The Importance of Dissolved Organic Matter to the Binding of Copper and the Release of Trace Elements from Coal Ash

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THE IMPORTANCE OF DISSOLVED ORGANIC MATTER TO THE BINDING OF COPPER AND THE RELEASE OF TRACE ELEMENTS FROM COAL ASH

By

Alison Marie Craven

B.S., The Pennsylvania State University, 2007

A thesis submitted to the
Faculty of the Graduate School of the
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of the requirement for the degree of
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This thesis entitled:

The Importance of Dissolved Organic Matter to the Binding of Copper and the Release of Trace Elements from Coal Ash

written by
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The final copy of this thesis has been examined by the signatories, and we find that both the content and the form meet acceptable presentation standards of scholarly work in the above mentioned discipline.
Craven, Alison Marie (Ph.D., Department of Chemistry and Biochemistry)

The Importance of Dissolved Organic Matter to the Binding of Copper and the Release of Trace Elements from Coal Ash

Thesis directed by Professor Joseph N. Ryan

Abstract

The bioavailability and toxicity of copper to aquatic life depends on its speciation. Dissolved organic matter (DOM) plays an important role in the speciation of copper, but there is still much uncertainty about what controls the strength and formation of the Cu$^{2+}$-DOM complex. The ratio of copper to DOM is known to affect the strength of Cu$^{2+}$-DOM binding, but previous methods to determine Cu$^{2+}$-DOM binding strength have generally not measured binding constants over the same Cu:DOM ratios, making these results difficult to directly compare. A competitive ligand exchange-solid phase extraction (CLE-SPE) method and a copper ion-selective-electrode (Cu-ISE) method were used to determine conditional stability constants for Cu$^{2+}$-DOM binding at near neutral pH and 0.01 M ionic strength over a range of Cu:DOM ratios that bridge the detection windows of previous measurements reported in the literature. As the Cu:DOM ratio increased from 0.0005 to 0.1 mg$_{Cu}$ mg$_{DOM}^{-1}$, the measured conditional binding constant ($K_{CuDOM}$) decreased from $10^{11.5}$ to $10^{5.6}$ M$^{-1}$. This behavior is consistent with the presence of Cu$^{2+}$ binding sites of higher affinity and lower abundance that become filled as the total copper concentration increases. A comparison of the binding constants measured using CLE-SPE with those measured by Cu-ISE and voltammetry methods demonstrates that the Cu:DOM ratio is an important factor controlling the Cu$^{2+}$-DOM binding strength for a variety of DOM isolates and whole water samples.
Using correct Cu$^{2+}$-DOM binding constants to accurately model copper speciation is important for predicting copper toxicity in cases like the waters draining the Pebble deposit in southwestern Alaska, where small increases in the dissolved copper concentration may be harmful to salmonids and other aquatic biota. Experimentally determined Cu$^{2+}$-DOM binding constants were used as inputs in Visual MINTEQ to model copper speciation and calculate Cu$^{2+}$ concentrations. The results were then compared to results from the biotic ligand model (BLM), a speciation and toxicity model recommended by U.S. EPA for calculation of stream copper standards, which uses the Windermere Humic Aqueous Model (WHAM) to model metal interactions with DOM. The BLM was found to over-estimate Cu$^{2+}$ at low total copper concentrations and under-estimate Cu$^{2+}$ at high total copper concentrations, which may result in over- or under-estimations of toxicity.

DOM also has also been shown to enhance the dissolution of soils, sediments and minerals, which could result in the release of toxic trace elements into aqueous systems. Coal ash contains high concentrations of toxic trace elements that may have the potential to be released into the water. Releases of coal ash to rivers and streams, such as that which occurred from the Kingston Fossil Plant in Kingston, TN, into the Emory and Clinch Rivers in December 2008, are a concern because of the potential for human health problems, as well as ecological effects. In order to understand the effect that DOM has on the release of trace elements from coal ash, a series of release experiments were performed using coal ash generated from the Kingston Fossil Plant as a function of DOM concentration, DOM aromaticity, and calcium concentration. Various DOM isolates and filtered site-water samples collected near the Kingston Fossil Plant were used to produce coal ash suspensions at a fixed ash:water ratio of 1:1000 and a near-neutral pH. The major and trace elemental composition of the solution, and specific trace metals
(mercury, lead, copper, aluminum) and metalloids (arsenic and selenium) were measured. The results indicate that DOM enhances the release of mercury, lead, copper, and aluminum from coal ash. The concentration of mercury, lead, copper and aluminum released from the coal ash was positively correlated with the SUVA$_{254}$ (ultraviolet absorbance at 254 nm divided by the dissolved organic carbon concentration) of the DOM. The release of arsenic and selenium from the coal ash was not dependent on the DOM concentration or SUVA$_{254}$. Calcium was shown to inhibit the release of mercury, lead, copper and aluminum from the coal ash, but did not have the same effect on the release of arsenic and selenium.
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Chapter 1

Introduction

Background

Mercury is a global pollutant because of widespread contamination caused by atmospheric deposition. In the water, microorganisms convert $\text{Hg}^{2+}$ to methylmercury, $\text{CH}_3\text{Hg}^+$, a neurotoxin that biomagnifies as it moves up the food chain (Ravichandran, 2004). Methylation depends on the speciation of mercury, so understanding the factors that affect speciation is important in order to assess the toxicity and uptake of mercury in the food chain. Copper is another trace metal that is toxic to aquatic organisms, depending on its speciation in water. The free copper ion ($\text{Cu}^{2+}$) and aqueous inorganic metal complexes ($\text{CuOH}^+$, $\text{CuCO}_3$) are the dominant species that are thought to control the toxicity of copper to organisms (Kalis et al, 2006; De Schamphelaere et al, 2004; Di Toro et al, 2001).

Dissolved organic matter (DOM) plays an important role in the speciation and bioavailability of aqueous metals and trace elements. DOM is a heterogeneous mixture of organic compounds which are functionally defined as the fraction of organic matter that passes through a 0.45-µm filter (Thurman, 1985). Understanding the chemistry of DOM is difficult due to the complexity of a single source and the variability between sources. The physical structure of DOM has been characterized by measurements of elemental composition, aromaticity, functional group content, and average molecular weight, but it is difficult to fully understand the reactivity of DOM due to the heterogeneity of the mixture (Waples et al, 2005; Stenson et al, 2005). Electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry, an analytical technique with ultrahigh resolution, was used to identify over 4,500 unique chemical
formulas for organic acids present in a water sample from the Suwannee River, which emphasizes the extent of heterogeneity of a single DOM source (Stenson et al, 2005).

There are many functional groups in DOM that can bind with dissolved metals, such as carboxyl, phenol, amine, and thiol groups. These functional groups exhibit a wide range of ionic character, which affects the binding preference for any particular metal. Carbon makes up about 50% of the mass of DOM, with the remainder of the mass made up of H, O, N, and S. Carboxyl and phenol groups are the most abundant and generally the weakest metal binding functional group, making up 15-20% of the carbon (by mass) (Waples et al, 2005). Nitrogen typically makes up 1-2% (by mass) of the total DOM (Vairavamurthy and Wang, 2002), while total sulfur makes up about 1% (by mass) of the DOM, with a large fraction (~20%) of the total sulfur in the reduced form (Vairavamurthy et al, 1997). Roughly estimating, every molecule of DOM has one functional group containing nitrogen, while only one in fifteen molecules of DOM contains reduced sulfur functional groups.

Different metals preferentially bind to different functional groups depending on the covalent index of the metal involved, which can result in large differences in the strength of metal binding to the DOM. Metal ions can be classified into two categories, hard and soft (Pearson, 1995). Hard metals, which include calcium, magnesium, and aluminum, prefer to bond with carboxyl and phenol functional groups, due to the low polarizability of the electron shell of both the metal and the functional group. This property gives these molecules spherical shapes and favors formation of weaker, ionic bonds (Rickard and Luther, 2006; Stumm and Morgan, 1970; Pearson, 1995). Soft metals, such as mercury, have a sphere of electrons in the outer shell with high polarizability, which gives these metals an affinity for forming stronger, more covalent-like bonds with the reduced sulfur functional groups in the DOM (Stumm and
Morgan, 1970). Nitrogen-containing functional group are softer than oxygen, but harder than reduced sulfur, so binding with transition metals of intermediate covalent index, such as Cu$^{2+}$, is favorable.

Metal Binding with Dissolved Organic Matter

Strong Hg$^{2+}$-DOM binding has been shown to occur at the reduced sulfur functional groups, which are present in the DOM at low concentrations (Gerbig et al, 2011). Haitzer et al. (2002) demonstrated that the binding constants between Hg$^{2+}$ and DOM depend on the Hg:DOM ratio. They found that for a DOM sample from the Florida Everglades, when the Hg:DOM ratio was lower than 1 µg Hg (mg of DOM)$^{-1}$, the mercury concentration was lower than the concentration of the strong binding sites and all of the mercury was bound to the strongest binding sites. At low Hg:DOM ratios, binding constants in the ranging from $10^{29.6}$ to $10^{33.5}$ M$^{-1}$ have been measured by various methods including competitive ligand exchange-solid phase extraction and equilibrium dialysis ligand exchange methods (Black et al, 2007; Hsu and Sedlak, 2003; Hsu-Kim and Sedlak, 2005; Haitzer et al, 2003). As the Hg:DOM ratio increased, the capacity of strong binding sites was exceeded and the effective binding constant decreased since mercury was now bound to the significantly weaker carboxyl containing sites (Figure 1.1).
Intermediate metals, such as Cu$^{2+}$ and others from the first row transition series, have behavior that falls in between the hard and soft metals. These metals form strong bonds with oxygen and nitrogen-containing functional groups, (Korshin et al, 1998; Karlsson et al, 2006) but may still be capable of bonding with reduced-sulfur sites in DOM (Karlsson et al, 2006). While there have been many studies that have measured Cu$^{2+}$-DOM binding constants, the variability of the Cu:DOM ratios under which the measurements were taken makes the results from different methods difficult to compare (Breault et al, 1996; Jin and Gogan, 2000; Witter et al, 1998; Bruland et al, 2000; Donat et al, 1994; McKnight et al, 1983; Kogut and Voelker, 2001; Brown et al, 1999). Cu$^{2+}$-DOM binding constants have been measured ranging from $10^{4.25}$ M$^{-1}$ to $10^{18.7}$ M$^{-1}$. These measurements have been predominately made using cupric ion-selective electrode (Cu-ISE) titrations or various voltammetry methods, which
have very little overlap in the range of Cu:DOM ratios that can be measured because of the differences in the analytical detection windows for both methods.

Chemical models are a useful tool for predicting aqueous metal speciation, but more work needs to be done to better understand and accurately incorporate the chemical interactions between dissolved metals and DOM. The importance of DOM on metal speciation is widely recognized and attempts have been made to incorporate metal interactions with DOM into chemical speciation models, such as the Windermere humic aqueous model (WHAM; Tipping, 1994) and the biotic ligand model (BLM; DiToro et al, 2002), but the lack of understanding of metal-DOM interactions across a wide range of environmentally relevant metal:DOM ratios often leads to misrepresentations in the chemical speciation. The BLM is a model that has been approved by the U. S. EPA to predict copper speciation and toxicity to various species of fish by using site-specific water quality data, such as alkalinity, hardness, pH, and DOM concentration (U. S. EPA 2007; Aiken et al, 2011; Paquin et al, 2002; Figure 1.2).

Figure 1.2. Schematic of the Biotic Ligand Model (Paquin et al, 2002)
The Pebble Mine is a world-class porphyry copper-gold-molybdenum deposit located in Southwestern Alaska. It is a topic of much debate because mining the deposit could impact the waters draining the deposit which may become a risk for aquatic life. Copper is a metal of concern because of its toxicity to fish and other aquatic biota (Meyer et al, 2007). Understanding the speciation of copper in the waters draining the Pebble deposit under current and elevated copper concentrations is important for predicting the bioavailability and toxicity of copper. Since DOM plays a significant role in copper speciation, it is important that accurate Cu$^{2+}$-DOM binding constants are being used in chemical speciation and toxicity models, such as the BLM, in order to accurately predict copper toxicity. While the BLM is an improvement over other aquatic life criteria for copper toxicity, previous researchers have discovered inconsistencies between predicted and observed toxicity values which may be caused by an over- or under-estimate of the Cu$^{2+}$-DOM binding constants used in the model (De Schamphelaere et al., 2004; Welsh et al., 2008).

**Release of Trace Elements from Coal Ash**

The release of coal ash into the environment is a concern because coal ash contains high concentrations of trace elements, such as arsenic, cadmium, cobalt, copper, lead, nickel, mercury, selenium and zinc that have the potential to be released into the water at elevated and possibly toxic concentrations (Fernández-Turiel et al., 1994; Hower et al, 2005). Coal ash primarily consists of an aluminosilicate core, and different varieties of coal ash contain either high calcium or iron concentrations. Some of the major elements present in coal ash have been shown to be readily leachable under a wide range of conditions, such as calcium, magnesium, sodium and potassium (Gitari et al, 2009). The release of trace elements from coal ash is known to be
affected by the water chemistry, such as pH, ionic strength, and redox conditions (Gitari et al, 2009; Kim and Hesbach, 2009; Jegadeesan et al, 2008), but DOM may also be important in enhancing the release of trace elements.

The role of DOM in the release of trace metals from minerals, soils, and sediments has been previously observed. The dissolution of cinnabar (HgS), a mineral with a very low solubility product constant, was enhanced in the presence of DOM (Ravichandran et al, 1998). It was also observed that the aromaticity of DOM isolated correlated with the rate of cinnabar dissolution (Waples et al, 2005; Figure 1.3).

While DOM increases the dissolution of minerals, it is still unknown if DOM increase the dissolution of trace elements from coal ash. Some work has been done to study the effect of organic ligands, such as citric acid and EDTA, on the leaching of metals from coal fly ash (Harris and Silberman, 1983; van der Bruggen et al, 1998), but no work has been done studying natural DOM on the release of metals from coal ash.

Figure 1.3. The dissolution rate of mercury from cinnabar in the presence of DOM isolates that range in % aromaticity (Waples et al, 2005).
Different trace elements in the coal ash may have different leaching behaviors in the presence of DOM. Hard metals, such as Ca$^{2+}$ and Al$^{3+}$, interact with oxygen-containing functional groups, which are present at high concentrations in the DOM. Other metals, such as Cu$^{2+}$ and Pb$^{2+}$, are classified as intermediate metals. Intermediate metals will bind with oxygen-containing functional groups at high metal:DOM ratios, but may interact with nitrogen- and reduced sulfur-containing functional groups as the metal:DOM ratio decreases (Pinheiro et al, 1999; Weng et al, 2002). Soft metals, such as Hg$^{2+}$, are known to bind very strongly with reduced-sulfur functional groups, which are present in the DOM in low concentrations (Haitzer et al, 2002; Gerbig et al, 2011). If metals preferentially bind to certain functional groups in the DOM, these differences may affect their leaching behaviors from coal ash.

Arsenic and selenium are elements of interest due to their toxicity. These metalloids are known to be present in various species in coal ash with the major species being the oxyanions arsenate and selenite (Huggins et al, 2009). DOM may be important for the release of these oxyanions from coal ash by forming aqueous complexes with arsenic and selenium and also through competition for surface adsorption sites resulting in desorption of the metalloids from the coal ash surface (Buschmann et al, 2006; Wang and Mulligan, 2006; Weng et al, 2009).

**Kingston Fossil Plant Coal Ash Spill**

In December 2008, the Kingston Fossil Plant operated by Tennessee Valley Authority and located near Harriman, Tennessee experienced a large containment system failure which resulted in the largest release of coal ash in the history of the United States (Ruhl et al, 2009; Bednar et al, 2010; U. S. EPA 2009; TVA 2009). The coal ash entered the Emory River at mile marker 2.5 and the areas that were affected by the coal ash spill continued upstream to mile marker 6 of the Emory River, and to mile marker 5 of the Clinch River (Ruhl et al, 2010; Figure
The release of 3.7 million cubic meters of wet coal ash is an ecological and public health concern due to the high concentrations of toxic elements in the ash, including mercury, arsenic and selenium that may have the potential to be released into the water.

![Map](image)

Figure 1.4. Map of the area surrounding the Kingston Fossil Plant (Ruhl et al, 2010).

**Description of Research**

**Measurement of Cu$^{2+}$-DOM Binding Constants**

In order to measure Cu$^{2+}$-DOM binding constants across a wide range of Cu:DOM ratios, a competitive ligand exchange-solid phase extraction (CLE-SPE) method was used that was similar to that of previous researchers (Black et al, 2007; Hsu and Sedlak, 2003; Hsu-Kim and Sedlak, 2005; Miller and Bruland, 1995; Gasper et al, 2007). This method uses C$_{18}$ resin as the
solid phase to separate DOM into two fractions operationally defined as the hydrophobic and hydrophilic fractions. The hydrophobic fraction of the DOM adsorbs to the resin, while the hydrophilic fraction remains in the eluent (Figure 1.5).

CLE-SPE makes use of a ligand with known stoichiometry and binding constant to compete with DOM for copper. If the Cu\(^{2+}\)-competing ligand complex is hydrophobic, it will increase the amount of copper that remains on the resin and allow for calculation of the binding strength of the hydrophilic DOM fraction. If a hydrophilic competing ligand is used, the amount of copper passing through the resin will increase and the binding strength of the hydrophobic DOM can be calculated.

The CLE-SPE method was used to measure the Cu\(^{2+}\)-DOM binding constants for a range of DOM isolates and whole water samples, including DOM isolated from the headwaters of three waters draining the Pebble deposit. Chapter 2 describes how the binding constants measured using the CLE-SPE method are able to bridge the gap in the detection windows of Cu-ISE.
titration and voltammetry methods. It also demonstrates that for a wide range of DOM isolates and whole water samples, the Cu$^{2+}$-DOM binding constants are dependent on the Cu:DOM ratio. Chapter 3 combines CLE-SPE and Cu-ISE methods to measure Cu$^{2+}$-DOM binding constants for DOM isolates collected from three site-waters draining the Pebble deposit. The map shown below highlights the three locations where water was collected for DOM isolation (Figure 1.6).

![Map of the Pebble deposit and the surrounding waters. The three locations (North Fork Koktuli, NK02; South Fork Koktuli, SK02; and Upper Talarik, UT02) where water was collected for DOM isolation and copper binding experiments.](image)

Once the Cu$^{2+}$-DOM binding constants were measured over a range of environmentally relevant Cu:DOM ratios, the experimentally determined binding constants were used to predict the Cu$^{2+}$ concentrations as the total copper concentration was increased using Visual MINTEQ.
The results were then compared to results produced by the BLM to evaluate fish toxicity in waters surrounding the Pebble deposit.

**Release of Trace Elements from Coal Ash**

In order to assess the role of DOM on the releases of trace elements from coal ash, a series of release experiments were done as a function of DOM concentration, aromaticity, and calcium concentration. DOM isolates of varying SUVA$_{254}$, which has been correlated with the aromatic carbon content (Weishaar et al, 2003), were added to coal ash suspensions in order to evaluate if DOM with greater aromaticity increased the release of trace elements from the coal ash. Calcium addition experiments were also performed to explore the role of calcium in trace element release. Chapter 4 describes the release of mercury from coal ash in the presence of DOM and Chapter 5 describes the release of other trace metals (copper, lead and aluminum) and metalloids (arsenic and selenium).
References


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Chapter 2

Copper(II) Binding by Dissolved Organic Matter: Importance of the Copper-to-Dissolved Organic Matter Ratio and Implications for the Biotic Ligand Model

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Abstract

The ratio of copper to dissolved organic matter (DOM) is known to affect the strength of copper-DOM binding, but previous methods to determine Cu\(^{2+}\)-DOM binding strength have generally not measured binding constants over the same Cu:DOM ratios. In this study, we used a competitive ligand exchange-solid phase extraction (CLE-SPE) method to determine conditional stability constants for Cu\(^{2+}\)-DOM binding at pH 6.6 and 0.01 M ionic strength over a range of Cu:DOM ratios that bridge the detection windows of copper-ion selective electrode and voltammetry measurements. As the Cu:DOM ratio increased from 0.0005 to 0.1 mg Cu (mg DOM\(^{-1}\)), the measured conditional binding constant (\(\delta K_{\text{CuDOM}}\)) decreased from \(10^{11.5}\) to \(10^{5.6}\) M\(^{-1}\).

A comparison of the binding constants measured using CLE-SPE with those measured by copper ion-selective electrode and voltammetry demonstrates that the Cu:DOM ratio is an important factor controlling Cu\(^{2+}\)-DOM binding strength even for DOM isolates of different types and different sources and for whole waters samples. The results were modeled using Visual MINTEQ and compared to results from the biotic ligand model (BLM). The BLM was found to over-estimate Cu\(^{2+}\) at low total copper concentrations and under-estimate Cu\(^{2+}\) at high total copper concentrations.
Introduction

The speciation of copper in water controls its bioavailability and aquatic toxicity (DiToro et al., 2001). Dissolved organic matter (DOM) plays an important role in the speciation of copper, but there is still much uncertainty about what controls the strength and formation of the copper-DOM complex. Many studies have shown that the toxicity of copper for fish, which is controlled by the interaction between free copper and the fish gill (DiToro et al., 2001), is reduced in the presence of DOM (Marr et al., 1999; Santore et al., 2001; De Schamphelaere and Janssen, 2004; Welsch et al., 2008). The biotic ligand model (BLM) uses site-specific water quality parameters such as pH, alkalinity, hardness, and DOM concentration to model copper speciation and predict copper toxicity. The BLM has been approved by the U.S. Environmental Protection Agency (U.S. EPA, 2007) to establish copper stream standards for the protection of aquatic life (Aiken et al., 2011; Paquin et al., 2002). Because DOM has been shown to significantly affect the speciation and toxicity of copper, the accurate treatment of copper-DOM binding constants, which are affected by factors such as pH, ionic strength, water chemistry, and the Cu:DOM ratio (Breault et al., 1996; Lu and Allen, 2002; Cabaniss and Shuman, 1988 I &II), in the BLM will improve assessments of copper toxicity in natural waters across a wide range of copper and DOM concentrations.

