P., Benedetti, M. F., Violleau, D. & Leenheer, J. A. Characterization and Copper Binding of Humic and Nonhumic Organic Matter Isolated from the South Platte River : Evidence for the Presence of Nitrogenous Binding Site. *Environ. Sci. Technol.* **37**, 328–336 (2003).
38. Aiken, G. R., McKnight, D. M., Wershaw, R. L. & MacCarthy, P. *Humic Substances in Soil, Sediment, and Water*. (John Wiley & Sons, Inc, 1985).
39. Tipping, E. WHAM -- A Chemical Equilibrium Model and Computer Code for waters, Sediments, and Soils Incorporating a Discrete Site/Electrostatic Model of Ion-Binding by Humic Substances. *Comput. Geosci.* **20**, 973–1023 (1994).
40. Tipping, E. Humic Ion-Binding Model VI : An Improved Description of the Interactions of Protons and Metal Ions with Humic Substances. *Aquat. Geochemistry* **4**, 3–47 (1998).
41. Miller, L. A. & Bruland, K. W. Competitive equilibration techniques for determining transition metal speciation in natural waters: Evaluation using model data. *Anal. Chim. Acta* **343**, 161–181 (1997).
42. US Environmental Protection Agency (EPA). Training materials on Copper BLM : Data Requirements. (2011).
43. Pernet-coudrier, B. *et al.* Dissolved organic matter from treated effluent of a major wastewater treatment plant: Characterization and influence on copper toxicity. *Chemosphere* **73**, 593–599 (2008).

44. Breault, R. F., Colman, J. a., Aiken, G. R. & Mcknight, D. Copper speciation and binding by organic matter in copper-contaminated streamwater. *Environ. Sci. Technol.* **30**, 3477–3486 (1996).
45. Craven, A. M., Aiken, G. R. & Ryan, J. N. Copper(II) Binding by Dissolved Organic Matter: Importance of the Copper-to-Dissolved Organic Matter Ratio and Implications for the Biotic Ligand Model. *Environ. Sci. Technol.* **46**, 9948–9955 (2012).
46. Ahmed, I. a M., Hamilton-Taylor, J., Bieroza, M., Zhang, H. & Davison, W. Improving and testing geochemical speciation predictions of metal ions in natural waters. *Water Res.* **67C**, 276–291 (2014).
47. Knepper, T. P. Synthetic chelating agents and compounds exhibiting complexing properties in the aquatic environment. *Trends Anal. Chem.* **22**, 708–724 (2003).
48. US Environmental Protection Agency (EPA). Training materials on Copper BLM : Implementation.
49. Nason, J. A., Sprick, M. S. & Bloomquist, D. J. Determination of copper speciation in highway stormwater runoff using competitive ligand exchange - Adsorptive cathodic stripping voltammetry. *Water Res.* **46**, 5788–98 (2012).
50. Chaminda, G. G. T., Nakajima, F., Furumai, H., Kasuga, I. & Kurisu, F. Comparison of metal (Zn and Cu) complexation characteristics of DOM in urban runoff, domestic wastewater and secondary effluent. *Water Sci. Technol.* **62**, 2044–50 (2010).
51. Oregon Department of Environmental Quality. Water Quality Assessment Database. *2012 Water Quality Report* (2012).
52. Sturdevant, D. Water Quality Copper Standards. *Oregon Department of Environmental Quality: DEQ Rules and Regulations* (2016).
53. Gustafsson, J. P. Visual MINTEQ. (2012).
54. Ingenloff, C. M. Isolation and Concentration of Dissolved Organic Matter in Stormwater Runoff through Reverse Osmosis. (University of Texas at Austin, 2011).

APPENDICES

Appendix A – Method Verification

A standard organic matter isolate, Suwannee River Fulvic Acid (SRFA, International Humic Substances Society) was used to verify the experimental and analytical methods used in this study. Determined values obtained from a 2-ligand model are plotted in Figure A1, and results are compared to those of two published studies also conducted on SRFA in Table A1. Determined stability constants from this study are similar to those published in the literature. Ligand densities are slightly lower in this study as compared to the two published studies, but given that conditions in these three studies (pH, ionic strength, Cu:DOC ratio) were not identical, differences in parameters are small enough to be accounted for by the effects of these differences.