The reported binding strength of copper-DOM complexes by different investigators vary by over ten orders of magnitude, with measured conditional binding constants ($^{c}K_{CuDOM}$) values ranging from $10^{4.25}$ to $10^{18.7}$ M$^{-1}$ (Breault et al., 1996; Jin and Gogan, 2000; Witter et al., 1998; Bruland et al., 2000; Donat et al., 1994; McKnight et al., 1983; Brown et al., 1999). Comparing these values is complicated because conditional binding constants are operationally defined for both the experimental conditions and detection windows of the method used to make the
measurement. Most of the measurements of copper-DOM binding constants have been made by two methods: copper ion-selective electrode (Cu-ISE) and voltammetric titration methods. The Cu-ISE titrations (Breault et al., 1996; Lu and Allen, 2002; McKnight et al., 1983; Brown et al., 1999) have been conducted over a range of Cu:DOM ratios of 0.004 to 0.4 mg Cu (mg DOM)$^{-1}$. The results of the Cu-ISE titrations are typically modeled as copper binding by strong and weak sites on the DOM with binding constants of $10^{4.25}$ to $10^{6.4}$ M$^{-1}$ for the weak sites and $10^{7.0}$ to $10^{8.1}$ M$^{-1}$ for the strong sites. The response of the Cu-ISE becomes non-linear at total copper concentrations less than $10^{-6}$ M (Thermo Fisher Scientific, 2008), therefore, the measurement of accurate binding constants at lower and more environmentally relevant Cu:DOM ratios, which typically ranges from $10^{-5}$ to $10^{-3}$ mg Cu (mg DOM)$^{-1}$, is not possible using Cu-ISE (Blaedel and Dinwiddie, 1974; Rozan et al., 1999).

Various types of voltammetric titrations (anodic stripping voltammetry, competitive ligand equilibrium-adsorptive cathodic stripping voltammetry, differential pulse anodic stripping voltammetry, cathodic stripping voltammetry) have been used to determine copper-DOM binding constants at lower Cu:DOM ratios ($5 \times 10^{-6}$ to $3 \times 10^{-3}$ mg Cu (mg DOM)$^{-1}$) because voltammetric methods are about three orders of magnitude more sensitive than the Cu-ISE. Copper binding constants determined by voltammetry are greater than those determined by Cu-ISE titrations – the conditional binding constants range from $10^{8.8}$ to $10^{18.7}$ M$^{-1}$, with some of the variability due to differences in the experimental conditions (Jin and Gogan, 2000; Witter et al., 1998; Bruland et al., 2000; Kogut and Voelker, 2001). While voltammetry methods are able to measure binding constants at low Cu:DOM ratios, these techniques become saturated when the total copper exceeds sub-micromolar concentrations (Rozan et al., 1999). Some studies have used both voltammetric and Cu-ISE measurements (Rozan et al., 1999; Xue and Sunda, 1997),
but the overlap between binding constants determined by the two methods is less than one order of magnitude, making the results difficult to compare.

Various competitive ligand exchange-solid phase extraction (CLE-SPE) methods have been successful in measuring metal-DOM binding constants at low metal:DOM ratios (Sunda and Hanson, 1987; Miller and Bruland, 1995; Hsu and Sedlak, 2003; Black et al., 2007). The detection window for CLE-SPE is limited by the analytical detection limit for copper, and can be increased by changing the experimental conditions. In this study, we used a CLE-SPE method to measure copper-DOM binding constants over a range of Cu:DOM ratios. We also measured copper binding to six different DOM isolates and two whole water samples with significantly different DOM concentrations, compositions, and nitrogen contents, and compared the results to measurements by Cu-ISE and voltammetry methods across a wide range of Cu:DOM ratios in order to bridge the gap between detection windows.
Methods

Overview

Binding constants for copper and DOM were measured by CLE-SPE. In brief, C$_{18}$ resin was used as the solid phase to separate the copper-DOM complexes into two fractions operationally defined as the hydrophobic and hydrophilic fractions. The hydrophobic fraction of the DOM sorbs to the resin and is retained, whereas the hydrophilic fraction of the DOM does not interact with the resin and passes through the column (Hsu and Sedlak, 2003; Black et al., 2007). The addition of a hydrophobic competing ligand decreases the amount of copper passing through the resin, while the addition of a hydrophilic competing ligand increases the amount of copper passing through the resin. By comparing the amount of copper passing through the resin with and without competing ligand additions, the binding strength of copper with the hydrophobic and hydrophilic fractions of the DOM can be calculated. In this study, well-characterized hydrophobic (benzoylacetone (Bz)) and hydrophilic (nitrilotriacetic acid (NTA)) ligands with known copper stoichiometries and binding strengths were used at different concentrations, or analytical competition strengths [$\alpha$(CuAL)], to effectively compete with the DOM to bind copper.

Materials

Copper stock solutions were prepared by serial dilution of a Cu(NO$_3$)$_2$ standard (National Institute of Standards and Technology, NIST 3114) and preserved in 10% HNO$_3$. Trace metal-grade HNO$_3$ was used in the preservation of copper samples and stock solutions. A 0.1 M benzoylacetone stock solution was prepared daily in methanol. In order to monitor potential degradation of the copper-binding keto-enol functional group in the benzoylacetone, the UV absorbance of the stock solution was monitored at 250 and 312 nm (Hewlett Packard, model
8453) before each benzoylacetonate addition (Shoji et al., 1976; Iglesias, 1996). Stock solutions of 1 M NTA, 1 M sodium dihydrogen phosphate (NaH$_2$PO$_4$), 3 M sodium perchlorate (NaClO$_4$), and 1 M sodium hydroxide (NaOH) were prepared by dissolving the chemicals into ultrapure water (> 18 MΩ resistivity; Barnstead, Nanopure). All chemicals used were obtained from Fisher Scientific.

The SPE was performed in a glass column (1 cm diameter; Spectrum Chromatography) cleaned and packed with 0.5 g of C$_{18}$ resin (Supelco ENVI-18) by suspending the resin in methanol. The resin was cleaned prior to every extraction by a series of sequential rinses using ultrapure water and 5 mM HCl rinses for 20 min each at a flow rate of 4 mL min$^{-1}$ (Hsu and Sedlak, 2003).

**Dissolved Organic Matter Isolates**

Six different DOM fractions of varying origin and chemical composition were obtained using the procedures outlined in Aiken et al., (1992). Briefly, DOM fractions were isolated by retention on XAD-8 (hydrophobic acids, HPoA) or XAD-4 (transphilic acids, TPiA) resins, back elution using 0.1 N NaOH, hydrogen saturation, and freeze-drying.

The primary fraction used in this study was the hydrophobic acid fraction of NOM isolated from the Suwannee River (SR HPoA). Five additional fractions were selected for this study based on nitrogen to carbon ratio to determine if nitrogen-containing functional groups play a significant role in the strength and capacity of copper binding by DOM. Four of the five additional DOM fractions were HPoA and TPiA isolates from three different sites in the Florida Everglades: F1, 2BS, and E0 (Ravichandran et al., 1998; Ravichandran et al., 1999). The fifth additional fraction was a TPiA isolate from the Gulf of Maine (Table 2.1).
Table 2.1. Nitrogen and carbon content and nitrogen-to-carbon molar ratio (N/C) for the DOM isolates used in this study.

<table>
<thead>
<tr>
<th>DOM isolate type and source</th>
<th>nitrogen (wt%)</th>
<th>carbon (wt%)</th>
<th>N/C (mol mol$^{-1}$)</th>
<th>SUVA$_{254}$ (L (mg C)$^{-1}$ m$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suwannee River hydrophobic acid</td>
<td>0.78</td>
<td>51.77</td>
<td>0.013</td>
<td>5.2</td>
</tr>
<tr>
<td>Gulf of Maine hydrophobic acid</td>
<td>1.11</td>
<td>47.40</td>
<td>0.017</td>
<td>4.2</td>
</tr>
<tr>
<td>Everglades F1 site hydrophobic acid</td>
<td>1.77</td>
<td>52.70</td>
<td>0.029</td>
<td>3.1</td>
</tr>
<tr>
<td>Everglades 2BS site hydrophobic acid</td>
<td>1.91</td>
<td>51.66</td>
<td>0.032</td>
<td>3.2</td>
</tr>
<tr>
<td>Everglades F1 site transphilic acid</td>
<td>2.72</td>
<td>48.04</td>
<td>0.046</td>
<td>2.6</td>
</tr>
<tr>
<td>Everglades E0 site transphilic acid</td>
<td>3.11</td>
<td>48.14</td>
<td>0.055</td>
<td>2.8</td>
</tr>
</tbody>
</table>
Whole Water Samples

Whole water samples were collected and filtered (0.45 µm membrane capsule filter; Geotech Environmental Equipment Inc.) from two locations: the F1 site (Ravichandran et al., 1998) in the Florida Everglades on August 18, 2010, and Williams Lake in Minnesota on September 10, 2010. Water quality measurements in the lab included pH, major cations, major anions, and alkalinity. Sub-samples were acidified using 1% trace metal grade nitric acid for total copper analysis. The dissolved organic carbon (DOC) concentration was measured using a persulfate oxidation method (Oceanography International, model 400). Ultraviolet (UV) light absorbance at 254 nm wavelength (UV$_{254}$) was also measured to calculate the specific UV absorbance ($\text{SUVA}_{254}$, L mg C$^{-1}$ m$^{-1}$) of the whole water samples by dividing the UV$_{254}$ (m$^{-1}$) by the DOC concentration (mg C L$^{-1}$) (Table A1).

Solution Preparation

Copper-DOM solutions (250 mL) were prepared in HDPE bottles from stock solutions of DOM isolates. DOM stock solutions (100 mg C L$^{-1}$) were prepared by addition of the DOM isolate to deionized water and filtration through a 0.45-µm membrane filter to verify complete dissolution. The DOM concentration in each stock solution was determined by measuring the DOC of each stock solution and multiplying the DOC by two, since DOM contains approximately 50% carbon by mass. The ionic strength of each solution was adjusted to 0.01 M using NaClO$_4$, and the pH was held constant at 6.6 ± 0.1 using a 3 mM NaH$_2$PO$_4$ buffer adjusted with 1 M NaOH.

The copper-DOM solutions were prepared in slightly different ways for each of the three experiments (Table 2.2). The first experiment was designed to measure the hydrophobic and hydrophilic DOM binding constants. To achieve this result, a solution was prepared with a
Cu:DOM ratio of 0.001 mg Cu (mg DOM)$^{-1}$ (20 µg L$^{-1}$ total copper; 20 mg L$^{-1}$ DOM) and titrated with either benzoylacetone (100-1,000 µM; $\alpha$(CuBz$_2^0$) = 4.1-6.1) or NTA (1-10 µM; $\alpha$(CuNTA$^-$) = 4.1-5.1). The second experiment was designed to study the effect of DOM isolates with different nitrogen contents on the copper-DOM binding constant. In this experiment, the Cu:DOM ratio was held constant at 0.001 mg Cu (mg DOM)$^{-1}$ with a benzoylacetone concentration of 100 µM ($\alpha$(CuBz$_2^0$) = 4.1) using all six DOM isolates (Table 1). The third experiment was designed to measure the effect of Cu:DOM ratio on the copper-DOM binding constant. In this experiment, the benzoylacetone concentration was held constant at 100 µM ($\alpha$(CuBz$_2^0$) = 4.1) and the Cu:DOM was varied from 0.001-0.1 mg Cu (mg DOM)$^{-1}$.

Table 2.2. Summary of the CLE-SPE experimental conditions.

<table>
<thead>
<tr>
<th></th>
<th>Experiment 1</th>
<th>Experiment 2</th>
<th>Experiment 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu (mg L$^{-1}$)</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02-0.2</td>
</tr>
<tr>
<td>DOM (mg L$^{-1}$)</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Cu:DOM (mg Cu (mg DOM)$^{-1}$)</td>
<td>1×10$^{-3}$</td>
<td>1×10$^{-3}$</td>
<td>1×10$^{-3}$ - 1×10$^{-1}$</td>
</tr>
<tr>
<td>benzoylacetone (µM)</td>
<td>100-1,000</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>NTA (µM)</td>
<td>1-10</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>DOM isolate</td>
<td>Suwannee River hydrophobic acid</td>
<td>Six isolates (Table 1)</td>
<td>Suwannee River hydrophobic acid</td>
</tr>
</tbody>
</table>

The copper-DOM solution was equilibrated for an excess of 24 ± 2 h before the competitive ligand was added. The competitive ligand was equilibrated with the copper-DOM solution for another 2 h before passing through the SPE column at a flow rate of 4 mL min$^{-1}$. These equilibration times are consistent with or in excess of the equilibration times used by previous researchers (Donat et al., 1994; Sunda and Hanson, 1987). Experiments were
conducted in duplicate for each set of conditions and the standard deviation between each measurement was reported.

**Copper-Dissolved Organic Matter Binding Constant Determination**

The copper-DOM binding constants were determined using a modified CLE-SPE method similar to that used by previous researchers (Sunda and Hanson, 1987; Miller and Bruland, 1995; Hsu and Sedlak, 2003; Black et al., 2007). A 1:1 complex was assumed:

\[
K_{CuLDOM}^{c} = \frac{[CuL_{DOM}]}{[Cu^{2+}][L_{DOM}]} \tag{1}
\]

where \(K_{CuLDOM}^{c}\) is the conditional copper-DOM binding constant (M\(^{-1}\)), \([CuL_{DOM}]\) is the concentration of copper bound to ligands in the DOM (M), \([Cu^{2+}]\) is the free copper concentration (M), and \([L_{DOM}]\) is the concentration of ligands in the DOM that are not bound to copper (M). NTA forms a 1:1 complex with copper, and the CuNTA\(^{-}\) complex has a conditional binding constant of \(K_{CuNTA}\) of \(10^{10.1}\) M\(^{-1}\) (pH=6.6, I=0.01 M) (Martell and Smith, 1977).

Conditional binding constants were corrected for ionic strength using the Davies equation.

Benzoylacetone forms a 2:1 complex with copper:

\[
K_{CuBz2} = \frac{[Cu(Bz)_{2}^{0}]}{[Cu^{2+}][Bz^{-}]^{2}} \tag{2}
\]

The Cu(Bz)\(^{2}\)\(^{0}\) complex has a conditional stability constant of \(K_{CuBz2}\) of \(10^{12.1}\) M\(^{-2}\) (pH=6.6, I=0.01 M) (Martell and Smith, 1977). At equilibrium equations [1] and [2] gives the equilibrium expression for the Cu-DOM binding constant as determined by the competition with benzoylacetone:

\[
K_{CuLDOM}^{c} = \frac{K_{CuBz2}[CuL_{DOM}][Bz^{-}]^{2}}{[Cu(Bz)_{2}^{0}][L_{DOM}]} \tag{3}
\]
where \([\text{CuL}_{\text{DOM}}]\) is the concentration of copper that passes through the C_{18} resin bound to hydrophilic DOM (M), \([\text{Bz}^-]\) is the concentration of the deprotonated form of benzoylacetone (M), which was calculated for pH 6.6 from the total concentration and the pKa of benzoylacetone, \([\text{Cu(Bz)}_2^0]\) is the concentration of hydrophobic copper-benzoylacetone complex (M), and \([\text{L}_{\text{DOM}}]\) is calculated by the difference in copper concentration passing through the resin before and after benzoylacetone addition.

The concentration of the copper-benzoylacetone complex, \([\text{Cu(Bz)}_2^0]\), is calculated as a range because copper retained on the resin may be bound to either benzoylacetone or the hydrophobic fraction of the DOM. This uncertainty results in a range of values for the calculated copper-DOM binding constants. The maximum value for \([\text{Cu(Bz)}_2^0]\) was estimated by assuming that all of the copper retained on the resin after ligand addition (\([\text{Cu}]_{\text{hydrophobic, Bz}}\)) was bound to benzoylacetone:

\[
[\text{Cu(Bz)}_2^0]_{\text{max}} = [\text{Cu}]_{\text{hydrophobic, Bz}} \tag{4}
\]

A minimum value for \([\text{Cu(Bz)}_2^0]\) was estimated by assuming that the copper retained on the resin before ligand addition (\([\text{Cu}]_{\text{hydrophobic, no Bz}}\)) continues to be bound to the hydrophobic DOM.

\[
[\text{Cu(Bz)}_2^0]_{\text{min}} = [\text{Cu}]_{\text{hydrophobic, no Bz}} - [\text{Cu}]_{\text{hydrophobic, no Bz}} \tag{5}
\]

A detailed explanation of calculations necessary for equation [3] is found in the appendix. The equations for benzoylacetone are shown here as an example and the same equations apply for NTA.

**Sample Analysis**

The total copper concentration in the column eluent was measured using a graphite furnace atomic absorption spectrometer (GF-AAS; Perkin Elmer, model 4110ZL) which had a detection limit of approximately 1µg L^{-1} total copper. Samples for copper analysis were
collected and stored in acid-cleaned HDPE bottles and preserved using 1% trace metal grade HNO₃. Standards for the GF-AAS were prepared from the NIST Cu(NO₃)₂ standard and preservation in 1% HNO₃. The recovery of certified U.S. Geological Survey reference samples for trace metals (T187 (2.04 µg L⁻¹ ± 0.133), T181 (7.79 µg L⁻¹ ± 0.452), T169 (14.3 µg L⁻¹ ± 0.8)) were within ± 15% of the verified concentration, and the relative standard deviation for triplicate analysis was less than 5%.
Results

Copper-Dissolved Organic Matter Binding Constant Determination

The distribution of copper between the hydrophilic and hydrophobic fractions of the Suwannee River HPoA (relative to the C\textsubscript{18} resin) was determined. In the absence of a competing ligand, 57.3 ± 2.8% of the total copper passed through the resin, indicating the fraction of copper that was bound to hydrophilic copper-DOM complexes (Figure 2.1).

Figure 2.1. Concentration of copper normalized to the influent copper concentration ([Cu]/[Cu\textsubscript{0}]) eluting from a C\textsubscript{18} resin column in the presence of Suwannee River hydrophobic acid (SR HPoA), NTA, and benzoylacetone.
As benzoylacetone was titrated into the Suwannee River copper-DOM solution, the copper concentration that passed through the C_{18} resin decreased (Figure 2.1). Increases in the benzoylacetone concentration resulted in over an order of magnitude increase for the copper-DOM\textsubscript{hydrophilic} conditional binding constant (Table 2.3). Although there is some overlap in the ranges of calculated $^{c}K_{\text{CuDOM, hydrophilic}}$ values, the average conditional binding constant increased from $10^{11.8}$ to $10^{12.9}$ M\textsuperscript{-1} as the benzoylacetone concentration increased from 100 to 1000 µM ($\alpha$(CuBz\textsubscript{2}) = 4.1 – 6.1). At higher ligand concentrations, benzoylacetone was able to compete for copper bound to stronger sites, which demonstrates the distribution of copper binding sites in the DOM.
Table 2.3. Binding constant \( (^{c}K_{CuDOM}) \) ranges for copper and Suwannee River hydrophobic acid as a function of nitrilotriacetic acid (NTA) and benzoylacetone (Bz) concentration. The competition strength of the copper and the added ligand complex \([\alpha(CuAL)]\) is also shown for each ligand concentration. Copper binding to both the hydrophobic and hydrophilic (relative to the C\(_{18}\) resin) fractions of the DOM was measured.

<table>
<thead>
<tr>
<th>[NTA] (µM)</th>
<th>log ( \alpha(CuNTA) )</th>
<th>( ^{c}K_{CuDOM} ) (M(^{-1}))</th>
<th>[Bz] (µM)</th>
<th>log ( \alpha(CuBz) )</th>
<th>( ^{c}K_{CuDOM} ) (M(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.1</td>
<td>10.4-11.1</td>
<td>100</td>
<td>4.1</td>
<td>11.2-12.4</td>
</tr>
<tr>
<td>10</td>
<td>5.1</td>
<td>11.2-11.7</td>
<td>500</td>
<td>5.5</td>
<td>12.0-13.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>750</td>
<td>5.9</td>
<td>12.2-13.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1000</td>
<td>6.1</td>
<td>12.2-13.2</td>
</tr>
</tbody>
</table>

The hydrophilic ligand NTA was used to determine copper-DOM\(_{\text{hydrophobic}}\) binding constants. The breakthrough curves show that the concentration of copper passing through the C\(_{18}\) resin increased as the NTA concentration was increased (Figure 1). Binding constants were calculated for the copper-DOM\(_{\text{hydrophobic}}\) complex at each of the NTA concentrations (Table 3).

**Dissolved Organic Matter Isolates**

The copper-DOM\(_{\text{hydrophilic}}\) binding constant was measured for the DOM isolates listed in Table 1. The ranges of calculated copper-DOM\(_{\text{hydrophilic}}\) binding constants overlapped for all of the isolates at a Cu:DOM ratio of 0.001 mg Cu (mg DOM\(^{-1}\)) (Figure A1). The average copper-DOM\(_{\text{hydrophilic}}\) binding constant for all of the DOM isolates, regardless of the source or type of isolate, was \( ^{c}K_{CuDOM} = 10^{11.6 \pm 0.1} \) (one standard deviation for \( n = 6 \)).

**Effect of the Cu:DOM Ratio**

In order to assess the effect of the Cu:DOM ratio on the strength of copper binding, binding constants were measured for the Suwannee River HPoA isolate at Cu:DOM ratios.
ranging from 0.001 to 0.1 mg Cu (mg DOM)$^{-1}$. The measured binding constants decreased as the Cu:DOM ratio increased (Figure 2).

Figure 2. Dependence of the logarithm of the $cK_{CuDOM}$ values on the Cu:DOM ratio for the Suwannee River hydrophobic acid (SR HPoA) isolate and whole waters from the Florida Everglades F1 site and the Williams Lake, MN, site. All points shown are the averages of the range of binding constant values calculated by CLE-SPE.
The binding constants measured for the whole water samples from the F1 site in the Florida Everglades and Williams Lake showed the same dependence on the Cu:DOM ratio as observed for the DOM isolates. Copper was added to the Williams Lake water to produce Cu:DOM ratios of 0.001 and 0.006 mg Cu (mg DOM)^{-1}, which resulted in binding constants of $10^{11.9}$ and $10^{10.8}$ M^{-1}, respectively. The copper binding constant of the DOM in the F1 water sample was measured at Cu:DOM ratios of 0.0003 and 0.004 mg Cu (mg DOM)^{-1}, which resulted in binding constants of $10^{13.3}$ and $10^{10.7}$ M^{-1}, respectively. The whole waters have different water chemistries, DOM concentrations, and SUVA$_{254}$ (a proxy for the aromaticity of the DOC; Weishaar et al., 2003) values (Table A1). The water from the F1 site had a DOC concentration of 25 mg C L^{-1} and a SUVA$_{254}$ value of 3.6 L mg C^{-1} m^{-1}. The Williams Lake water had a DOC concentration of 3.5 mg C L^{-1} and SUVA value of 1.2 L mg C^{-1} m^{-1}. 
Discussion

Role of Cu:DOM Ratio

The Cu$^{2+}$-DOM binding constants were measured over a range of Cu:DOM ratios that overlap those measured by voltammetric and Cu-ISE titrations. The measured binding constants displayed a strong dependence on the Cu:DOM ratio consistent with those of previous studies (Figure 3). A similar relationship has been observed between conditional binding constants and complexation capacities for a variety of DOM sources (Town and Filella, 2000). While this empirical relationship between operationally defined binding constants and the Cu:DOM ratio does not identify specific binding site concentrations and capacities, it provides a framework for studying and modeling the interactions between copper and DOM from various sources. We compared the binding constants we determined using CLE-SPE to binding constants determined using voltammetric titrations (Jin and Gogan, 2000; Witter et al., 1998; Bruland et al., 2000), Cu-ISE titrations (Breault et al., 1996; McKnight et al., 1983; Brown et al., 1999) and those obtained by an approach similar to CLE-SPE, where ligand competition and SEP-PAK C$_{18}$ cartridge adsorption was used to measure the Cu$^{2+}$ concentration in seawater samples (Sunda and Hanson, 1987). The studies used in this comparison span the range of Cu$^{2+}$-DOM binding constants reported in the literature ($10^{4.25}$ to $10^{18.7}$ M$^{-1}$) (Breault et al., 1996; Jin and Gogan, 2000; Witter et al., 1998; Bruland et al., 2000; Donat et al., 1994; McKnight et al., 1983; Kogut et al., 2001; Brown et al., 1999) and results were chosen based upon availability of provided copper and DOM concentrations. For simplicity, only seven studies were included, but the excluded results reported similar dependence of the Cu$^{2+}$-DOM binding constants on the Cu:DOM ratio (Figure A2; Breault et al., 1996; Lu and Allen, 2002; Jin and Gogan, 2000; Witter et al., 1998; Bruland et
al., 2000; McKnight et al., 1983; Kogut et al., 2001; Brown et al., 1999; Sarathy and Allen, 2005; Louis et al., 2009).

![Figure 2.3](image)

Figure 2.3. Summary of copper-DOM binding constants from the literature as a function of the Cu:DOM ratio. The voltammetry results are shown as open symbols and the Cu-ISE results are shown as horizontal striped symbols. The results from the SEP-PAK sorption study are shown with the diagonally striped symbols. The results measured by CLE-SPE in this study for both the DOM isolates and whole waters are shown with the solid squares.

The Cu:DOM ratio was not explicitly reported in any of the previous studies, so we calculated the Cu:DOM ratios using the copper and DOM concentrations reported in these studies, when available. The three voltammetric titration studies and the SEP-PAK study reported concentrations of copper-binding ligands (M). If it is assumed that all sites of a given binding strength are occupied by Cu$^{2+}$, the stoichiometry of Cu:L$_{DOM}$ is 1:1, and that Cu$^{2+}$ and inorganic copper complexes are negligible, the concentration of copper-binding ligands is equal to the bound copper concentration. Using this assumption, the reported ligand concentration was
converted to a total copper concentration. If the DOC concentration was reported, the DOM concentration was estimated to be twice that value. The DOC or DOM concentration was not reported in three of the sources used in the comparison, so we estimated values based on reported values for water samples collected from similar locations (Table A2) (Louis et al., 2009; Korshin et al., 1998; Croue et al., 2003; Harvey, 1983; Zhou and Wangersky, 1989; Retamal et al., 2007). Estimation of the DOM concentration might result in errors in the estimated Cu:DOM ratio of about a factor of two, but these errors will not significantly change the results because the results cover such a wide range of Cu:DOM ratios. The three Cu-ISE studies used in this comparison reported the concentration of copper-binding sites (M) per mg of DOC. The concentration of binding sites was converted to the maximum concentration of copper, and the DOC value was converted to DOM.

The same dependence of copper-DOM binding constants on the Cu:DOM ratio as seen with the binding constants measured by CLE-SPE was observed for the seven other published copper-DOM binding studies. These studies include three different analytical methods over a Cu:DOM range of approximately five orders of magnitude (Figure 3). The binding constants measured using the CLE-SPE method span a much greater range of Cu:DOM (0.0005 to 0.1 mg Cu (mg DOM)$^{-1}$) than the overlap in Cu:DOM between voltammetric (DPASV) and Cu-ISE titrations of only a single order of magnitude examined by a previous study (0.0003 to 0.001 mg Cu (mg DOM)$^{-1}$) (Rozan et al., 1999).

The results used in the binding constant comparison (Figure 3) were collected under widely varying experimental conditions, including using DOM derived from different sources, whole waters and isolates, and varying water quality (pH, ionic strength, hardness) conditions. The pH for the CLE-SPE results was 6.6 and the pH for the comparison studies ranged from 6 to
Differences in pH are expected to cause variation in binding constant values due to protonation of binding sites and increased competition with protons at lower pH. Other variations may be caused by differences in ionic strength (I) from 0.001 to approximately 0.7 M and water hardness (expressed as CaCO$_3$) from <1 to approximately 800 mg L$^{-1}$. In hard waters, high concentrations of calcium and magnesium may compete for binding sites in the DOM and reduce the concentration of available sites for copper binding (Breault et al., 1996).

At low Cu:DOM ratios, copper binds to the strongest ligands in the DOM, which may be nitrogen-containing functional groups (Korshin et al., 1998; Croue et al., 2003; Frenkel et al., 2000). Model compounds containing nitrogen, such as 8-hydroxyquinoline ($K_1=10^{12.0}$, $K_2=10^{22.9}$; 25°C, I = 0.1 M; Martell and Smith, 2004) and EDTA ($K_1= 10^{18.87}$; 25°C, I = 0.1 M; Martell and Smith, 2004) have similar binding constants with copper as what was measured for DOM at low Cu:DOM ratios. Strong binding to reduced sulfur functional groups may also be important at low Cu:DOM ratios. For example, strong binding has been observed for Cu$^+$-thiol complexes with glutathione ($K= 10^{13.2}$; pH=8.35, seawater; Leal and van der Berg, 1998) and cysteine ($K= 10^9$; pH=8.35, seawater; Leal and van der Berg, 1998) consistent with Cu-thiol measurements in natural waters (Leal and van der Berg, 1998; Laglera and van der Berg, 2003). Since Cu$^{2+}$ is not known to interact with thiols (Martell and Smith, 2004; Leal and van der Berg, 1998), Cu-thiol binding is thought to result from Cu(II) reduction to Cu(I) and strong Cu$^+$-thiol binding (Leal and van der Berg, 1998). The binding strength of the weaker sites suggests that they consist of oxygen-containing functional groups, such as carboxyl and phenol groups. The binding constants measured at high Cu:DOM ratios are comparable to those measured for copper binding to carboxyl-containing compounds, such as phthalic acid ($K_1= 10^{3.22}$, $K_2=10^{5.5}$; 25°C, I = 0.1 M; Martell and Smith, 2004). Copper binds to phenol groups more strongly than
carboxyl groups, but less strongly than some nitrogen-containing functional groups, consistent with the decrease in the binding constant as the Cu:DOM ratio increases. For example, copper and salicylic acid, which contains both a carboxyl and phenol functional group, has a binding constant of $10^{10.62}$ (25°C, I= 0.1 M; Martell and Smith, 2004).

The CLE-SPE method has the potential to measure copper-DOM binding constants over a larger Cu:DOM range than measured in this study by changing the concentration or the type of the competing ligand. Because of the ability to use CLE-SPE over a wide range of Cu:DOM ratios, CLE-SPE is a valuable method for studying metal-DOM interactions in many environments ranging from natural waters with extremely low copper concentrations to waters affected by industrial or mining activities that may contain high copper concentrations (Sunda and Hanson, 1987; Miller and Bruland, 1995; Hsu and Sedlak, 2006; Black et al., 2007).

Effects of DOM Type

Our data show that the binding strength at a given Cu:DOM ratio is similar regardless of the DOM source or characteristics. When both the hydrophilic and hydrophobic fractions of the same DOM isolate were measured at the same Cu:DOM ratio, the ranges of calculated binding constants overlapped. This indicates that the two DOM fractions contain copper binding sites of similar strength and that the strongest binding sites are distributed throughout both fractions of the DOM. The calculated ranges of binding constants from CLE-SPE measurement of DOM isolates with nitrogen:carbon ratios ranging from 0.013 to 0.055 all overlapped, which indicates that there is not a detectable dependence on the nitrogen content at a fixed Cu:DOM ratio of 0.001 mg Cu (mg DOM)$^{-1}$ (Figure A1). While there may be a difference in copper binding strength at lower Cu:DOM ratios than reported here, at the ratio studied, the DOM isolate with the lowest nitrogen:carbon ratio had nitrogen-containing binding sites in excess of the copper
concentration by almost an order of magnitude. These findings are consistent with a past study which reported that differences due to DOM type are small compared to differences in ionic strength and pH (Cabaniss and Shuman, 1988 II).

Even though the nitrogen:carbon ratio did not affect the strength of copper binding to the DOM isolates measured, there may be a specific form of nitrogen that could coordinate copper in specific conformations to result in very strong metal binding. The types of nitrogen-containing functional groups in DOM have been studied using nitrogen K-edge x-ray absorption near edge structure (XANES) spectroscopy. Vairavamurthy and Wang, (2002) detected four peaks in a series of marine sediments and humic acid isolates that corresponded to four nitrogen-containing functional groups: pyridines, oxidized pyridines, amide compounds, and nitro compounds. The amide compounds were the dominant group in all measured samples. Thorn and Cox, (2009) also identified amides and aminoquinones as the dominant nitrogen-containing functional group using $^{15}$N NMR on soil humic and fulvic acid samples.

In order to determine which forms of nitrogen may be responsible for strong copper binding, copper-DOM binding constants were compared to binding constants for model compounds with the most abundant nitrogen-containing functional groups found in natural organic matter samples (asparagine and 8-hydroxyquinoline). Whereas model compounds provide a frame of reference for copper binding constants, they have not been explicitly identified in DOM and are only used in comparison in order to gain mechanistic insight into types of binding sites that are likely present in DOM. Asparagine, which contains an amide functional group, has copper binding constants of $K_1=10^{7.8}$, and $K_2=10^{14.4}$ (25°C, I=0.1 M; Martell and Smith, 2004). In contrast, copper binds more strongly ($K_1=10^{12.0}$, $K_2=10^{22.9}$; 25°C, I=0.1 M; Martell and Smith, 2004) with an aminoquinone compound, 8-hydroxyquinoline.
Porphyrin compounds which contain nitrogen as amino groups have also been reported to be an important class of compounds present in DOM at low abundance (Vairavamurthy and Wang, 2002). These compounds have highly specific metal coordination sites, which may result in very strong binding of copper at low Cu:DOM ratios, and their low abundance in DOM could explain their absence in the XANES results. EDTA ($K_1 = 10^{18.87}$, 25°C, I=0.1 M; Martell and Smith, 2004) is a synthetic model compound that provides an example how strong copper binding can result from a highly specific coordination site that may be present in DOM at very low concentrations.

**Implications for the Biotic Ligand Model**

The results presented here have implications for the BLM and its prediction of copper-DOM binding in natural waters (Di Toro et al., 2001; Santore et al., 2001). A number of studies have compared BLM-predicted toxicity results with results from laboratory toxicity studies (Ng et al., 2010; Chen et al., 2010) and found that the BLM tends to under-predict copper toxicity (Welsch et al., 2005; De Schamphelaere and Janssen, 2004). The copper binding constants measured by CLE-SPE in this study were used to predict Cu$^{2+}$ concentrations for the F1 site in the Florida Everglades using Visual MINTEQ (Gustafsson, 2009) and the calculated Cu$^{2+}$ concentrations were compared to the results from the BLM, which uses the Windermere Humic Aqueous Model (WHAM; Tipping, 1994) to model Cu-DOM interactions (Figure 4). Visual MINTEQ was chosen to model the experimentally determined binding constants over a range of Cu:DOM ratios, because WHAM does not allow for the input of binding constants. A model ligand was used in the MINTEQ calculations to represent DOM, and its binding constant was adjusted according to the observed relationship with the Cu:DOM ratio for each total copper concentration. The water quality parameters for the Everglades site were used as inputs to both
codes (Table A3). Binding constants for the major copper-inorganic complexes (Santore et al., 2001; Gustafsson, 2009) are shown for both models in Table A4 and any differences between the inorganic binding constants used in the BLM and Visual MINTEQ are small enough to not cause a significant difference in the calculated Cu\(^{2+}\) concentration.

Figure 2.4. Free copper concentration ([Cu\(^{2+}\)]) as a function of the total dissolved copper ([Cu\(_T\)]) with free copper concentrations calculated using the biotic ligand model (BLM) with its copper-DOM binding constants and Visual MINTEQ with copper-DOM binding constants determined by CLE-SPE in this study for the water at the Florida Everglades F1 site. Water chemistry inputs for both models are shown in Table A3 and binding constants for the inorganic species are shown in Table A4.

At total dissolved copper concentrations less than 20 \(\mu g\) L\(^{-1}\) (3\(\times\)10\(^{-7}\) M), the BLM estimated higher Cu\(^{2+}\) concentrations than the MINTEQ model developed using copper-DOM binding constants determined in this study (Figure 4). At total dissolved copper concentrations greater than 20 \(\mu g\) L\(^{-1}\), the opposite was found – the BLM estimated lower Cu\(^{2+}\) concentrations.
This result is consistent with the observed under-prediction of copper toxicity at Cu:DOM ratios greater than approximately $10^{-3}$ mg Cu (mg DOM)$^{-1}$ (De Schamphelaere and Janssen, 2004). This cross-over point will change based on the DOM concentration in different environments, but for the Everglades F1 site water with a low Cu:DOM ratio of $4 \times 10^{-5}$ mg Cu (mg DOM)$^{-1}$, the BLM predicted higher Cu$^{2+}$ concentrations than the MINTEQ model. The over-estimation of Cu$^{2+}$ by WHAM at low total copper concentrations is consistent with results from a previous study (Ndungu, 2012). By refining the DOM component of the BLM consistent with the continuous Cu:DOM ratio dependence found in this study, the accuracy of the BLM toxicity predictions could be improved over all Cu:DOM ratios.
References


Chapter 3

Laboratory Measurement of Copper-Dissolved Organic Matter Complexation and Its Relevance to Fish Toxicity in Streams Draining the Pebble Deposit in Alaska

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Abstract

Cupric ion-selective electrode (Cu-ISE) titrations and competitive ligand exchange-solid phase extractions (CLE-SPE) were used to estimate Cu$^{2+}$ binding affinities and capacities of dissolved organic matter (DOM) isolated from headwater streams draining the Pebble deposit in southwestern Alaska. The Cu-ISE titrations were conducted over a Cu:DOM range of 0.0016-0.26 mg$_{\text{Cu}}$ mg$_{\text{DOM}}^{-1}$ and the CLE-SPE experiments were conducted over a Cu:DOM range of 0.0005-0.011 mg$_{\text{Cu}}$ mg$_{\text{DOM}}^{-1}$. The Cu-ISE titrations, which were fit to a discrete, two-ligand model using FITEQL, resulted in conditional binding constants of $10^{6.9}$-$10^{7.9}$ ($cK_1$) and $10^{4.7}$-$10^{6.0}$ M$^{-1}$($cK_2$). The CLE-SPE experiments were determined as a single conditional binding constant with a range of $10^{8.6}$-$10^{11.3}$ M$^{-1}$. For both methods, the affinity of copper binding by DOM increased as the Cu:DOM ratio decreased, which indicates an increasing abundance of copper-binding sites of decreasing affinity in the DOM. Two alternative modeling frameworks, the biotic ligand model (BLM) and Visual MINTEQ, were used to evaluate the potential site-specific toxicity of Cu$^{2+}$ to rainbow trout. At copper concentrations greater than 5 µg L$^{-1}$, Cu$^{2+}$ concentrations predicted by the BLM were lower those predicted by Visual MINTEQ, which suggests that the BLM over-estimates the amount of organically-complexed Cu$^{2+}$ and under-estimates free copper toxicity to salmonids in the Pebble watersheds under conditions of increasing copper concentrations that could exist during or after mine development.
Introduction

The toxicity of copper to aquatic life depends on the speciation of copper. Free copper (Cu\(^{2+}\)) is the form of copper that is thought to be the most bioavailable and to have the greatest influence on the toxicity of copper to salmonids and other aquatic biota (Campbell, 1995; Paquin et al., 2002). Although a number of dissolved constituents can influence the speciation of copper, the primary controlling variables in freshwater include calcium, pH, and dissolved organic matter (DOM) (MacRae et al., 1999b; Welsh et al., 2008). These substances influence copper toxicity because of the formation of less toxic organic and inorganic complexes and competitive interactions with metal uptake sites on gill tissues (Meyer et al., 2007). As a result of these processes, copper toxicity tends to be mitigated in harder waters at near-neutral pH values and in waters with higher DOM concentrations.

The Pebble project, a world-class porphyry copper-gold-molybdenum deposit in southwestern Alaska, is located in the headwaters of the Nushagak River and Kvichak River watersheds that drain into Bristol Bay. The Pebble deposit contains copper, gold, and molybdenum in sulfide minerals, including pyrite and chalcopyrite, and silver, arsenic, antimony, and other metals and metalloids occur irregularly in the surrounding soils (Eppinger et al., 2010). Many metals (e.g., cadmium, zinc, lead) and metalloids (e.g., arsenic, selenium) could be released to the environment from Pebble project wastes and could harm salmonids and other aquatic biota. Copper is a special concern because the concentrations of copper in Pebble waste leachates are generally higher than any other metal or metalloid (PLP, 2011a) and because copper is particularly toxic to fish and invertebrates (Meyer et al., 2007). Bristol Bay is the largest sockeye salmon fishery in the State of Alaska (Clark et al., 2006) and is also an important subsistence fishery for native communities (Westing, 2006). In addition to lethality to aquatic
biota that can be caused at higher concentrations, small increases in copper concentrations can adversely affect the olfactory systems of salmonids and inhibit prey avoidance and the return to home spawning drainages (McIntyre et al., 2008; 2012). An understanding of the bioavailability and toxicity of copper to salmonids in site waters in the vicinity of the Pebble project is critical to the evaluation of the potential environmental consequences of mine development.

To reflect the influence of site-specific water quality conditions on copper toxicity to aquatic biota, the U.S. Environmental Protection Agency (EPA) recommends the use of the Biotic Ligand Model (BLM) to calculate site-specific water quality standards (U.S. EPA, 2007). The BLM is comprised of CHESS (Santore and Driscoll, 1995), a geochemical speciation code that calculates inorganic metal speciation; WHAM V (Tipping, 1994), a code that calculates the degree of Cu$^{2+}$ interaction with DOM; binding constants for the Cu$^{2+}$-biotic ligand (e.g., the fish gill) complex; and copper lethal accumulation ($LA_{50}$) values, which remain constant for a given aquatic biota regardless of water quality (Di Toro et al., 2001). The main purpose of the BLM is to predict the concentration of total dissolved copper that would cause toxicity to aquatic life under a range of water quality conditions. Although the BLM is an improvement over the hardness-based aquatic life criteria because it considers all major dissolved ions, the treatment of DOM in the model has been questioned by a number of researchers, based in part on inconsistencies between predicted and observed toxicity values (De Schamphelaere et al., 2004; Welsh et al., 2008).