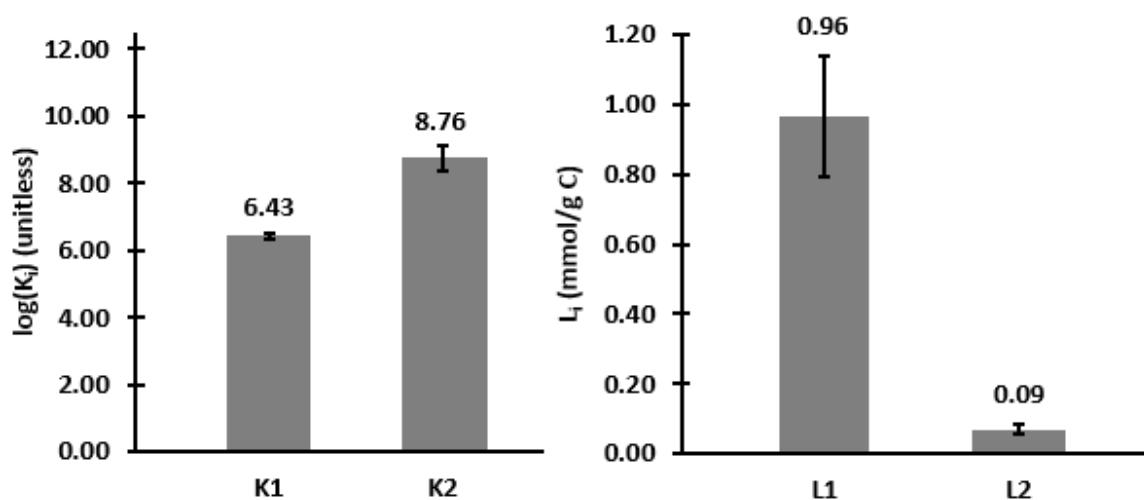


Figure A1: Determined stability constants (left) and ligand densities (right) for Suwannee River Fulvic Acid (SRFA) analyzed using a 2-ligand Langmuir-type model. Data labels represent average values and error bars represent 95% confidence intervals (n=7).

Table A1: A comparison of SRFA parameters related to copper binding determined by this study to those in published literature.

| | $\log(K_1)$ | $\log(K_2)$ | L_1 (mmol/g C) | L_2 (mmol/g C) |
|-----------------------------------|-------------|-------------|------------------|------------------|
| This Study | 6.43 | 8.76 | 0.96 | 0.09 |
| Ma, 2001⁷ | 7.45 | 9.40 | 1.14 | 0.35 |
| McKnight, 1983⁸ | 5.9 | 7.7-7.8 | 1.3 | 0.3 |

Appendix B – Willamette River Dilution Study

An anecdotal study on water samples obtained from the Willamette River was performed in conjunction with this study. Water samples were obtained from the Taylor Drinking Water Treatment Plant (TTP) in Corvallis, Oregon, and the discharged effluent stream from the Wastewater Treatment Facility (WWTF) in Corvallis, Oregon. Copper titrations identical in procedure to those described in the main body of this thesis were conducted on pure WWTF water (100% WW), pure TTP water (0% WW), and both a 50/50 and a 25/75 mixture of WWTF/TTP water (50% WW and 25% WW, respectively) to simulate the conditions that would occur downstream of the WWTF outfall as a result of the mixing of these waters. The results from this experiment are presented in Figure B1.

Results show that, like the Tualatin River samples, the binding strength of the stronger ligand-copper complex is several orders of magnitude stronger in the WWTF effluent than in the river or mixed waters. Otherwise results were rather sporadic, without a consistent pattern evident in the mixed water samples. The strength of the stronger stability constant is significantly greater in the 25% WW sample than in the 0% WW sample, but, although the average value for this parameters is more than an order of magnitude greater in the 50% WW sample than in the 0% WW sample, the difference was not significant. It is possible that more repetitions of these titrations could lead to decreased variance in sample parameters, which could lead to additional significant differences, but this is not certain.