The objective of this research was to measure the Cu$^{2+}$-DOM binding characteristics in streams that drain the Pebble deposit and to use the measurements to evaluate the implications for copper toxicity to salmonids. The studies were conducted to simulate a range of copper concentrations and Cu:DOM ratios that would be representative of current background
conditions and those that could result if stream copper concentrations increased as a result of mining. We used the results from the Cu$^{2+}$-DOM binding studies and water chemistry results in two codes, the BLM and Visual MINTEQ (Gustafsson, 2009), to evaluate potential fish toxicity in the Pebble deposit area.
Methods

Controlled laboratory studies were performed to assess the Cu$^{2+}$ binding characteristics of DOM in the three main drainages at the site: North Fork Koktuli River, South Fork Koktuli River, and Upper Talarik Creek (Figure 3.1). Adding copper to site-specific DOM simulated the addition of dissolved copper to the streams under mining conditions and allowed us to estimate the strength, abundance, and stability of Cu$^{2+}$-DOM complexes. We used the BLM (Version 2.2.3; HydroQual, 2007) and the geochemical speciation code Visual MINTEQ (Gustafsson, 2009) to estimate the effect of Cu$^{2+}$ binding to DOM on the concentration of free copper and copper toxicity.
Sample Collection and Analysis

Existing information on the geographic distribution of dissolved copper, hardness, and dissolved organic carbon (DOC) concentrations, salmonid habitat and population data, and information about the location of the mineral deposit were used to select water sampling locations for the study. Samples were collected from each major watershed at the site (Figure 3.1): North Fork Koktuli River (NK), South Fork Koktuli River (SK), and the Upper Talarik Creek (UT). Water samples for the copper binding studies were collected on June 7-8, 2010.
flown by helicopter to Anchorage, and shipped on ice to the U. S. Geological Survey in Boulder, Colorado, USA, for DOM isolation and copper binding experiments and to Columbia Analytical Services (CAS) in Kelso, Washington, USA, for chemical analysis. Samples for copper binding studies (50 L at each location) were collected in pre-washed high-density polyethylene bottles (10 L). Samples for chemical analysis were either unfiltered for determination of total metals, major cations, total suspended solids, and total cyanide, or were filtered in the field (0.45 µm acrylic copolymer coated membrane capsule filter; Geotech Environmental Equipment, Inc.) for determination of dissolved metals, major anions, DOC, and total dissolved solids. Dissolved and total metal and major cation samples were preserved with trace metal-grade nitric acid to pH < 2, and DOC samples were preserved with sulfuric acid to pH < 2.

Unfiltered samples were analyzed for total metals and metalloids (Al, As, Cd, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sb, Se, U, and Zn). Filtered samples were analyzed for dissolved metals and metalloids (same as total), major cations and anions (Na⁺, K⁺, Ca²⁺, Mg²⁺, Cl⁻, SO₄²⁻, and alkalinity), DOC, nitrate, nitrite, ammonia, and total dissolved solids. Specific conductance, pH, and temperature were measured in the field.

Na, K, Ca, Mg, and Fe were determined using inductively coupled plasma-atomic emission spectroscopy (ICP-AES; EPA Method 200.7; U.S. EPA, 2001); Al, As, Cd, Cr, Cu, Mn, Mo, Ni, Pb, Sb, Se, U, and Zn were determined by inductively coupled plasma-mass spectrometry (ICP-MS; EPA Method 200.8; U.S. EPA, 1994). Chloride and sulfate were determined using ion chromatography (Method 300.0; U.S. EPA, 1993a). Total, bicarbonate, and carbonate alkalinity were determined by titration (Standard Method 2320B; APHA, 1998). DOC was determined using the persulfate oxidation method on a total organic carbon analyzer (Oceanography International, model 700). Automated colorimetry was used to determine
cyanide and nitrate-nitrite concentrations (EPA Methods 335.4 and 353.2; U.S. EPA, 1993b, 1993c). Ammonia was determined using the automated phenate method (Standard Method 4500-NH3 G; APHA et al., 1998). Total dissolved and total suspended solids were determined by weight after filtration and drying at 180°C (Standard Methods 2540C and D; APHA et al., 1998). Filtered surface water sub-samples were concentrated by rotary evaporation (approximately 10×) for use in copper titration experiments (Table 3.1). The precipitation of solids was not observed in the concentrated water samples. The possibility of solid precipitation was checked for each concentrated water sample using Visual MINTEQ; no solids exceeded their solubility in the concentrated samples. Trip, laboratory, and equipment blanks were collected and analyzed as part of the water quality sampling effort.

Table 3.1. Experimental conditions for titration of whole-water samples (concentrated and buffered)

<table>
<thead>
<tr>
<th>Sample site</th>
<th>Initial DOM concentration&lt;sup&gt;a&lt;/sup&gt; (mg L&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Concentrated DOM concentration (mg L&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Concentration Factor</th>
<th>Cu concentration added (µg L&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Cu:DOM ratio (mg&lt;sub&gt;Cu&lt;/sub&gt; mg&lt;sub&gt;DOM&lt;/sub&gt; &lt;sup&gt;-1&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>North Fork Koktuli</td>
<td>4.0</td>
<td>38.4</td>
<td>9.6</td>
<td>64–6,400</td>
<td>1.7×10&lt;sup&gt;-3&lt;/sup&gt;–170×10&lt;sup&gt;-3&lt;/sup&gt;</td>
</tr>
<tr>
<td>Upper Talarik</td>
<td>2.6</td>
<td>24.6</td>
<td>9.5</td>
<td>64–6,400</td>
<td>2.6×10&lt;sup&gt;-3&lt;/sup&gt;–260×10&lt;sup&gt;-3&lt;/sup&gt;</td>
</tr>
<tr>
<td>South Fork Koktuli</td>
<td>4.4</td>
<td>39.8</td>
<td>9.0</td>
<td>64–6,400</td>
<td>1.6×10&lt;sup&gt;-3&lt;/sup&gt;–160×10&lt;sup&gt;-3&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>DOM concentration is based on the assumption that the DOC makes up 50% of the DOM.

**Characterization and Isolation of DOM**

The absorbance of light at 254 nm was measured on filtered samples using an ultraviolet/visible spectrophotometer (Hewlett Packard, model 8453). The specific ultraviolet
absorbance (SUVA$_{254}$) was calculated by dividing the ultraviolet absorbance at 254 nm by the DOC concentration (L mg$_c$-1 m$^{-1}$). DOM was isolated from the Pebble site waters using XAD resins (XAD-8, acrylic ester; XAD-4, styrene divinylbenzene; Amberlite, Rohm & Haas), following the method of Aiken et al. (1992). The XAD-8 resin retains hydrophobic acids (HPoA; aquatic humic substances). The XAD-4 resin retains transphilic acids (TPiA). The resins were cleaned with three successive rinses of 0.1 N NaOH and 0.1 N HCl immediately before use. The resin columns were arranged in series and connected with Teflon® tubing. Each of the three samples was acidified to pH 2 and passed through the column pair. The columns were disconnected and back-eluted with 0.1 N NaOH, and the eluates were acidified with concentrated HCl to pH 2. Cations were removed using a hydrogen-saturated cation-exchange resin. The isolated fractions from each resin were freeze-dried and stored in jars at room temperature. The hydrophobic acid DOM isolates from the three sample sites were used for the laboratory copper binding studies.

**Laboratory Copper Binding Studies**

Copper titration and competitive binding studies were conducted to evaluate Cu$^{2+}$-DOM complexation at the three sampling locations. Titration studies were performed on site water samples and on laboratory waters with isolated site organic matter. Competitive ligand exchange-solid phase extraction (CLE-SPE) experiments were performed using laboratory waters with different concentrations of isolated site organic matter. All calculated binding constants are conditional for the specific pH and ionic strength (I) conditions of the experiment (Table 3.2).
Table 3.2. Cu:DOM ratio, pH, and ionic strength conditions for Cu-ISE titration and CLE-SPE measurements.

<table>
<thead>
<tr>
<th>Cu-ISE Titrations</th>
<th>Cu:DOM (\text{mg}<em>{\text{Cu}} \text{mg}</em>{\text{DOM}}^{-1})</th>
<th>pH</th>
<th>Ionic Strength (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentrated, site water</td>
<td>1.6\times10^{-3} - 260\times10^{-3}</td>
<td>7.3</td>
<td>0.010 - 0.015</td>
</tr>
<tr>
<td>Isolated DOM</td>
<td>1.6\times10^{-3} - 160\times10^{-3}</td>
<td>6.0</td>
<td>0.010 - 0.015</td>
</tr>
</tbody>
</table>

| CLE-SPE | 0.0005, 0.002, 0.011 | 6.0 | 0.010 |

Copper titrations

Copper titrations were performed on (1) filtered water samples from all three drainages that were rotary-evaporated to increase DOM concentrations and (2) laboratory waters with isolated DOM from the three sampling locations.

The copper titration solution was prepared by making sequential dilutions of a 1,000 mg L\(^{-1}\) (15.9 mM) Cu(NO\(_3\))\(_2\) solution preserved in 10% trace metal grade nitric acid. A cupric ion-selective electrode (Cu-ISE; Thermo Scientific Orion, model 94-29), reference electrode (Thermo Scientific Orion double-junction Ag/AgCl), and an ISE meter (Orion, model 290 A+) were used to measure Cu\(^{2+}\) in solution as the total copper concentration was increased. The reference electrode contained an inner filling solution of saturated AgCl in KCl and an outer filling solution of 10% KNO\(_3\). The Cu-ISE was solid-state; the electrode surface was polished before each titration sequence using polishing strips.

The Cu-ISE was calibrated with an ethylenediamine solution according to the method outlined by Avdeef et al. (1983). Changes in pH were used to lower the Cu\(^{2+}\) concentration.
while maintaining a total copper concentration of 1 mM. This calibration provided a Nerstian response over a range of \( pCu^{2+} \) from 3-17. The calibration was checked weekly; it consistently held a Nernstian response with a slope of \( 29.8 \pm 0.3 \text{ mV decade}^{-1} \). The detection limit for the Cu-ISE was controlled by the total copper concentration, which must be \( 10^{-6} \) M or greater to obtain an accurate \( Cu^{2+} \) measurement (Thermo Fisher Scientific, 2008).

Each sample to be titrated was added to a glass beaker that was acid-cleaned using trace metal-grade 10% HCl and 10% HNO\(_3\) before each titration. The beaker was covered in foil to block light and prevent photo-induced interferences at the electrode surface. The solution was stirred constantly using a magnetic bar and stir plate. The electrode potential was monitored during each \( Cu(NO_3)_2 \) addition. After approximately 2-3 min, the change in the electrode potential reading was < 0.2 mV; equilibrium readings were recorded after 5 min. The pH was monitored using a pH electrode (Beckman Coulter, calomel pHree electrode) and meter (Beckman Coulter, 400 series). The pH was recorded at the same time as the electrode potential.

**Concentrated site water titrations**

After concentration by rotary-evaporation, samples were buffered at pH 7.3 using 5 mM 3-(N-morpholino)propanesulfonic acid (MOPS) (\( pK_a = 7.2 \)), and the ionic strength was adjusted (\( I = 0.01 \) M) using sodium perchlorate (NaClO\(_4\)) and increased to \( I = 0.015 \) M due to the addition of \( Cu(NO_3)_2 \) over the course of the titration. The pH was chosen because it is similar to the measured field pH of the three water samples. The copper was added as 20 or 200 \( \mu L \) additions of 5 mM \( Cu(NO_3)_2 \) in 3.2% trace metal-grade nitric acid to result in total copper concentrations ranging from \( 10^{-6} \) to \( 10^{-4} \) M. The program FITEQL was used to fit the titration curves and to calculate the conditional \( Cu^{2+} \)-DOM binding constants (\( ^c K \)) and binding site or ligand concentrations ([L]).
Titrations with isolated DOM

For the experiments measuring copper binding to DOM isolates, each experimental solution was buffered to pH 6.0 to eliminate the formation of copper hydroxide precipitates. Titrations were conducted on 100 mL of solution containing 20 mg L$^{-1}$ of isolated DOM (about 10 mg$_C$ L$^{-1}$ DOC) and buffered to pH 6.0 with 5 mM 2-(N-morpholino)ethanesulfonic acid (MES). The buffer was selected based on its acid dissociation constant ($pK_a = 6.5$) and inability to bind copper (Mash and Chin, 2003). Copper was added in 20 or 200 µL increments of 5 mM Cu(NO$_3$)$_2$ in 3.2% trace metal-grade nitric acid to create a range of total dissolved copper concentrations from 0.064 to 6.4 mg L$^{-1}$ ($10^{-6}$ to $10^{-4}$ M) and Cu:DOM ratios of 0.0016 to 0.16 mg$_{Cu}$ L$^{-1}$: mg$_{DOM}$ L$^{-1}$. Sodium hydroxide (NaOH) was added after each copper addition to maintain a constant pH of 6.0 over the course of the titration. The ionic strength of the solution was adjusted to 0.01 M with NaClO$_4$ and increased to 0.015 M over the course of the titration. The ionic strength increase was calculated using Visual MINTEQ. The elevated ionic strength was necessary to obtain accurate readings from the Cu-ISE. Each titration was conducted in duplicate.

Determination of conditional binding constants for Cu-ISE titrations

Results from the titrations using concentrated site water at pH 7.3 and laboratory water with isolated DOM at pH 6.0 were modeled using the program FITEQL (Westall, 1982) to determine conditional binding constants. A discrete two-ligand model was found to provide the minimum number of ligands ($L_1, L_2$) and conditional binding constants ($^cK_1, ^cK_2$) that produced good fits for the DOM from each of the three streams. For each experimental fit, the weighted sum of squares divided by the degrees of freedom (WSOS/DF) was calculated in FITEQL.
Typically, WSOS/DF values of less than 20 are considered good fits (Herbelin and Westall, 1999). A two-ligand model is also consistent with previous copper titration studies, which makes results comparable between studies (McKnight et al., 1983; Breault et al., 1996). Inorganic Cu$^{2+}$-hydroxide and Cu$^{2+}$-carbonate species were also included in the FITEQL model. The results obtained from the model are conditional for pH = 6.0 and $I = 0.015$ M for titrations with isolated DOM, and pH 7.3 and $I = 0.015$ for titrations with concentrated site water.

**CLE-SPE**

The Cu$^{2+}$-DOM binding constants for the three isolated site water DOM samples were also measured using a competitive ligand exchange-solild phase extraction (CLE-SPE) method (Chapter 2). This method allows evaluation of copper binding at total copper concentrations lower than the 10$^{-6}$ M detection limit of the Cu-ISE method. This method uses a C$_{18}$ resin to separate DOM into two fractions operationally defined as the hydrophobic and hydrophilic fractions. The hydrophobic fraction of the DOM adsorbs to the resin and is retained, while the hydrophilic fraction passes through the resin with the eluent.

Three 250 mL solutions (one solution for each isolate obtained from the three sampling sites) were prepared using the same isolated DOM as in the laboratory water copper titration experiments (Table 3.3). The ionic strength of each solution was adjusted to 0.01 M using NaClO$_4$, and the pH was held constant at 6.0 ± 0.1 using a 3 mM sodium dihydrogen phosphate (NaH$_2$PO$_4$) buffer and NaOH adjustments.

Due to limitations on the amount of water collected (samples were flown out from the remote site by helicopter), there was not enough isolated organic matter to measure binding constants for all three sample locations at each Cu:DOM ratio. The variability allowed us to
explore the effect of increasing copper concentration and Cu:DOM ratio on the strength of Cu$^{2+}$-DOM binding.

Table 3.3. Cu:DOM ratios used for each DOM isolate in the CLE-SPE measurements

<table>
<thead>
<tr>
<th>Cu:DOM (mgCu mgDOM$^{-1}$)</th>
<th>$K_{CuDOM}$ (M$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper Talarik Creek</td>
<td>0.011</td>
</tr>
<tr>
<td>North Fork Koktuli River</td>
<td>0.002</td>
</tr>
<tr>
<td>South Fork Koktuli River</td>
<td>0.0005</td>
</tr>
</tbody>
</table>

The binding constant of copper with ligands contained in the natural DOM can be expressed as:

$$K_{CuLDOM} = \frac{[CuL_{DOM}]}{[Cu^{2+}][L_{DOM}]}$$  \[1\]

Each sample was equilibrated for 24 ± 2 h at the Cu:DOM ratio selected before the competitive ligand, benzoylacetone, was added. Benzoylacetone forms a 2:1 complex with Cu$^{2+}$:

$$K_{CuBz^2_0} = \frac{[CuBz^2_0]}{[Cu^{2+}][Bz^-]^2}$$  \[2\]

The CuBz$^2_0$ complex has a conditional stability constant of $10^{11.2}$ at pH 6.0 (Martell and Smith, 1977). The combination of Equations [1] and [2] yields the final equilibrium expression for the Cu-DOM binding constant as determined by the competition with benzoylacetone:

$$K_{CuBz_{DOM}} = \frac{K_{CuBz^2_0}[CuL_{DOM}][Bz^-]^2}{[Cu(Bz)^2_0][L_{DOM}]}$$  \[3\]

where $K_{CuBz_{DOM}}$ is the conditional binding constant for CuL$_{DOM}$; [CuL$_{DOM}$] is the concentration of copper that passes through the column; [Bz$^-$] is the concentration of deprotonated benzoylacetone; [CuBz$^2_0$] is the concentration of copper retained on the column, determined by
difference; and $[L_{DOM}]$ is the concentration of copper-binding ligands in the DOM. All concentrations are in moles per liter.

The benzoylacetone was added to the solution and allowed to equilibrate for another 2 h before solid phase extraction (SPE) extraction. The amount of benzoylacetone added to each solution varied depending on the Cu:DOM ratio of the experiment. For the lowest Cu:DOM ratio of 0.0005, 500 µM benzoylacetone was added, and for the higher Cu:DOM ratios of 0.002 and 0.011, 100 µM benzoylacetone was added.

Total copper was measured in the eluent from each CLE-SPE experiment using either a graphite furnace atomic absorption spectrometer (GF-AAS; Perkin Elmer, model 4110ZL; about 1 µg L$^{-1}$ detection limit) or an ICP-MS (about 0.1 µg L$^{-1}$ detection limit) for measurements using the South Fork Koktuli sample. U.S. Geological Survey standard reference water samples (T187, T181, T169; USGS, 2010) were used as laboratory control samples; when analyzed by GF-AAS, all had recoveries within ±15% of the reported concentration. The relative standard deviation values for triplicate analysis of all samples were less than 5%.

**BLM and MINTEQ modeling**

The stream water quality and copper binding results were used to estimate the speciation of copper in site water using two modeling approaches: the BLM and Visual MINTEQ. We compared BLM-predicted Cu$^{2+}$ concentrations (using site water quality as inputs) to those calculated by Visual MINTEQ (using site water quality and the experimentally-determined Cu$^{2+}$-DOM binding constants).

The BLM (Version 2.3.3) was used to model copper speciation and toxicity under a range of water quality conditions. The water quality data from the North Fork Koktuli River water was
used for all the model calculations because its alkalinity, hardness, UV absorbance, DOC, and SUVA$_{254}$ values are intermediate between those of the Upper Talarik Creek and South Fork Koktuli Rivers. Using measured major element, total dissolved copper, and DOC concentrations, the BLM calculates a copper LC$_{50}$ value for a number of aquatic species, including rainbow trout, the only fish species in the BLM that is present in streams that drain the Pebble project area. The BLM was used to calculate LC$_{50}$ values for the North Fork Koktuli River under a range of pH, hardness, and DOC values. The BLM was also used to calculate concentrations of dissolved species in solution, including Cu$^{2+}$, for the North Fork Koktuli River site conditions (measured in June 2010) at different total dissolved copper concentrations. All other inputs (pH, DOC, cations, and anions) were held constant.

Visual MINTEQ was used to calculate Cu$^{2+}$ concentrations at pH 6.0 using the experimentally-determined Cu$^{2+}$-DOM binding constants (Figure 3.3), and the results were compared to the Cu$^{2+}$ concentrations predicted by the BLM. In order to model the Cu$^{2+}$ concentrations at a higher pH value (7.13; pH of North Fork Koktuli site water), the relationship between Cu$^{2+}$-DOM binding constants and the Cu:DOM ratio for a range of binding constants reported in the literature (pH ranging from 6 to 8; Chapter 2) was used to calculate the model inputs to Visual MINTEQ. The Visual MINTEQ modeling approach differs slightly from the BLM modeling approach because it does not account for electrostatic interactions between species. The inorganic inputs to Visual MINTEQ were the same as those used in the BLM (Table 3.4). Visual MINTEQ allows the addition of binding constants for metal-organic complexes, whereas the BLM does not. We added Cu$^{2+}$-DOM binding to Visual MINTEQ as a series of experimentally-determined Cu$^{2+}$-DOM binding constants as a function of Cu:DOM ratios (Table 3.4; binding constants determined from the results from Figure 3.3 and the results
from Chapter 2). Each Cu\(^{2+}\) concentration was calculated individually at each total copper concentration using Cu\(^{2+}\)-DOM binding constants and ligand concentrations (L\(_{\text{DOM}}\)) derived from the relationship with the Cu:DOM ratio. Visual MINTEQ calculated the speciation of copper at each total copper concentration (8×10\(^{-10}\) to 2×10\(^{-5}\) M, or 0.05 to 1,000 \(\mu\)g L\(^{-1}\)) and each L\(_{\text{DOM}}\) concentration (modeled as a 1:1 complex at 8×10\(^{-10}\) to 2×10\(^{-5}\) M).

The Cu\(^{2+}\) concentrations from both model outputs were plotted against total dissolved copper concentrations to examine the effect of increasing total copper concentration on the concentration of Cu\(^{2+}\) and to compare the results from this study to predictions made using the BLM.
Table 3.4. Model input values

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value (range used in modeling)</th>
<th>Input used for BLM or MINTEQ?</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.00 and 7.13 (6.0–9.0) (^a)</td>
<td>Both</td>
</tr>
<tr>
<td>Cu</td>
<td>8E (^{-10})–2E (^{-5}) M (0.05–1,000 (\mu g) L (^{-1}))</td>
<td>Both</td>
</tr>
<tr>
<td>DOC</td>
<td>2.17 mgc L (^{-1}) (0.25–10.0) (^a)</td>
<td>BLM</td>
</tr>
<tr>
<td>(L_{DOM}) (“DOM ligand”)</td>
<td>8E (^{-10})–2E (^{-5}) M (^b)</td>
<td>MINTEQ</td>
</tr>
<tr>
<td>(\log^c K_{CuL})</td>
<td>Cu total ((\mu g) L (^{-1}))</td>
<td>(\log^c K) (M (^{-1}))</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>15.9</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>15.1</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>13.2</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>12.4</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>10.6</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>9.8</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>7.9</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>7.1</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>5.3</td>
</tr>
<tr>
<td></td>
<td>1,000</td>
<td>4.5</td>
</tr>
<tr>
<td>Humic acid (HA)(^%)</td>
<td>10</td>
<td>BLM</td>
</tr>
<tr>
<td>Calcium</td>
<td>4.09 mg L (^{-1}) (1.4–55.5) (^a)</td>
<td>Both</td>
</tr>
<tr>
<td>Magnesium</td>
<td>1.10 mg L (^{-1}) (0.4–14.9) (^a)</td>
<td>Both</td>
</tr>
<tr>
<td>Hardness</td>
<td>14.7 mg L (^{-1}) as CaCO(_3) (5–200 at constant Ca:Mg = 3.72) (^a)</td>
<td>BLM</td>
</tr>
<tr>
<td>Sodium</td>
<td>1.79 mg L (^{-1})</td>
<td>Both</td>
</tr>
<tr>
<td>Sulfate</td>
<td>1.70 mg L (^{-1})</td>
<td>Both</td>
</tr>
<tr>
<td>Chloride</td>
<td>0.50 mg L (^{-1})</td>
<td>Both</td>
</tr>
<tr>
<td>Alkalinity</td>
<td>22.3 mg L (^{-1}) as CaCO(_3)</td>
<td>Both</td>
</tr>
</tbody>
</table>

\(^a\) For “titration” in BLM.
\(^b\) For “titration” in MINTEQ.
\(^c\) The \(\log^c K\) was calculated from the regression of measured binding constant as a function of the Cu:DOM ratio (Figure 3.3) at pH 6. These values were used to model Cu\(^{2+}\)-DOM binding in MINTEQ at pH 6.
\(^d\) The \(\log^c K\) was calculated from the regression of measured binding constant as a function of the Cu:DOM ratio for Cu\(^{2+}\)-DOM binding constants measured at pH values 6 to 8 (Chapter 2). These values were used to model Cu\(^{2+}\)-DOM binding in MINTEQ at pH 7.13.
\(^e\) A default input of 10% HA is recommended for the BLM when actual percent is not known.
Results

Water quality results

The three streams where water was used for the copper binding studies have similar water quality data (Table 3.5). The dissolved copper was the highest in the South Fork Koktuli sample at 1.3 µg L\(^{-1}\); this sample also had the highest DOC concentration at 2.2 mg\(_C\) L\(^{-1}\). The SUVA\(_{254}\) values were similar for all three water samples and ranged from 2.7 to 3.2 L mg\(_C\) L\(^{-1}\) m\(^{-1}\).

Water quality data from 2004 to 2008 were used to evaluate background stream chemistry near the Pebble deposit area (Figure 3.1). Headwater streams (representative mainstem locations only) near the deposit contain low solute concentrations, have neutral to slightly acidic pH values, and can be characterized as calcium-bicarbonate-type waters (Table 3.6). Dissolved copper concentrations were highest near the deposit and in the upper South Fork Koktuli River basin, where the cover of glacial outwash material over the western deposit area is thin. Mean dissolved copper values at any one site did not exceed 3 µg L\(^{-1}\) in the South Fork Koktuli River and values were 1 µg L\(^{-1}\) or less in the North Fork Koktuli River and Upper Talarik Creek. The highest copper concentrations were located at a site directly over the western side of the Pebble deposit. Hardness values were generally < 25 mg L\(^{-1}\) as CaCO\(_3\) and were higher on the western side of the deposit area and in the Upper Talarik Creek drainage. Sites with higher copper concentrations generally had higher hardness values, reflecting weathering of the deposit. DOC concentrations were generally ≤ 2 mg\(_C\) L\(^{-1}\). The maximum DOC concentration (8.2 mg\(_C\) L\(^{-1}\)) was measured in the headwaters of Upper Talarik Creek. DOC concentrations were generally higher in the upper reaches of the three streams, decreased with distance downstream, and peaked during spring snowmelt and fall rain events.
Table 3.5. Water quality and DOM characterization results for Cu\(^{2+}\) binding study samples

<table>
<thead>
<tr>
<th></th>
<th>North Fork Koktuli River (NK-02)</th>
<th>Upper Talarik Creek (UT-02)</th>
<th>South Fork Koktuli River (SK-02)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH (field)</td>
<td>7.1</td>
<td>7.6</td>
<td>7.2</td>
</tr>
<tr>
<td>specific conductance</td>
<td>36.6</td>
<td>64.4</td>
<td>37.9</td>
</tr>
<tr>
<td>alkalinity</td>
<td>(mg L(^{-1}) as CaCO(_3))</td>
<td>22.3</td>
<td>33.9</td>
</tr>
<tr>
<td>hardness</td>
<td>(mg L(^{-1}) as CaCO(_3))</td>
<td>14.7</td>
<td>28.4</td>
</tr>
<tr>
<td>dissolved Cu</td>
<td>(µg L(^{-1}))</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>DOC</td>
<td>(mg(_c) L(^{-1}))</td>
<td>2.0</td>
<td>1.3</td>
</tr>
<tr>
<td>SUVA(_{254})</td>
<td>(L mg(_c)(^{-1}) m(^{-1}))</td>
<td>3.1</td>
<td>2.7</td>
</tr>
<tr>
<td>HPOA</td>
<td>%</td>
<td>38</td>
<td>37</td>
</tr>
<tr>
<td>HPOA SUVA(_{254})</td>
<td>(L mg(_c)(^{-1}) m(^{-1}))</td>
<td>3.9</td>
<td>3.4</td>
</tr>
<tr>
<td>TPiA</td>
<td>%</td>
<td>15</td>
<td>17</td>
</tr>
<tr>
<td>TPiA SUVA(_{254})</td>
<td>(L mg(_c)(^{-1}) m(^{-1}))</td>
<td>2.3</td>
<td>1.8</td>
</tr>
<tr>
<td>Ca(^{2+})</td>
<td>(mg L(^{-1}))</td>
<td>4.09</td>
<td>8.44</td>
</tr>
<tr>
<td>Mg(^{2+})</td>
<td>(mg L(^{-1}))</td>
<td>1.10</td>
<td>1.73</td>
</tr>
<tr>
<td>Na(^{+})</td>
<td>(mg L(^{-1}))</td>
<td>1.79</td>
<td>2.49</td>
</tr>
<tr>
<td>K(^{+})</td>
<td>(mg L(^{-1}))</td>
<td>-</td>
<td>0.4</td>
</tr>
<tr>
<td>SO(_4^{2-})</td>
<td>(mg L(^{-1}))</td>
<td>1.70</td>
<td>3.06</td>
</tr>
<tr>
<td>Cl(^{-})</td>
<td>(mg L(^{-1}))</td>
<td>0.50</td>
<td>0.61</td>
</tr>
</tbody>
</table>

DOC=dissolved organic carbon; SUVA\(_{254}\)= specific ultraviolet absorbance at 254 nm; HPOA=hydrophobic acids; TPiA=triphillic acids
Table 3.6. Ranges and mean water quality values for mainstem headwater streams draining the Pebble deposit

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>DOC (mg C L(^{-1}))</th>
<th>Hardness (mg L(^{-1}) as CaCO(_3))</th>
<th>Alkalinity (mg L(^{-1}) as CaCO(_3))</th>
<th>Dissolved Cu (mg L(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>South Fork Koktuli River</strong></td>
<td>Min</td>
<td>4.39</td>
<td>0.08</td>
<td>7.68</td>
<td>1.55</td>
</tr>
<tr>
<td></td>
<td>Max</td>
<td>8.85</td>
<td>4.09</td>
<td>42.6</td>
<td>40.0</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>6.68</td>
<td>1.58</td>
<td>19.7</td>
<td>16.5</td>
</tr>
<tr>
<td><strong>Upper Talarik Creek</strong></td>
<td>Min</td>
<td>4.90</td>
<td>0.08</td>
<td>14.9</td>
<td>12.0</td>
</tr>
<tr>
<td></td>
<td>Max</td>
<td>8.16</td>
<td>8.18</td>
<td>62.2</td>
<td>56.0</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>7.08</td>
<td>1.38</td>
<td>33.9</td>
<td>34.6</td>
</tr>
<tr>
<td><strong>North Fork Koktuli River</strong></td>
<td>Min</td>
<td>4.99</td>
<td>0.69</td>
<td>7.52</td>
<td>6.00</td>
</tr>
<tr>
<td></td>
<td>Max</td>
<td>8.36</td>
<td>3.37</td>
<td>36.4</td>
<td>38.0</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>6.83</td>
<td>1.66</td>
<td>20.2</td>
<td>23.7</td>
</tr>
</tbody>
</table>

SFK sites: SK100G, SK100F, SK100C, SK100A
UT sites: UT100D, UT100B
NFK sites: NK100C, NK100B, NK100A (see Figure 1 for site locations)
Hardness, alkalinity, dissolved copper through 2007 (updated using EBD); others through 2008.

Copper titrations

**Titrations with concentrated site water**

The Cu\(^{2+}\)-DOM binding characteristics were measured for the concentrated and buffered (pH 7.3) site waters (Table 3.7). At high total copper concentrations, the Cu\(^{2+}\) concentration remained constant as the total copper concentration increased. This behavior may have been caused by Cu\(^{2+}\)-hydroxide precipitation at a pH above 7. As a result, the six data points for total copper concentrations greater than \(5 \times 10^{-5}\) M were excluded from the two-ligand FITEQL fit,
and only 13 of the 19 titration points (1×10^{-6} to 5×10^{-5} M total copper) were used in determining the Cu^{2+}-DOM binding constants.

A stronger-binding ligand (L_1) was observed in samples from all three steams, with conditional binding constants (cK_1) ranging from 10^{6.9} to 10^{7.9} M^{-1}. The weaker-binding ligand (L_2) in the South Fork Koktuli samples was slightly weaker than L_1, with a conditional binding constant (cK_2) of 10^{6.0} M^{-1}. The North Fork Koktuli and Upper Talarik samples had much lower conditional binding constant for L_2, (cK_2 = 10^{4.9} M^{-1}). Cu^{2+} binding ligand concentrations (µmol L^{-1}) were calculated using FITEQL and divided by the total DOC concentration (µmol mgC^{-1}) to account for differences in DOC concentration (see Table 3.7).

### Table 3.7. Conditional Cu^{2+}-DOM binding constants at pH 7.3 and I = 0.015 M for titration data from concentrated, buffered Pebble Project site waters modeled with a discrete two-ligand model in FITEQL.

<table>
<thead>
<tr>
<th>Sample</th>
<th>log K_1</th>
<th>log K_2</th>
<th>µM</th>
<th>µmol mgC^{-1}</th>
<th>WSOS/DF</th>
</tr>
</thead>
<tbody>
<tr>
<td>NK</td>
<td>6.9</td>
<td>4.9</td>
<td>11.0</td>
<td>182.2</td>
<td>0.57</td>
</tr>
<tr>
<td>UT</td>
<td>7.4</td>
<td>4.9</td>
<td>2.54</td>
<td>215.3</td>
<td>0.21</td>
</tr>
<tr>
<td>SK</td>
<td>7.9</td>
<td>6.0</td>
<td>3.17</td>
<td>41.4</td>
<td>0.16</td>
</tr>
</tbody>
</table>

WSOS/DF: weighted sum of squares/degrees of freedom.

**Titrations with isolated DOM**

For the DOM isolates from the three sample locations, the modeled fit agreed well with the experimental data (Figure 3.2), as shown by the low WSOS/DF values (Table 3.8). The results for the three DOM isolates were similar, with a higher binding constant (cK_1) ranging from 10^{6.9} to 10^{7.2} M^{-1} and a lower binding constant (cK_2) ranging from 10^{4.7} to 10^{5.4} M^{-1}. The ligand concentrations (µmol mgC^{-1}) were converted to Cu:DOM ratios (ranging from 0.02 - 0.2
mg$_{\text{Cu}}$ mg$_{\text{DOM}}^{-1}$) by setting the ligand concentration (L$_1$ or L$_2$) equal to the maximum amount of 
Cu$^{2+}$ that is bound to the DOM for each calculated binding strength ($\nu K$). The DOC 
concentration was measured for each copper titration experiment, and the DOM concentration 
was calculated by multiplying the DOC concentration by a factor of 2, based on the estimate that 
approximately half of the DOM is carbon. The conditional binding constants for Cu$^{2+}$-DOM 
increased as the Cu:DOM ratio decreased (Figure 3.3).

Table 3.8. Conditional Cu$^{2+}$-DOM binding constants at pH 6.0 and I = 0.015M for titration data 
from laboratory waters using isolated Pebble Project site DOM modeled with a discrete two-
ligand model in FITEQL.

<table>
<thead>
<tr>
<th>Sample</th>
<th>log $K_1$</th>
<th>log $K_2$</th>
<th>$\mu$M</th>
<th>$\mu$mol mg$_C^{-1}$</th>
<th>WSOS/DF</th>
</tr>
</thead>
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<td>7.67</td>
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</tbody>
</table>
Figure 3.2. Cu-ISE titration experimental curves and FITEQL two-ligand fit for DOM isolated from the North Fork Koktuli River, Upper Talarik Creek, and South Fork Koktuli Rivers. The Cu$^{2+}$ concentration was measured as the total copper concentration increased from $10^{-6}$ to $10^{-4}$ M.
Figure 3.3. Cu$^{2+}$-DOM binding constants as a function of Cu:DOM ratio for Cu-ISE titration using laboratory waters with DOM isolates from the three sites and CLE-SPE experiments using laboratory waters with DOM isolates from the three sites; pH 6.0, I 0.01 M.

**CLE-SPE**

Solutions of the three DOM isolates were created using different total copper and DOM concentrations (approximately $5 \times 10^{-4}$ to $1 \times 10^{-4}$ mg$_{Cu}$ mg$_{DOM}^{-1}$) to evaluate the effect of the Cu:DOM ratio on binding strength (see Table 3.3). The conditional binding constants measured for the isolated DOM samples using the CLE-SPE method had the same relationship observed for the Cu-ISE titration method; binding constants were found to increase as the Cu:DOM ratio decreased (Figure 3.3).
Comparison between MINTEQ and BLM

MINTEQ and the BLM were used to calculate Cu$^{2+}$ over a range of total dissolved copper concentrations (Figure 3.4a and b). At pH 6, when the total dissolved copper concentrations was less than $1.6 \times 10^{-7}$ M (10 µg L$^{-1}$), the BLM estimated higher Cu$^{2+}$ concentrations than the MINTEQ model that used experimental Cu$^{2+}$-DOM binding constant inputs. At total dissolved copper concentrations greater than $1.6 \times 10^{-7}$ M (10 µg L$^{-1}$), the BLM estimated lower Cu$^{2+}$ concentrations. The crossover point between the two models shifted to a slightly lower total copper concentrations ($7.8 \times 10^{-8}$ M (5 µg L$^{-1}$) vs. $1.6 \times 10^{-7}$ M (10 µg L$^{-1}$)) as the pH was increased from 6.0 to 7.13, the ambient pH of the North Fork Koktuli sample. At pH 7.13, the BLM Cu$^{2+}$ concentration estimates were only 10-20% of the MINTEQ-estimated values. For example, at a total dissolved copper concentration of $1.6 \times 10^{-7}$ M (about 10 µg L$^{-1}$), the BLM estimated that only 0.006 µg L$^{-1}$ would be Cu$^{2+}$, while MINTEQ estimated that 0.03 µg L$^{-1}$ would be Cu$^{2+}$, a 5-fold difference. At a total dissolved copper concentration of $1.6 \times 10^{-6}$ M (about 100 µg L$^{-1}$), the BLM estimated that only 0.5 µg L$^{-1}$ would be Cu$^{2+}$, while MINTEQ estimated that 5.2 µg L$^{-1}$ would be Cu$^{2+}$, an approximately 10-fold difference (see Figure 3.4b).
Figure 3.4. (a) BLM and MINTEQ model calculations at pH 6.0 of Cu$^{2+}$ using results from the North Fork Koktuli site water chemistry and experimental Cu$^{2+}$-DOM binding experiments; (b) BLM and MINTEQ model calculations of Cu$^{2+}$ at pH 7.13 using water quality results from the North Fork Koktuli site water chemistry and modeling the relationship between the measured Cu$^{2+}$-DOM binding constants and the Cu:DOM ratio for published values ranging in pH from 6 to 8 (Chapter 2).
Discussion

Water quality

The results of the field water quality analyses demonstrated that the streams near the Pebble deposit area have low concentrations of constituents that reduce the toxicity of copper to aquatic biota (e.g. calcium, alkalinity, and DOM). The acute toxicity of copper to rainbow trout increases as hardness and alkalinity decrease (Miller and Mackay, 1980; Reid and McDonald, 1988; Welsh et al., 2000; Hansen et al., 2002a; Meyer et al., 2007). A loss of calcium from gills through displacement by Cu\(^{2+}\) increases the permeability of the gills and facilitates the loss of ions and a fish’s susceptibility to copper exposure. Increasing hardness increased the toxicity for rainbow trout exposed to copper across all alkalinity values.

The bioavailability and subsequent toxicity of copper to aquatic biota can be modified by the presence of DOM. The influence of DOM on copper toxicity is a function of the strength and capacity of Cu\(^{2+}\)-DOM binding sites (MacRae et al. 1999a, 1999b; Marr et al. 1999; Meyer et al., 2007). Investigators have attributed the toxicity of Cu\(^{2+}\)-organic complexes to the relative binding strength of Cu\(^{2+}\) with the organic ligand relative to that of the fish gill (Meyer et al., 2007). For example, ethylenediaminetetraacetic acid (EDTA) and nitrilotriacetic acid (NTA), which have high binding constants with Cu\(^{2+}\), decreased copper toxicity to freshwater shrimp and other aquatic biota (Daly et al., 1990; Playle et al., 1992). MacRae et al. (1999b) and Playle et al. (1993) estimated the apparent binding constant for Cu\(^{2+}\) and the gill (\(K_{gill}\)) for rainbow trout (10\(^{7.5}\)), brook trout (10\(^{7.25}\)), and fathead minnow (10\(^{7.4}\)), and these values are used in the BLM (Meyer et al., 2007).
The Cu$^{2+}$-DOM binding constants determined using the Cu-ISE titration and CLE-SPE methods were found to be inversely related to the Cu:DOM ratio (Figure 3.3). Higher binding constants were measurable using the CLE-SPE method because the detection window of the method is controlled by the strength and concentration of the competitive ligand. In the CLE-SPE method, the concentration of the competitive ligand was varied to allow for measurement of lower-capacity, higher-affinity ligands that are able to bind Cu$^{2+}$ at low Cu:DOM ratios. The results indicate that at low Cu:DOM ratios, available Cu$^{2+}$ is bound to less abundant, higher-affinity binding sites in the DOM. As the total dissolved copper concentration increases relative to the DOM concentration, as could occur during mining at the Pebble deposit, Cu$^{2+}$ will fill weaker binding sites in the DOM. This inverse relationship between Cu$^{2+}$-DOM binding constants and Cu:DOM ratio is an important factor in understanding the potential influence of DOM on the toxicity of copper to aquatic organisms at the Pebble Project site. The relationship suggests that even small increases in dissolved copper concentrations without a commensurate increase in DOM concentrations will reduce the effective Cu$^{2+}$-DOM binding constant, which will increase the amount of bioavailable copper. Under these conditions, the BLM could underestimate copper toxicity, as shown in Figure 3.4.

Other investigators have suggested that the BLM underestimates the toxicity of copper to aquatic biota in the presence of DOM (e.g., De Schamphelaere et al., 2004; Welsh et al., 2008). One potential reason for this under-prediction may involve the way Cu$^{2+}$-DOM complexation is handled in the BLM (e.g., Lipton et al., 2006; Welsh et al., 2008). The BLM uses the WHAM code (Tipping, 1994), which uses a proton exchange model to estimate the binding of dissolved metals among eight idealized organic ligands in humic and fulvic acids (Tipping, 1994; Di Toro
et al., 2001). A recent review comparing BLM predictions with laboratory toxicity results for metal mixtures (lead and copper) found that the BLM predicted a straightforward antagonistic competition between the two metals, whereas laboratory tests demonstrated that low concentrations of Cu$^{2+}$ actually had a synergistic effect on the uptake of lead by a green algae (Chen et al., 2010).