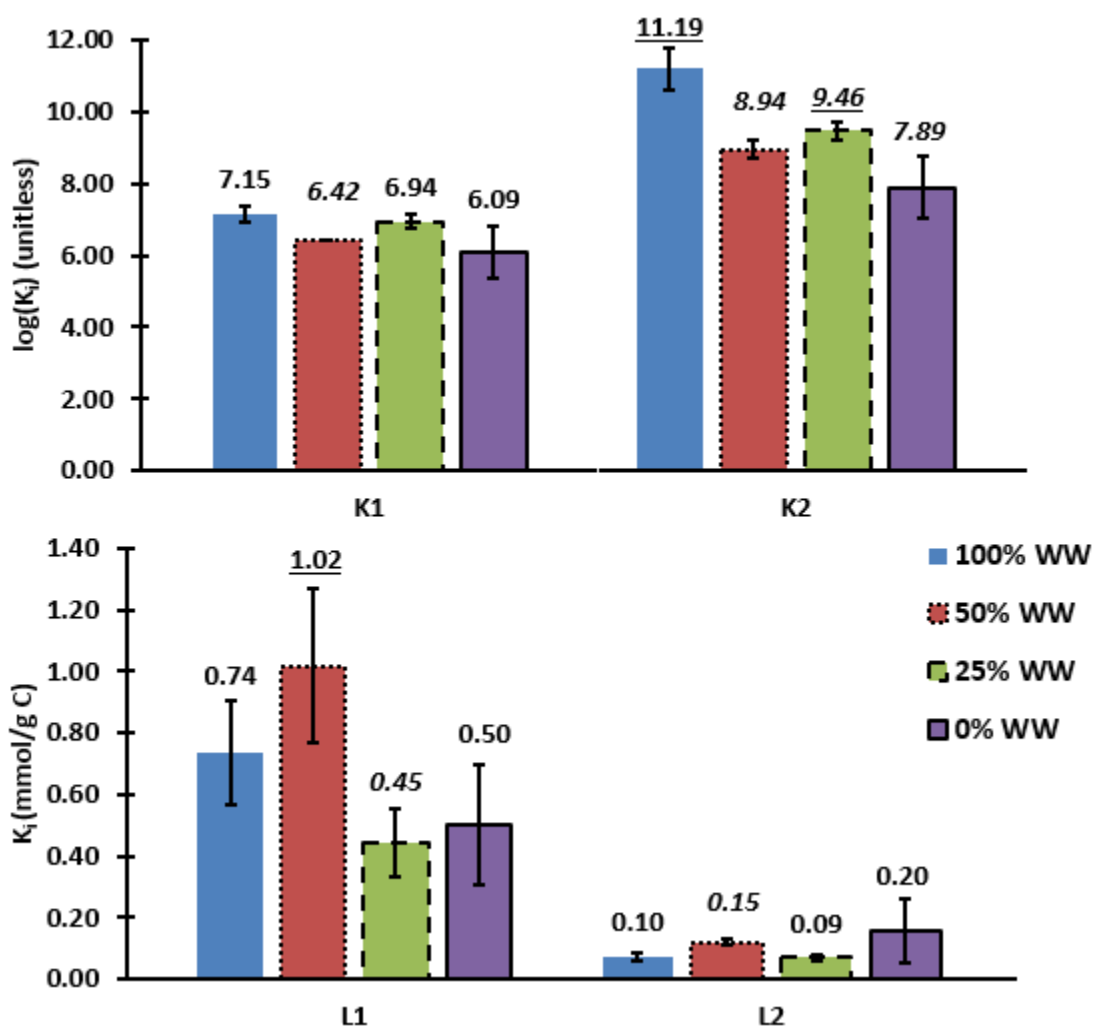


Figure B1: Results from the Willamette River dilution experiments. Determined binding strengths are on top and ligand densities are on the bottom. Data labels represent average values, and error bars represent 95% confidence intervals. Italic data label represent a significant difference from 100% WW, and underlined data labels represent a significant difference from 0% WW. For the 100% WW samples $n=4$, and for the other samples $n=3$. Significant differences were determined using two-tailed unpaired t-tests, where unequal variance was assumed where n was different and equal variance was assumed where n was the same.