The discrepancy in the Cu$^{2+}$ concentration between the BLM and the MINTEQ results at pH 7.13 (Figure 3.4b) can be reconciled by adjusting the DOC input concentration in the BLM. Decreasing the DOC concentration effectively simulates a smaller binding constant for Cu$^{2+}$ and DOM by reducing the concentration of strong Cu$^{2+}$-binding ligands. When the same water quality (see Table 3.4) but lower DOC concentrations are used as BLM inputs, the Cu$^{2+}$ concentrations predicted by the BLM approach those predicted by MINTEQ. The curve obtained from MINTEQ for environmentally relevant copper concentrations (1 to 100 μg L$^{-1}$) falls in between the two BLM curves for when the DOC concentrations were decreased by a factor of four (from 2.2 to 0.54 mg C L$^{-1}$), and by a factor of two (from 2.2 to 1.1 mg C L$^{-1}$) (Figure 3.5). Other investigators have suggested that using one-half the measured DOC concentration in the BLM yields a better empirical fit with fish toxicity data in some cases (De Schamphelaere et al., 2004; Welsh et al., 2008). These findings are consistent with our study results and suggest that the BLM appears to overestimate Cu$^{2+}$-DOM complexation at total copper concentrations generally associated with toxicity to aquatic biota.
Figure 3.5. DOC “titrations” using the BLM at pH 7.13 and site water chemistry for the North Fork Koktuli River. The DOC concentration was decreased incrementally in the model from the measured value of 2.17 mg C L$^{-1}$ to 0.27 mg C L$^{-1}$ to show how lowering the DOC concentration creates a better fit with the MINTEQ calculated Cu$^{2+}$ concentrations.

We used the BLM to show the relationship between predicted salmonid toxicity, represented by the copper LC$_{50}$ values for rainbow trout, and three water quality parameters: pH, DOC, and hardness (Figure 3.6). As shown in Figure 3.6a, the increase in the LC$_{50}$ with pH is largely caused by the decrease in Cu$^{2+}$ and concomitant increase in organically-bound Cu$^{2+}$ with increasing pH. With increasing DOC concentrations, the BLM predicts that copper LC$_{50}$ values for rainbow trout increase by approximately 100 µg L$^{-1}$ for every 2 mg C L$^{-1}$ increase in DOC (Figures 3.6b). At low hardness values, the BLM predicts that copper is less toxic to rainbow trout than at hardness values above 20 mg L$^{-1}$ as CaCO$_3$ (Figure 3.6c). The higher LC$_{50}$ values...
appear to be related to competition for Cu$^{2+}$ by the gill and DOC. The BLM predicts that Cu$^{2+}$ will preferentially bind to DOC at very low hardness values, and LC$_{50}$ values consequently increase. However, as hardness increases, up to about 20 mg L$^{-1}$ as CaCO$_3$, the BLM predicts that Cu$^{2+}$ will shift to the gill, resulting in lower LC$_{50}$ values due to cation competition on the gill surface.

The $K_{gill}$ value of the gill is set at $10^{7.4}$ for rainbow trout in the BLM. However, if that value is poorly known or varies in different fish species or under different water quality conditions, the LC$_{50}$ can change dramatically (Figure 3.6d). At hardness values above about 20 mg L$^{-1}$ as CaCO$_3$, the LC$_{50}$ is predicted to rise by about 4 or 5 $\mu$g L$^{-1}$ for each 20-mg$_C$ L$^{-1}$ increase in hardness. The U.S. EPA’s hardness relationship (U.S. EPA, 2009) does not show this type of behavior at low hardness values.
(a) and (b) illustrate the relationship between LC50 (μg/L) and pH, as well as LC50 (μg/L) and DOC (mg/L) for different metal concentrations. The graphs show a clear increase in LC50 with decreasing pH and increasing DOC concentration.

(a) LC50 (pH) vs. DOC (mg/L)

(b) LC50 (μg/L) vs. DOC (mg/L)
Figure 3.6. BLM relationship between LC$_{50}$ and (a) pH (and relationship between Cu$^{2+}$ and total organic-Cu$^{2+}$ concentrations and pH), (b) DOC, (c) hardness, and (d) LC$_{50}$ for total dissolved copper as a function of the log K of the fish gill for DOC values ranging from 0.05 to 5 mg$_c$ L$^{-1}$. 
Environmental implications

Our results indicate that streams in the vicinity of the Pebble Project have a very low assimilative capacity for copper without creating potentially adverse effects to salmonids. The surface water streams near the Pebble deposit have low hardness, alkalinity, and DOC concentrations. Although the natural DOM in streams close to the Pebble deposit contains organic ligands with high Cu$^{2+}$ binding affinity, their concentrations are low. An increase in the total copper concentration relative to the DOM concentration, as would likely occur during mining, will reduce the net binding affinity of natural DOM by shunting added copper to ligands with lower binding strengths. As a consequence of this relationship, natural DOM present at the Pebble Project site is unlikely to be strongly protective against copper toxicity to aquatic organisms. Our findings indicate that DOM from streams around the Pebble deposit area has a lower Cu$^{2+}$ binding ability than predicted by the BLM at environmentally relevant concentrations (i.e. 1 $\mu$g L$^{-1}$ or higher). The results from this study suggest that using the BLM to predict copper toxicity under mining conditions could under-estimate copper toxicity to salmonids in the Pebble deposit watersheds. Site-specific toxicity studies using native fishes should be conducted to expand the BLM and explore the effects of low copper concentrations on local aquatic biota.
References


Chapter 4

Effect of Dissolved Organic Matter on the Release of Mercury from Coal Ash

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Abstract

Releases of coal ash to rivers and streams, such as that which occurred from the Kingston Fossil Plant in Kingston, TN, into the Emory and Clinch Rivers in December 2008, are a concern because of the potential for human health problems as well as ecological effects that may be caused by the presence of ash in the environment. Coal ash contains high concentrations of toxic trace metals present in the ash that may have potential to be released into the environment. In order to understand the effect that dissolved organic matter (DOM) has on metal leaching from coal ash, a series of mercury release experiments were performed using coal ash generated from the Kingston Fossil Plant in the presence and absence of DOM. Various DOM isolates and filtered water samples collected near the Kingston Fossil Plant were used to produce coal ash suspensions at a fixed ash:water ratio of 1:1000 and a near-neutral pH. Results indicate that DOM plays an important role in the leaching of mercury from the coal ash. DOM isolates with higher aromatic carbon content were found to release more mercury from the ash. The addition of calcium inhibits the release of mercury from the coal ash. The results observed for mercury release from coal ash exposed to two river surface waters samples were consistent with the relationships observed with the isolated DOM.
Introduction

The containment failure which resulted in the release of 3.8 million cubic meters of coal ash from the Tennessee Valley Authority’s Kingston Fossil Plant in December 2008 was the largest release of coal ash in U.S. history (EPA 2009; TVA 2009; Bednar et al., 2010). This spill was of an environmental concern due to high concentrations of toxic metals in the ash that may be released into surrounding waters. Coal contains toxic trace elements, such as arsenic, selenium and mercury that are concentrated in the ash during combustion (Wadge and Peterson, 1986; Fernández-Turiel et al., 1994; Hower et al., 2005). Studies have shown that many of the elements present in coal ash are available for release and transport in aqueous systems (Jegadeesan et al., 2008; Gitari et al., 2009; Kim and Hesbach, 2009). Understanding the release and mobility of trace elements is important in evaluating the risk for human health and ecological problems as a result of coal ash releases.

Mercury is an element of particular concern due to its potential to methylate and bioaccumulate. Mercury released from the ash could result in increased mercury concentrations in both the surface waters and river sediments, and the potential for mercury release depends on the speciation of mercury in the coal ash. Numerous studies have correlated the unburned organic matter fraction of coal ash with mercury concentrations (Hower et al., 2000; Lopez-Anton et al., 2007; Suarez-Ruiz et al., 2007; Hower et al., 2010). A recent survey of the Clinch and Emory Rivers reported elevated mercury concentrations in sediments downstream of the Kingston coal ash spill (Ruhl et al., 2009), which may have been cause by release of mercury from coal ash and adsorption onto the sediments or the accumulation of coal ash in the sediments. While mercury is not readily leachable from coal ash under many conditions (Noel et al., 2007), dissolved organic matter (DOM) is known to strongly bind mercury at low mercury:

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DOM ratios (Haitzer et al., 2002). Exposure of coal ash to DOM could result in release of mercury from the coal ash, but the environmental factors controlling the long-term fate of mercury in the coal ash are not well understood.

Solution pH is known to be a primary factor controlling trace metal release from minerals, including ash, due to pH-dependent dissolution of minerals and proton exchange of surface-bound metals (Otero-Rey et al., 2005; Baba et al., 2008; Jegadeesan et al., 2008; Gitari et al., 2009; Kim and Hesbach, 2009). At low pH, proton exchange with surface adsorbed cations will release metals from the coal ash. Dissolution of oxide minerals at low pH will also lead to the increase of trace metals that are associated with these minerals in the coal ash. At high pH values, hydroxide may compete with oxyanions, such as arsenic and selenium, for binding sites on the coal ash surface. The pH dependent release of trace metals can be affected by the coal ash type. There are two basic types of coal ash: (1) alkaline, which contains high calcium concentrations which are readily leachable and produce a basic pH in water; and (2) acidic, which contains higher iron oxide concentrations, and produces a lower pH when suspended in water.

Limited work has been done to study the effect of organic ligands, such citric acid and ethylenediaminetetraacetic acid (EDTA), on the leaching of metals from coal ash (Harris and Silberman, 1983; van der Bruggen et al., 1998). In these studies, organic ligands were added to coal ash suspensions and the concentrations of metals released from the coal ash increased in the presence of the complexing ligands. But since model compounds lack the diversity and complexity of natural DOM, the knowledge of the effect of simple organic ligands on metal release from coal ash may not provide insight to the role of DOM.
Dissolved organic matter (DOM) also plays an important role in the dissolution of oxide, sulfide, and aluminosilicate minerals, with oxides and aluminosilicates making up the majority of the coal ash composition. Past studies have shown that DOM enhances the dissolution of cinnabar (HgS), a mineral with very low solubility (Ravichandran et al., 1998). Additionally, properties of DOM, such as aromaticity, increased the rate of cinnabar dissolution (Waples et al., 2005). There have also been studies that show that DOM is also important for the dissolution of lead oxides (Lin and Valentine, 2009; Shi and Stone, 2009), and the release of trace elements, such as arsenic, from the oxide mineral surfaces (Bauer and Blodau, 2006; Wang and Mulligan, 2006). DOM is also important in the aqueous speciation of mercury due to its ability to form strong complexes via reduced sulfur functional groups at low mercury: DOM ratios (Haitzer et al., 2002; Gerbig et al., 2011). While DOM is known to play a role in mineral dissolution and metal complexation, little is known about the effect of DOM on the dissolution and release of mercury from coal ash.

The presence of calcium has been shown to inhibit the release of mercury from solids, such as cinnabar minerals, soils, and sediments (Ravichandran et al., 1998; Suess, 2006; Langner, 2009). Adding calcium reduced the concentration of dissolved mercury, while removing calcium using by cation exchange methods was shown to increase the concentration of dissolved mercury (Ravichandran et al., 1998). The role of calcium on the release of mercury from coal ash is important to understand because calcium concentrations in natural waters can greatly vary depending on the geology of the site. Also, the calcium content of the coal ash itself can greatly vary, depending on the coal source, and calcium is a major element that is readily leachable from coal ash under many conditions, which may influence the release of other trace elements from coal ash (Wang et al., 2009).
In this study, mercury release experiments were conducted using isolated DOM from the Everglades in Florida and Williams Lake in Minnesota, and filtered surface water samples from the Emory and Clinch Rivers in order to understand the role of DOM on the release of mercury from Kingston coal ash. Chemical properties of the DOM were also explored in order to uncover mechanisms for mercury release. In addition, the effect of calcium on the role of DOM in releasing mercury from the coal ash was studied.
Methods

Characterization of Coal Ash

The coal ash used in this study was a freshly produced sample collected from the ash hopper at the Kingston Fossil Plant in Kingston, TN, on October 19, 2010. Elemental composition of the whole coal ash was determined by an HCl/HNO$_3$/HF digestion at the LEGS laboratory at the University of Colorado. The digest was analyzed for major elements (Al, Ca, Fe, K, Mg, Mn, Na, P, Si, Ti) using inductively coupled plasma-atomic emission spectrometry (ICP-AES; Applied Research Laboratories, Model 3410+) and trace elements (As, Co, Cr, Cu, Ni, Pb, Zn) were analyzed using inductively coupled plasma-mass spectrometry (ICP-MS; Varian 810MS). Solid phase mercury was measured using a thermal decomposition method with a gold trap and atomic adsorption detector on a DMA-80 direct mercury analyzer (Milestone, Inc.). The organic matter content of the coal ash was determined by loss on ignition (LOI) by heating approximately 1 g of ash at 550° C for 1 h. Surface area measurements were made using Brunauer, Emmett and Teller (BET) multi-point surface area analysis (Micromeritics Instrument Corp., model Gemini V2.00) at the Chemical and Biological Engineering Department at the University of Colorado. To evaluate the size distribution of mercury and other elements in the coal ash, the coal ash was dry sieved by hand into three different size fractions (<53µm, 53-106 µm, and >106 µm) using 270 mesh and 140 stainless steel mesh sieves. LOI and surface area measurements were done for the three size fractions and the whole ash sample.

Selective sequential extractions for mercury were done for the whole ash sample, the <53µm fraction, and the >106 µm fraction by Frontier Geosciences Inc. according to the method outlined by Bloom et al. (2003). Sequential extractions were performed on each coal ash sample first using 1 M sodium hydroxide (NaOH) to remove organo-complexed mercury, followed by
12 M nitric acid (HNO₃) to remove strongly-complexed mercury, including Hg⁰, and the last extraction was done using aqua regia to remove the metacinnabar-like mercury (HgS, HgSe) from the coal ash.

**Dissolved Organic Matter Isolates**

DOM from two sites (Everglades in Florida and Williams Lake in Minnesota) were isolated according to the procedures of Aiken et al. (1992). DOM fractions were obtained by retention on XAD-8 (hydrophobic acids; HPoA) and XAD-4 (transphilic acids, TPiA), back-elution using NaOH, and freeze-drying. The HPoA fraction generally contains larger, more aromatic compounds than the TPiA fraction, which typically contains more oxygen and nitrogen than the HPoA. The four isolates used in this study were selected to span a range of specific UV absorbances measured at 254 nm (SUVA₂₅₄) values to represent the range of values found in natural waters systems (Table 4.1). The specific UV absorbance at 254 nm (SUVA₂₅₄; L mgC⁻¹ m⁻¹) was determined by dividing the absorption of ultraviolet light at 254 nm (UV₂₅₄) to the dissolved organic carbon (DOC) concentration.
Table 4.1. DOM isolates used in this study and their corresponding $\text{SUVA}_{254}$ values.

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<th>DOM Isolate</th>
<th>$\text{SUVA}<em>{254}$ (L mg$</em>{C^{-1}}$ m$_{-1}$)</th>
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<td>Williams Lake TPiA</td>
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</tr>
<tr>
<td>Williams Lake HPoA</td>
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<tr>
<td>Everglades TPiA</td>
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</tr>
<tr>
<td>Everglades HPoA</td>
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</table>

**Surface Water Samples**

Surface water samples (10 L) were collected and filtered (0.45 µm membrane capsule filter; Geotech Environmental Equipment Inc.) from the Emory ($35^\circ$ 56' 22.1562", -84° 31' 3.9498") and Clinch ($35^\circ$ 53' 40.9338", -84° 27' 49.4604") Rivers upstream on the Kingston Fossil Plant in Kingston, TN and the coal ash spill affected waters (Figure 4.1). Samples from both locations were collected on in June 2011 by Tennessee Valley Authority personnel and shipped on ice overnight. A second sampling was done for the same two sites in September 2011 to observe the effects of seasonal variability of the water samples on mercury release from coal ash. Water quality measurements included pH, major cations, major anions, alkalinity, DOC, $\text{SUVA}_{254}$, and mercury.
Mercury Release Experiments

Mercury release experiments were performed in trace metal grade 10% hydrochloric/10% nitric acid-cleaned 125 mL glass flasks at an ash:water ratio of 0.1:100 g:mL using ultrapure water (>18 MΩ resistivity; Barnstead, Nanopure). At this ratio, the release of alkaline components of the coal ash in DI water resulted in a pH of 9.2. In order to achieve a neutral pH, which would better represent the pH of the Emory and Clinch Rivers, the pH was slowly titrated down to $6.8 \pm 0.5$ using a series of five additions of 0.01 M trace metal-grade nitric acid over a period of 5 h. The neutralized ash solution was allowed to equilibrate for $22 \pm 2$ h on a shaker.
table set to a speed of 200 rpm. After the coal ash was neutralized, DOM was added from stock solutions (100 to 200 mg C L$^{-1}$; prepared by dissolving DOM isolates in DI water, adjusting the pH to 5.8±0.1, and passing the stock solution through a 0.45 µM membrane filter) to result in final DOM concentrations ranging from 0 to approximately 40 mg L$^{-1}$. The coal ash-DOM suspension was mixed on a shaker table for 4 d at 200 rpm, unless otherwise indicated. This equilibration time was selected based on mercury release kinetics experiments, which showed that the mercury release reached equilibrium for all of the isolates studied between 2 and 4 d. The glass flasks were covered with aluminum foil to prevent any light from reaching the solution. After 4 d, the coal ash was allowed to settle and the solution was passed through a membrane filter (0.45 µm filter opening; Supor membrane, Pall Life Sciences) and collected for analysis (pH, mercury, trace metals). The DOC concentration was measured after the 4 d equilibration time to determine the concentration adsorbed onto the coal ash surface (Oceanography International, model 700). All batch leaching experiments were conducted in duplicate.

For mercury release experiments using the Emory and Clinch River water samples, the same ash: water ratio was used as described above. Kinetics experiments were done to determine the equilibrium time for mercury release from coal ash exposed to the river waters. The release of mercury in both surface water samples reached equilibrium between 2-4 d of leaching; results at 4 d are reported.

Select sequential batch experiments were performed under constant DOM conditions (Everglades HPoA isolate at a concentration of about 10 mg C L$^{-1}$) in order to assess the potential for long-term release of mercury from the coal ash. These experiments were set up identically to those described above. After the initial equilibration time of 4 d, the coal ash was
allowed to settle and 90 mL of the solution was removed and replaced with an equal volume of fresh DOC solution at the same DOC concentration. This process was repeated for six batches, each with a 4 d equilibration time. The solution that was removed from the coal ash during each step, filtered (0.45 μm Supor) and collected for analysis. Filters were weighed before and after filtration to measure the mass of coal ash lost during each batch. The mercury concentrations were normalized to the mass of coal ash (ng Hg g ash⁻¹) in order to account for any decreases in mercury due to the lost ash. Measured mercury concentrations were corrected to account for the mercury in the remaining 10 mL that was not removed. This was done by subtracting the previously measured mercury concentration after adjusting for dilution.

To study the effect of calcium on DOM-facilitated mercury release from coal ash, batch experiments were done under identical pH and DOM conditions, but with varying concentrations of added calcium (10⁻³.₅-₁₀⁻².₀M). Calcium was added to experimental solutions from a 1 M Ca(NO₃)₂ stock solution. Calcium addition experiments contained 8.8 mg C L⁻¹ Everglades HPoA isolate and mercury release experiments were performed at the same conditions as above.

**Mercury Release Sample Analysis**

Aqueous mercury measurements were performed in analytical duplicate by cold vapor atomic fluorescence spectrophotometry following EPA method 1631 (CVAFS; PS Analytical, Millennium Merlin). Samples were preserved using 1% BrCl by volume in trace metal-clean glass vials (I-Chem 200 series). Mercury standards for the CVAFS were prepared by dilution from a Hg(NO₃)₂ standard (National Institute of Standards and Technology) and preservation in 1% BrCl. The analytical detection limit was calculated as three times the standard deviation of seven analyses of a 2.5 ng L⁻¹ mercury standard and was determined to be approximately
1 ng L$^{-1}$. Major and trace metals were analyzed in the leachate using ICP-AES and ICP-MS. Samples were collected in acid-cleaned high density polyethylene bottles and preserved by adding 1% trace metal-grade HNO$_3$. Major anions (SO$_4^{2-}$, NO$_3^-$, Cl$^-$) were analyzed by ion chromatography. DOC concentrations were measured using persulfate oxidation on a total organic carbon analyzer (Oceanography International, model 700). The UV absorbance of samples and stock solutions were measured using an UV/visible spectrophotometer (Hewlett Packard, model 8453). Electrophoretic mobility measurements were done by microelectrophoresis (ZetaPALS, Brookhaven Instruments Corp.) in triplicate on the unfiltered bulk ash sample (pH = 6.8, I = approximately 0.1 M) after the ash was leached. Electrophoretic mobility measurements were converted to zeta potentials using the Smoluchowski equation. The zeta potential of the coal ash after equilibration with the Everglades HPoA isolate was measured in order to verify that the loss of DOC from solution was due to adsorption of the DOC to the ash surface. To make these measurements, the coal ash was shaken to re-suspend all of the coal ash particles.
Results

Coal Ash Characterization

The Kingston coal ash primarily consists of silica and alumina for both the whole ash and the size fractions (Table 4.2). The major elemental composition reported as mass percent is based on the assumption that all of the major elements are present in the coal ash as oxides, and that the oxidation states of iron and manganese are assumed to be Fe (III) and Mn (II), respectively. Approximately 6.2% of the whole ash is unburned organic matter, and the organic matter content increases as the size of the coal ash fractions increase (Table 4.3). The surface area also increased as the size of the coal ash fraction increased (Table 4.3). Mercury was found to be concentrated in the largest size fraction (>106 μm), with the highest mercury concentration found in the 53-106 μm fraction.

The selective sequential extraction of mercury from the coal ash revealed similar results for the whole coal ash and the different size fractions of the coal ash when the normalized percentage released (mercury released by a single extraction divided by the total mercury released by all of the extraction steps) was compared (Table 4.3). The majority of mercury was extracted by the 12 M nitric acid (strongly-complexed, Hg\(^0\)), with a smaller portion extracted by aqua regia (metacinnabar-like, HgSe) fraction. No mercury was present in the 1M sodium hydroxide extraction (organo-complexed) fraction for any of the samples.
Table 4.2. Kingston Fossil Plant coal ash characterization by total acid digestion and analysis by ICP-AES (major elements), ICP-MS (trace elements), and CVAFS (mercury).

<table>
<thead>
<tr>
<th>Major Elements (mass % oxides)</th>
<th>Trace Elements (µg g⁻¹)</th>
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<tbody>
<tr>
<td>SiO₂</td>
<td>49.2 ± 1.3</td>
</tr>
<tr>
<td>Al₂O₃</td>
<td>25.3 ± 0.35</td>
</tr>
<tr>
<td>CaO</td>
<td>7.31 ± 0.07</td>
</tr>
<tr>
<td>Fe₂O₃</td>
<td>5.27 ± 0.10</td>
</tr>
<tr>
<td>K₂O</td>
<td>2.02 ± 0.07</td>
</tr>
<tr>
<td>MgO</td>
<td>1.89 ± 0.005</td>
</tr>
<tr>
<td>TiO₂</td>
<td>1.49 ± 0.03</td>
</tr>
<tr>
<td>Na₂O</td>
<td>0.68 ± 0.008</td>
</tr>
<tr>
<td>P₂O₅</td>
<td>0.49 ± 0.03</td>
</tr>
<tr>
<td>MnO</td>
<td>0.02 ± 0.0003</td>
</tr>
<tr>
<td>LOI¹</td>
<td>6.20 ± 0.0002</td>
</tr>
</tbody>
</table>

Total 99.9

¹ loss on ignition at 550°C.
Table 4.3. Characterization data for the whole ash and sieved ash size fractions (<53 μm, 53-106 μm, >106 μm) of Kingston Fossil Plant coal ash. Sequential extraction data was normalized to the total recovered mercury and is reported as a percentage (%).

<table>
<thead>
<tr>
<th></th>
<th>Whole Coal Ash</th>
<th>&lt;53 μm</th>
<th>53-106 μm</th>
<th>&gt;106 μm</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass (%)</td>
<td>100</td>
<td>87.1</td>
<td>10.6</td>
<td>1.5</td>
<td>99%</td>
</tr>
<tr>
<td>Surface area (m$^2$ g$^{-1}$)</td>
<td>6.2 ± 0.06</td>
<td>4.0 ± 0.06</td>
<td>12.8 ± 0.22</td>
<td>16.5 ± 0.30</td>
<td>82%</td>
</tr>
<tr>
<td>LOI (mass %)</td>
<td>6.2 ± 2×10$^{-4}$</td>
<td>4.3 ± .03</td>
<td>16.8 ± 0.7</td>
<td>25.4 ± 5.2</td>
<td>95%</td>
</tr>
<tr>
<td>Total Hg (ng g$^{-1}$)</td>
<td>357 ± 1.4</td>
<td>259 ± 1.3</td>
<td>860 ± 145</td>
<td>706 ± 18.9</td>
<td>92%</td>
</tr>
<tr>
<td>Organo-complexed Hg (extracted by 1 M NaOH)</td>
<td>BDL</td>
<td>BDL</td>
<td>nm$^1$</td>
<td>BDL</td>
<td>N/A</td>
</tr>
<tr>
<td>Strong-complexed Hg, Hg$^0$ (extracted by 12 M HNO$_3$)</td>
<td>95.7</td>
<td>95.3</td>
<td>nm</td>
<td>93.9</td>
<td>N/A</td>
</tr>
<tr>
<td>Metacinnabar-like Hg, HgSe (extracted by aqua regia)</td>
<td>4.3</td>
<td>4.8</td>
<td>nm</td>
<td>6.1</td>
<td>N/A</td>
</tr>
</tbody>
</table>

$^1$ Not measured, $^2$loss on ignition at 550°C
**Mercury Release from Coal Ash**

In the absence of DOM, mercury was released from the Kingston coal ash at non-detectable concentrations (≤1 ng L\(^{-1}\)). In the presence of DOM, the release of mercury increased with increasing DOM concentration (Figure 4.2). At the highest concentration of the Everglades HPoA isolate added (19.5 mg C L\(^{-1}\)), 5.4% of the total mercury present in the coal ash was released, which was the maximum release for all of the DOM concentrations added (Figure 4.2). Under similar conditions, more mercury was released when the solution contained the Everglades HPoA compared to the Williams Lake HPoA. At higher F1 HPoA concentrations (≥ 8.8 mg C L\(^{-1}\)), the mercury concentration released from the ash approaches a plateau. When the F1 HPoA concentration increased from 8.8 mgC L\(^{-1}\) to 19.5 mgC L\(^{-1}\), the mercury only increased from 17.8±0.3 ng L\(^{-1}\) to 18.6±0.6 ng L\(^{-1}\). A similar plateau was not observed in the presence of the Williams Lake HPoA. As the WL HPoA concentration increased from 8.5 mgC L\(^{-1}\) to 17.0 mgC L\(^{-1}\), the mercury concentration increased to a much greater extent from 10.3±0.7 ng L\(^{-1}\) to 14.3±0.8 ng L\(^{-1}\).
Figure 4.2. Mercury released from coal ash after a 4-d equilibration time as a function of DOM concentration for the HPoA isolates from the Everglades in Florida and Williams Lake in Minnesota. Error bars show one standard deviation of the duplicate measurements.
Mercury release from the Kingston coal during the sequential release experiments decreased rapidly with increasing batch number until it reached a plateau after three batches (Figure 4.3). The mercury concentration released after the plateau was reached was approximately four times less than the initial mercury released. The mercury release was normalized to the ash mass to account for coal ash lost during filtration (approximately 1-2% by mass). Low concentrations of mercury were still being released from the coal ash after six sequential batches.
Figure 4.3. Mercury released normalized to the mass of coal ash after each sequential experiment (batch number). Error bars show one standard deviation of the duplicate measurements.
Leaching experiments were done using four different DOM isolates of varying SUVA$_{254}$ values in order to determine if chemical properties that vary between DOM sources affect the release of trace metals from coal ash. SUVA$_{254}$ has been strongly correlated with the aromatic carbon content in DOM (Weishaar et al., 2003). The concentration of mercury released had a strong positive correlation with the SUVA$_{254}$ (Figure 4.4). This result is consistent with the F1 HPoA isolate releasing higher mercury concentrations than the WL HPoA isolate in the DOM addition experiments (Figure 4.2).
Figure 4.4. Mercury concentration released from the Kingston coal ash as a function of SUVA$_{254}$ of the DOM isolates (Table 4.1).
DOM Adsorption to Coal Ash

The DOC remaining in solution after equilibration with the coal ash for 4 d was measured and the DOC concentration adsorbed to the ash surface (in mg C per kg ash) was determined by difference to produce DOC adsorption isotherms for the Everglades HPoA and the Williams Lake HPoA isolates (Figure 4.5). More of the Everglades HPoA adsorbed to the coal ash surface than the Williams Lake HPoA across the entire range of DOC concentrations added. Larger differences are observed between the two isolates as the DOC concentration increases.
Figure 4.5. Carbon adsorbed on the coal ash surface determined by difference as a function of the DOC concentration in solution after a 4-d equilibration time.
In the absence of DOC, the zeta potential of the coal ash surface after leaching was -16.6 ± 1.4 mV. This measurement indicates that the surface of the coal ash is negatively charged under the given conditions (pH = 6.8, I = approximately 0.1 M). As the concentration of the added Fl HPoA isolate increased, the zeta potential of suspended ash particles became more negative, decreasing to -26.7 ± 0.3 mV at the maximum DOC concentration of 16.9 mgC L\(^{-1}\) (Figure 4.6).

**Figure 4.6**. The zeta potential of the coal ash surface as a function of the DOC concentration. The error bars show the standard deviation of triplicate measurements.

**Effect of Calcium on Mercury Release**

Calcium additions were made to solutions containing constant concentrations of Everglades HPoA (8.8 mg C L\(^{-1}\)) in order to study the effect that calcium has on the release of mercury from coal ash. Before additional calcium (as Ca(NO\(_3\))\(_2\)) was added to the coal ash-DOM suspension, a background calcium concentration of 10\(^{-3}\) M was released from the coal.
ash following the 4-d equilibration time. As the calcium concentration was increased, the concentration of mercury released from the ash decreased (Figure 4.7). As the calcium concentration was increased from the background level to $10^{-2.0}$ M, the dissolved mercury concentration in solution decreased by approximately 35%. A trend of decreasing mercury release with increasing calcium concentration was observed. The difference between measurements at the highest and lowest calcium concentrations was significant ($t= 8.5$), and the concentration of mercury released across the measured range had a strong negative correlation ($R^2 = 0.88$) (Figure 4.7). The average zeta potential of the coal ash surface at the highest Ca(NO$_3$)$_2$ concentration measured ($10^{-2.55}$M) was within the standard deviation of the zeta potential measured when $10^{-3.55}$ M Ca(NO$_3$)$_2$ was added to the system (Figure 4.8).

![Figure 4.7](image)

Figure 4.7. Mercury release from the Kingston coal ash in the presence of 8.8 mg C L$^{-1}$ Everglades HPoA as a function of total calcium concentration. The lowest calcium concentration shown ($10^{-3.55}$ M) is the background calcium concentration released from the coal ash.
Figure 4.8. The zeta potential of the coal ash surface as a function of the calcium concentration. The error bars show the standard deviation of triplicate measurements.
Mercury Release by Emory and Clinch River Waters

For the samples collected in June, both of the river water samples had low, and very similar, DOC concentrations – the Emory River contained 2.1 mg C L\(^{-1}\) and the Clinch River contained 1.9 mg C L\(^{-1}\). The Emory River water had a higher SUVA\(_{254}\) value (2.3 L mg C\(^{-1}\) m\(^{-1}\)) than the Clinch River water (1.8 L mg C\(^{-1}\) m\(^{-1}\)). The Clinch River water had an overall greater conductivity and calcium concentration that was approximately three times that of the Emory River (Table 4.4). The release of mercury from the coal ash exposed to filtered whole water samples collected from the Emory and Clinch Rivers in June, 2011, was measured after the 4 d equilibration time. The pH was unadjusted for the whole water release experiments in order to represent the conditions during the coal ash spill. Both of the whole water solutions reached a pH of 8.2 ± 0.2, which remained constant over the course of the experiment. The release of mercury from coal ash exposed to Emory River water was 3.4 ± 0.1 ng L\(^{-1}\), while coal ash in contact Clinch River water released 2.3 ± 0.2 ng L\(^{-1}\) (Figure 4.9).
Table 4.4. Characterization of filtered surface waters collected from the Emory and Clinch Rivers.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Emory River June 2011</th>
<th>Clinch River June 2011</th>
<th>Emory River September 2011</th>
<th>Clinch River September 2011</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.6</td>
<td>7.5</td>
<td>6.8</td>
<td>7.5</td>
</tr>
<tr>
<td>DOC (mg C L⁻¹)</td>
<td>2.1</td>
<td>1.9</td>
<td>3.3</td>
<td>2.8</td>
</tr>
<tr>
<td>SUVA₂₅⁴ (L mg C⁻¹ m⁻¹)</td>
<td>2.3</td>
<td>1.8</td>
<td>2.4</td>
<td>2.4</td>
</tr>
<tr>
<td>Ca (mg L⁻¹)</td>
<td>13.9</td>
<td>35.3</td>
<td>10.9</td>
<td>32.3</td>
</tr>
<tr>
<td>Mg (mg L⁻¹)</td>
<td>3.9</td>
<td>9.4</td>
<td>2.5</td>
<td>8.2</td>
</tr>
<tr>
<td>Na (mg L⁻¹)</td>
<td>3.9</td>
<td>7.2</td>
<td>3.7</td>
<td>4.8</td>
</tr>
<tr>
<td>K (mg L⁻¹)</td>
<td>1.2</td>
<td>1.6</td>
<td>1.8</td>
<td>1.9</td>
</tr>
<tr>
<td>Cl⁻ (mg L⁻¹)</td>
<td>2.9</td>
<td>7.5</td>
<td>3.7</td>
<td>2.0</td>
</tr>
<tr>
<td>NO₃⁻ (mg L⁻¹)</td>
<td>0.8</td>
<td>2.5</td>
<td>1.5</td>
<td>2.1</td>
</tr>
<tr>
<td>SO₄²⁻ (mg L⁻¹)</td>
<td>24.1</td>
<td>46.2</td>
<td>12.0</td>
<td>16.8</td>
</tr>
<tr>
<td>Alkalinity (mg L⁻¹ as CaCO₃)</td>
<td>45.9</td>
<td>114</td>
<td>nm¹</td>
<td>nm</td>
</tr>
</tbody>
</table>

¹ not measured.
In September, the calcium concentration decreased by 3 mg L\(^{-1}\) for both water samples, the DOC concentrations for both waters increased in the September samples – the Emory River contained 3.3 mg C L\(^{-1}\) and the Clinch River contained 2.8 mg C L\(^{-1}\). The SUVA\(_{254}\) values for both waters collected in September were 2.4 L mg C\(^{-1}\) m\(^{-1}\) (Table 4.4). There was greater mercury release from the coal ash when it was exposed to both the Emory and Clinch River waters collected in September, 2011, with the mercury release from the coal exposed to the Clinch water increasing to a greater extent (Figure 4.9).

![Bar graph showing mercury release from coal ash after 4 days in filtered surface water samples from the Emory and Clinch Rivers from June and September 2011.](image)

Figure 4.9. Mercury release from coal ash after 4 d in filtered surface water samples from the Emory and Clinch Rivers from June and September 2011.
Discussion

Effect of DOM on Mercury Release

The results indicate that DOM has a significant effect on the release of mercury from coal ash. While there have not been any previous studies that have directly looked at the role of DOM on the release of mercury, there has been work done looking at the role of organic ligands on the leaching of metals from coal ash, soils, and sediments. In these studies, it was shown that organic ligands, such as citric acid and EDTA, promoted increased dissolution (Harris and Silberman, 1983; Janos et al., 2002). Some researchers thought that very strong complexing ligands, such as EDTA, were able to extract the total concentration of leachable metals from coal ash (Garrabants and Kosson, 2000; Jegadeesan et al., 2008). This led some researchers to suggest the use of strong chelating ligands as a method for detoxification of fly ash (Van der Bruggen et al., 1998; Hong et al., 2000; Zhang and Itoh, 2006). Another study looked at the leaching of trace metals in the presence of humic acids, and while mercury was not one of the measured metals, it was shown that humic acids significantly increased the total leachable trace metal concentrations (Janos et al., 2004).

Enhanced dissolution by DOM has been observed previously for some minerals (Lin and Valentine, 2009; Shi and Stone, 2009), while it was shown to inhibit the dissolution of other minerals (Banerjee and Nesbitt, 2001; Jacobson et al., 2005). DOM was shown to enhance the dissolution of cinnabar, a mercury sulfide mineral with very low solubility (Ravichandran et al., 1998; Waples et al., 2005). It was observed that DOM concentration positively correlated with aqueous mercury concentration under equilibrium conditions until it reached a plateau where increasing the DOM concentration did not result in an increase in dissolved mercury (Ravichandran et al., 1998). Results shown here for coal ash follow the same behavior as the
A surface adsorption dissolution mechanism is consistent with mercury release from the coal ash for the isolates studied because higher dissolved mercury concentrations were released by the DOM isolate that had greater adsorption to coal ash surface (Everglades HPoA). The zeta potential results for the coal ash support DOM adsorption to the coal ash surface because DOM is negatively charged and adsorption to the ash surface would result in a more negatively charged surface (Figure 4.6). It has been shown that dissolution of cinnabar, resulting in release of mercury into solution, requires direct contact between the DOM and the mineral surface (Waples et al., 2005). While a similar experiment was not done here, we believe that a similar release
mechanism is responsible for the release of mercury from coal ash based on the limited DOC adsorption and mercury release data.

The SUVA$_{254}$ of the DOM was positively correlated with the release of mercury from the coal ash (Figure 4.4). The dissolution rate of cinnabar (Waples et al., 2005) and the amount of mercury dissolved at equilibrium (Ravichandran et al., 1998) were shown to correlate with the aromatic carbon content of the DOM present in solution, which is consistent with what we observed for the release of mercury from coal ash. SUVA$_{254}$ is also known to co-vary with the average molecular weight of DOM samples (Chin et al., 1994; Waples et al., 2005). Many studies have shown that larger, more aromatic molecules preferential adsorption to oxide minerals (McKnight et al., 1992; Gu et al., 1994; Meier et al., 1999), and adsorption of DOM to the coal ash surface may contribute to mercury release. Our findings are inconsistent with those of Waples et al. (2005) where it was observed that the concentration of irreversibly adsorbed DOM was correlated with lower cinnabar dissolution rates. In this study, the Everglades HPoA, which had greater adsorption to the coal ash, released greater mercury concentrations.

**Effect of Calcium on Mercury Release**

The results indicate that calcium inhibits the release of mercury from coal ash in the presence of DOM. As the calcium concentration in solution increased, the concentration of dissolved mercury in solution decreased. This behavior has been observed for other mercury-containing solids in the presence of calcium and DOM (Ravichandran et al., 1998; Suess, 2006; Langner, 2009). Low concentrations of calcium (2.5×10$^{-4}$ M) were shown to reduce mercury dissolution from cinnabar by approximately 85% in the presence of DOM (Ravichandran et al., 1998). Adding calcium to solution was shown to promote the aggregation and precipitation of mercury sulfide in solutions that were previously stabilized by DOM (Ravichandran et al., 1999).
Mercury release studies were also done on contaminated soils and marine sediments in the presence and absence of calcium and magnesium additions. It was found that the addition of calcium reduced the release of mercury from contaminated soils (Suess, 2006), as well as decreased the concentration and SUVA of the DOM being released from marine sediments (Langner, 2009). In these studies, it was suggested that changes in the charge distribution due to Ca\(^{2+}\)-DOM interactions could change DOM characteristics important for interacting with surfaces. This could result in a reduction of the interaction between DOM and the coal ash surface, which is important for the release of mercury. Calcium may also occupy binding sites on the coal ash surface important for ligand exchange, such as hydroxyl functional groups, thus reducing the total sites available for DOM binding which could result in a decrease in the release of mercury. Zeta potential measurements of the coal ash surface after the Ca\(^{2+}\) additions indicate that Ca\(^{2+}\) addition did not result in significant Ca\(^{2+}\) adsorption to negatively-charged coal ash surface (Figure 4.8). This suggests that Ca\(^{2+}\)-DOM associations were likely responsible for the observed reduced mercury release. These findings are in contrast to those of Ca\(^{2+}\)-DOM-cinnabar systems by Ravichandran et al. (1998), where the zeta potential became more positive after the addition of calcium to DOM-cinnabar suspensions, indicating Ca\(^{2+}\) interactions with the cinnabar surface.

**Mercury Release by Emory and Clinch River Waters**

The mercury release from coal ash exposed to the two river water samples is consistent with results observed for the experiments using DOM isolates. Specifically, experiments with isolated DOM exhibited a positive relationship between DOC concentration and SUVA\(_{254}\) and mercury release (Figure 4.2 and 4.4). A negative relationship between calcium concentration and
mercury release was also observed (Figure 4.7). In Figure 4.9, coal ash that was exposed to both Emory River, which had higher DOC concentrations, SUVA<sub>254</sub> values, and lower calcium concentrations than the Clinch, released more mercury. The mercury concentration released from the ash exposed to the river water samples (Emory and Clinch collected in June and September 2011) showed a positive correlation with DOC concentration (Figure 4.10a). DOC concentrations of all river water samples were below 5 mg C L<sup>-1</sup>, which is significantly lower than the DOM isolate concentrations (as mg C L<sup>-1</sup>) that resulted in a plateau for the same ash:water ratio (Figure 4.2). It was also demonstrated with the isolates that DOM sources with higher SUVA<sub>254</sub> values will release more mercury than those with lower SUVA<sub>254</sub> values at an equal DOC concentrations. When the SUVA<sub>254</sub> for each whole water sample was plotted against the mercury released per mg DOC, the relationship was not as clear (Figure 4.10b). While the samples with higher SUVA appeared to release greater mercury per mg C, the error between measurements makes any correlation difficult to determine.

The calcium concentrations of the Emory and Clinch Rivers were different – the Clinch River has a calcium concentration approximately three times higher than the Emory River (Table 4.4). Based on results seen with the Everglades HPoA isolate that calcium inhibits the release of mercury, it could be predicted that the high Ca<sup>2+</sup> concentration in the Clinch River would result in lower mercury release than with the Emory River. While both samples from the Emory River released higher concentrations of mercury, it is difficult to attribute this difference to any one factor (DOC concentration, SUVA<sub>254</sub>, calcium). In an attempt to determine which factor plays the greatest role in controlling mercury release, the mercury concentration released from the coal ash for the September 2011 river water samples were normalized to the measured DOC concentration (Figure 4.11). Because the two water samples had the same SUVA<sub>254</sub>, this reveals
that the difference in calcium concentration between the two river water samples did not have a measurable effect on mercury release. Since the difference in the calcium concentration between river water samples was only slightly greater than the background calcium concentration released from the ash ($10^{-3.3}$ M compared to $10^{-3.6}$ M), calcium would not be expected to significantly inhibit the release of mercury from the coal ash.
Figure 4.10. (a) mercury release as a function of the DOC concentration of filtered surface waters; and (b) mercury release as a function of SUVA$_{254}$ of the filtered surface waters.
Environmental Impact

Based on the results from the DOM isolates and the Emory and Clinch water samples, the conditions most favorable for mercury release from coal ash into natural surface waters will be in environments where the water contains high concentrations of aromatic DOM and low dissolved calcium concentrations. The waters surrounding the Kingston Fossil Plant, the Emory and Clinch Rivers, both had low concentrations of DOC and low SUVA\textsubscript{254} values. Our work suggests that there is a low risk for initial mercury release from coal ash into the surface waters surrounding the Kingston Fossil Plant, but release of mercury can take place over long timescales, as indicated by the sequential release experiments. This slow release may lead to much greater amount of mercury being released from the coal ash than what was predicted by the equilibrium experiments. Coal ash exposed to waters containing higher concentrations of DOM, more aromatic DOM, or low calcium concentrations would be at a higher risk for mercury release into the surface waters. Also, coal ash that contains lower calcium concentrations may
behave differently than what was observed for the Kingston Fossil Plant sample. For example, a coal ash sample that contains less calcium and more iron oxides may release different concentrations of mercury in the presence of DOM. Spatial and temporal variability in DOM concentration and composition should be taken into account when assessing the mercury leaching potential.
References


Chapter 5

Release of Trace Elements from Coal Ash Produced from the Kingston Fossil Plant in the Presence of Dissolved Organic Matter

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Abstract

The release of coal ash because of a containment system failure at the at Tennessee Valley Authority’s Kingston Fossil Plant in December 2008 are a concern because of the potential for human health problems as well as ecological effects that may occur if high concentrations of toxic trace metals present in the ash are released into the environment. The purpose of this work was to determine if dissolved organic matter (DOM) enhances the release of other trace metals and metalloids from coal ash and how differences between element (cationic vs. anionic) and DOM aromaticity effect this release. A series of release experiments were carried out on the coal ash produced at the Kingston Fossil Plant in Harriman, TN, as a function of DOM concentration, DOM aromaticity, and calcium concentration. The major and trace elemental composition of the solution, and specific trace metals (lead, copper, aluminum) and metalloids (arsenic and selenium) were measured. The results indicate that DOM enhances the release of lead, copper and aluminum from coal ash. The concentration of lead, copper and aluminum released from the coal ash had a positive correlation with the SUVA$_{254}$. The release of arsenic and selenium from the coal ash was not dependent on the DOM concentration or source. Calcium was shown to inhibit the release of lead, copper and aluminum from the coal ash, but did not have the same effect on the release of arsenic and selenium.
Introduction

Coal used in power production contains high concentrations of many trace elements that become even more concentrated in the coal ash upon combustion (Wadge et al., 1986; Fernández-Turiel et al., 1994). In December 2008, a coal ash spill at the Tennessee Valley Authority’s Kingston Fossil Plant released 3.8 million cubic meters of coal ash into the Emory River (Ruhl et al., 2009; U.S. EPA, 2009; TVA, 2009; Bednar et al., 2010). This unprecedented spill raised many concerns about the potential for trace elements, in particular arsenic, selenium, and mercury, as well as other trace metals which are concentrated in the ash during combustion (Wadge and Peterson, 1986; Fernández-Turiel et al., 1994; Hower et al., 2005) to be released into the surrounding waters at concentrations harmful to the aquatic ecosystem (Jegadeesan et al., 2008; Ruhl et al., 2009; Gitari et al., 2009; Kim and Hesbach, 2009). Understanding the release and mobility of trace elements is important in evaluating the risk for human health and ecological problems as a result of coal ash releases.

Coal ash contains many trace elements that are available to be released, but the “available” fractions vary between different leaching procedures, water conditions, and coal ash types (Kim and Hesbach, 2009; Gitari et al., 2009). Coal ash is primarily composed of an aluminosilicate core and can contain high concentrations of calcium and iron, depending on the origin of the coal. Calcium and iron are easily released from the coal ash under a range of conditions (Gitari et al., 2009). Trace metals, such as cadmium, cobalt, copper, lead, nickel, mercury, and zinc, are concentrated in the coal ash (Fernández-Turiel et al., 1994; Hower et al., 2005) and have been observed to be released at low pH values because of mineral dissolution and proton exchange mechanisms (Otero-Rey et al., 2005; Baba et al., 2008; Jegadeesan et al., 2008; Gitari et al., 2009; Kim and Hesbach, 2009). The major form of arsenic and selenium in the coal ash are the
oxyanion species arsenate and selenite (Huggins et al, 2009), which are released at neutral to slightly alkaline pH values because of competition with hydroxide ions for surface adsorption sites and precipitation with dissolved calcium at low pH values (Jankowski et al., 2006; Gitarti et al., 2009).

Dissolved organic matter (DOM) may increase the concentration of trace elements released from the coal ash. It has been shown that the presence of organic ligands, such as EDTA and citric acid cause an increase in the concentrations of trace metals (e.g. lead, copper, iron, zinc) released from coal ash (Harris and Silberman, 1983; van der Bruggen et al., 1998; Janos et al., 2002; Jegadeesan et al., 2008). DOM may also be important for the release of oxyanions from coal ash because it forms aqueous complexes with arsenate and selenite and is also known to compete for surface adsorption sites, which may result in displacement of the oxyanions (Buschmann et al, 2006; Wang and Mulligan, 2006; Weng et al, 2009). DOM concentration and aromaticity were shown to increase the concentration of mercury released from the coal ash (Chapter 4). This observation was consistent with the increased dissolution of cinnabar (HgS), seen in the presence of DOM (Ravichandran et al., 1998), as well as results from a later study, where the aromatic carbon content of DOM was correlated with the rate of cinnabar dissolution (Waples et al., 2005). Based upon the enhanced release of mercury from coal ash in the presence of DOM shown in Chapter 4, we expect that a similar enhanced release will be observed for other metal cations, but the amount of enhancement may vary between soft, intermediate and hard metals.

Strong metal-DOM interactions are a function of both metal and ligand properties. Soft metals, such as Hg$^{2+}$, are known to bind very strongly with reduced sulfur functional groups, which are present in DOM in low concentrations (Haitzer et al., 2002; Gerbig et al., 2011). Hard
metals, such as Ca$^{2+}$ and Al$^{3+}$, do not bind strongly with reduced sulfur functional groups; instead, they interact with oxygen-containing functional groups, which are present in much higher concentrations in the DOM. Other metals, such as Cu$^{2+}$ and Pb$^{2+}$, are intermediate metals; these metals will bind with oxygen-containing functional groups at high metal:DOM ratios, but may interact with nitrogen- and reduced sulfur-containing functional groups as the metal:DOM ratio decreases (Pinheiro et al, 1999; Weng et al, 2002; also see Chapter 2 and 3). Preferentially binding based on metal hardness to functional groups in the DOM of varying strengths and concentrations may result in differences in the amount of metal released from the coal ash.

The presence of calcium has been shown to inhibit the release of mercury from coal ash (see Chapter 4), minerals, soils, and sediments (Ravichandran et al., 1998; Suess, 2006; Langner, 2009). If the inhibition of mercury release was because of Ca$^{2+}$ occupying binding sites in the DOM (Chapter 4), the release of other trace metals could be affected differently because of the preferences for binding sites that vary in strength and capacity between soft, intermediate and hard metals.

While there has been much work that has studied the leaching of trace elements from coal ash using different standard leaching procedures (Otero-Rey et al., 2005; Gitari et al., 2009; Wang et al., 2009; Bednar et al., 2010), the role of DOM on the release of trace elements remains in question. The purpose of this study is to identify the role of DOM on the release of the trace metals aluminum, copper, lead, which range from hard to soft cations, and metalloids (arsenate and selenite) which exist as oxyanions in the coal ash. Dissolved concentrations of these elements were measured in the presence and absence of DOM in order to test the hypothesis that DOM enhances the release of trace elements from coal ash. The release of the same elements was also measured for six DOM isolates which covered a range of SUVA$_{254}$ values found to
different environments in order to determine if the releases of these trace elements correlate with the aromaticity of the DOM, similarly to the release of mercury (Chapter 4). Lastly, the role of calcium was also examined to determine if calcium affects the release of trace elements from coal ash and how this effect varies between the hard and soft metals and the oxyanions.
Methods

Materials

The freshly-produced coal ash sample used in these release experiments was collected from the ash hopper at the Tennessee Valley Authority’s Kingston Fossil Plant on October 19, 2010 by TVA personnel and shipped to the University of Colorado. Stock solutions of 0.1 M nitric acid (HNO$_3$) were diluted from concentrated trace metal grade nitric acid and used to neutralize the coal ash leachate before the DOM was added. A 0.1 M sodium hydroxide (NaOH) solution was used to neutralize the pH of the acidic DOM stock solutions to a pH of 5.8 ± 0.1 before addition to the coal ash suspensions. All solutions were prepared by dissolving the chemicals into ultrapure water (> 18 MΩ resistivity; Barnstead, Nanopure). All chemicals used were obtained from Fisher Scientific.

Dissolved Organic Matter Isolates

Organic matter from three sites – the Everglades in Florida, Williams Lake in Minnesota, and the Penobscot River in Maine – were isolated from the water according to the procedures of Aiken et al. (1992). DOM fractions were obtained by retention on XAD-8 (hydrophobic acid; HPoA) and XAD-4 (transphilic acid; TPiA) resins, back-elution using NaOH, and freeze-drying. The HPoA fraction of the DOM was retained on the XAD-8 resin and generally contains larger, more aromatic compounds than the TPiA fraction, which was retained on the XAD-4 resin. The six isolates used in this study were the Williams Lake HPoA, Williams Lake TPiA, Everglades HPoA, Everglades TPiA, Penobscot River HPoA and Penobscot River TPiA (Table 5.1). These isolates were selected based on their specific UV absorbance measured at 254 nm (SUVA$_{254}$), which were selected to cover a wide range of SUVA$_{254}$ values found in natural waters. Aromaticity is positively correlated with SUVA for a wide range of DOM isolates (Weishaar et
al., 2003) and has also increased the concentrations of mercury released from coal ash under similar conditions (Chapter 4). All DOM isolates were prepared as approximately 200 mg L\(^{-1}\) stock solutions by dissolving the freeze-dried DOM in ultrapure water (> 18 MΩ resistivity; Barnstead, Nanopure). The DOM stock solutions were adjusted to a pH of 5.8 ± 0.1 using a 0.1 M NaOH solution, filtered (0.45-µm Supor membrane, Pall Life Sciences), and refrigerated until addition to the coal ash suspensions.
Table 5.1. DOM isolates and their corresponding elemental composition and SUVA$_{254}$ values.

<table>
<thead>
<tr>
<th>DOM Isolate</th>
<th>carbon (wt%)</th>
<th>hydrogen (wt%)</th>
<th>oxygen (wt%)</th>
<th>nitrogen (wt%)</th>
<th>sulfur (wt%)</th>
<th>SUVA$_{254}$ (L mgC$^{-1}$ m$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Williams Lake transphilic acid (TPiA)</td>
<td>49.63</td>
<td>5.41</td>
<td>40.60</td>
<td>3.36</td>
<td>1.00</td>
<td>1.4</td>
</tr>
<tr>
<td>Williams Lake hydrophobic acid (HPoA)</td>
<td>55.23</td>
<td>5.73</td>
<td>36.53</td>
<td>1.76</td>
<td>0.75</td>
<td>1.8</td>
</tr>
<tr>
<td>Everglades TPiA</td>
<td>48.64</td>
<td>4.39</td>
<td>43.08</td>
<td>2.29</td>
<td>1.60</td>
<td>3.0</td>
</tr>
<tr>
<td>Everglades HPoA</td>
<td>53.09</td>
<td>4.44</td>
<td>39.25</td>
<td>1.33</td>
<td>1.89</td>
<td>4.6</td>
</tr>
<tr>
<td>Penobscot River TPiA</td>
<td>52.71</td>
<td>4.32</td>
<td>41.42</td>
<td>1.08</td>
<td>0.47</td>
<td>3.1</td>
</tr>
<tr>
<td>Penobscot River HPoA</td>
<td>47.05</td>
<td>4.23</td>
<td>46.99</td>
<td>1.73</td>
<td>N/A</td>
<td>4.5</td>
</tr>
</tbody>
</table>
Characterization of Ash

The coal ash used in this study was a sample of freshly produced coal ash collected from the Kingston Fossil Plant in Kingston, TN, on October 19, 2010. The coal ash sample was the same as the sample used in a previous study on the release of mercury from coal ash (Chapter 4). The coal ash was sieved into three different size fractions (<53µm, 53-106 µm, and >106 µm) and the chemical analyses were done on the size fractions in addition to the whole coal ash sample. Briefly, elemental composition was determined by an HCl/HNO₃/HF digestion at the LEGS laboratory at the University of Colorado. Elemental analysis of the digest was performed for the major elements using an inductively coupled plasma-atomic emission spectrometry (ICP-AES; Applied Research Laboratories, Model 3410+). Trace elements (As, Cu, Pb, Se) were analyzed using inductively coupled plasma-mass spectrometry (ICP-MS; Varian 810MS). Loss on ignition (LOI) was conducted at 550°C for 1 h to measure the organic matter content. Surface area measurements were made using Brunauer, Emmett and Teller (BET) multi-point surface area analysis (Micromeritics Instrument Corp.; model Gemini V2.00) at the Chemical and Biological Engineering Department at the University of Colorado. Images of the coal ash were collected using a field emission scanning electron microscopy (FESEM; JEOL JSM 7401F, 5kV accelerating voltage).

Trace Element Release Experiments

Batch release experiments were performed according to the procedure described in Chapter 4 for the trace elements. To briefly describe, 0.1 g of the coal ash was added to 100 mL ultrapure water (> 18 MΩ resistivity; Barnstead, Nanopure), neutralized to a pH of 6.8 ± 0.5 using a series of five additions of 0.01M trace metal-grade nitric acid, and equilibrated for 22 ± 2 h. After neutralization, the DOM isolates were added from stock solutions (100 to 200 mgC L⁻¹;
prepared by dissolving DOM isolates in DI water, adjusting the pH to 5.8±0.1, and passing the stock solution through a 0.45 µM membrane filter) to produce different coal ash-DOM suspensions with final DOC concentrations ranging from 0 to 19.5 mg C L⁻¹. The suspensions were allowed to equilibrate for 4 d before filtration (0.45-µm Supor membrane, Pall Life Sciences) and analysis of the leachate. This equilibration time was determined from kinetics experiments (Chapter 4). All release leaching experiments were conducted in duplicate.

In order to study the effect of calcium on the release of trace elements from coal ash, the concentration of the Everglades HPoA was held constant (8.8 mg C L⁻¹) as Ca(NO₃)₂ was added to the suspensions. Ca(NO₃)₂ was added (10⁻³.55 to 10⁻².54 M) to a neutralized (pH = 6.8 ± 0.5) Everglades HPoA solution. The ash-DOM-calcium suspensions were equilibrated for 4 d, filtered, and the leachate was collected for analysis.

**Sample Analysis**

Major and trace metals that were released from the coal ash were using ICP-AES (Applied Research Laboratories, Model 3410+) and ICP-MS (Varian 810MS). Filtered samples for metal analyses were collected in acid-cleaned high-density polyethylene bottles and preserved using 1% trace metal-grade HNO₃. Dissolved organic carbon (DOC) concentrations were measured using the persulfate oxidation method on a total organic carbon analyzer (Oceanography International, model 700). The UV absorbance of samples and stock solutions were measured using an UV/visible spectrophotometer (Hewlett Packard, model 8453).
Results

Characterization of the Coal Ash

The Kingston coal ash primarily consists of silica and alumina for both the whole ash and the size fractions (Table 5.2). The organic matter content increases as the size of the coal ash fractions increase (Table 5.2). Most of the trace metals are concentrated in the smallest (<53 µm) size fraction of the coal ash, with mercury and selenium as the exceptions (Table 5.3). The highest mercury concentration was found in the 53-106 µm size fraction, and the highest selenium concentration was found in the >106 µm size fraction (Table 5.3). SEM images of the largest (>106 µm) and smallest (<53µm) size fractions of the coal ash are shown in Figure 5.1. The smallest size fraction coal ash particles are more spherical and uniform compared to the largest size fraction particles which are more amorphous and porous, which leads to the greater surface area of these particles. This result is consistent with what has been observed for other coal ash samples (Schure et al., 1985; Mollah et al., 1994; Kutchko and Kim, 2006). The results from LOI determined that the largest coal ash fraction (>106 µm) was the most organic-rich.
Table 5.2. Major elements as oxides and organic matter content in the whole coal ash and the coal ash size fractions.

<table>
<thead>
<tr>
<th>major elements</th>
<th>&lt;53 µm</th>
<th>53-106 µm</th>
<th>&gt;106 µm</th>
<th>Whole</th>
</tr>
</thead>
<tbody>
<tr>
<td>SiO$_2$</td>
<td>40.4</td>
<td>38.9</td>
<td>33.2</td>
<td>40.4</td>
</tr>
<tr>
<td>Al$_2$O$_3$</td>
<td>25.1</td>
<td>22.3</td>
<td>17.5</td>
<td>24.4</td>
</tr>
<tr>
<td>CaO</td>
<td>13.2</td>
<td>7.2</td>
<td>6.2</td>
<td>12.6</td>
</tr>
<tr>
<td>Fe$_2$O$_3$</td>
<td>7.0</td>
<td>6.9</td>
<td>6.7</td>
<td></td>
</tr>
<tr>
<td>K$_2$O</td>
<td>2.1</td>
<td>1.9</td>
<td>1.6</td>
<td>2.0</td>
</tr>
<tr>
<td>MgO</td>
<td>2.4</td>
<td>1.4</td>
<td>1.1</td>
<td>2.3</td>
</tr>
<tr>
<td>TiO$_2$</td>
<td>1.7</td>
<td>1.2</td>
<td>1.0</td>
<td>1.6</td>
</tr>
<tr>
<td>Na$_2$O</td>
<td>1.1</td>
<td>0.7</td>
<td>0.5</td>
<td>1.1</td>
</tr>
<tr>
<td><strong>Organic Matter</strong></td>
<td><strong>4.3</strong></td>
<td><strong>16.8</strong></td>
<td><strong>25.4</strong></td>
<td><strong>6.2</strong></td>
</tr>
<tr>
<td><strong>total</strong></td>
<td><strong>97.3</strong></td>
<td><strong>96.2</strong></td>
<td><strong>93.4</strong></td>
<td><strong>97.3</strong></td>
</tr>
</tbody>
</table>

*Organic matter content was determined by the loss on ignition at 550°C for 1 h*
Table 5.3. Trace element concentration in the whole coal ash and the coal ash size fractions.

<table>
<thead>
<tr>
<th>Trace elements</th>
<th>(&lt;53 μm)</th>
<th>53-106 μm</th>
<th>&gt;106 μm</th>
<th>Whole</th>
</tr>
</thead>
<tbody>
<tr>
<td>As</td>
<td>57.5 ± 6.7</td>
<td>40.4 ± 0.7</td>
<td>41.8 ± 0.9</td>
<td>57.6 ± 4.5</td>
</tr>
<tr>
<td>Cd</td>
<td>1.27 ± 0.09</td>
<td>0.71 ± 0.06</td>
<td>0.49 ± 0.14</td>
<td>1.19 ± 0.01</td>
</tr>
<tr>
<td>Cu</td>
<td>148 ± 3.3</td>
<td>99.0 ± 2.3</td>
<td>118 ± 7.7</td>
<td>144 ± 8.1</td>
</tr>
<tr>
<td>Hg</td>
<td>0.26 ± 0.001</td>
<td>0.86 ± 0.02</td>
<td>0.71 ± 0.02</td>
<td>0.36 ± 0.01</td>
</tr>
<tr>
<td>Ni</td>
<td>105 ± 3.1</td>
<td>73.8 ± 1.2</td>
<td>94.6 ± 4.3</td>
<td>102 ± 2.9</td>
</tr>
<tr>
<td>Pb</td>
<td>57.5 ± 1.5</td>
<td>27.1 ± 0.6</td>
<td>21.4 ± 1.8</td>
<td>53.1 ± 0.6</td>
</tr>
<tr>
<td>Se</td>
<td>2.8 ± 0.2</td>
<td>7.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>46.7 ± 0.4</td>
<td>4.8 ± 0.3</td>
</tr>
<tr>
<td>Zn</td>
<td>125 ± 5.4</td>
<td>77.4 ± 3.6</td>
<td>66.4 ± 8.8</td>
<td>121 ± 3.3</td>
</tr>
</tbody>
</table>

<sup>a</sup>Only a single analysis was done.
Figure 5.1. SEM images of different size fractions of the Kingston Fossil Plant coal ash: (A) >106 µm coal ash fraction (retained on a 106 µm sieve). The magnification is 400 times, and the accelerating voltage is 5.0 kV; (B) <53 µm coal ash fraction (passed through a 53 µm sieve). Scale bars showing the length of 10 µm are given at the lower right hand corner of each image. The magnification is 700 times, and the accelerating voltage is 5.0 kV.
Effect of DOM on the Release of Trace Elements from Coal Ash

Lead, Copper, and Aluminum

Before the addition of DOM, concentrations of lead, copper, and aluminum released from the coal ash were very low. The concentrations of lead and copper released were both below the analytical detection limits, and the aluminum concentration released from the coal ash was $6.5 \pm 0.81 \mu g L^{-1}$, or 0.005% of the total aluminum in the whole coal ash (129 mg g$_{ash}^{-1}$). The concentration of all three metals in solution increased as the DOM concentration was increased (Figure 5.2). When the added concentration of the Everglades HPoA isolate was 36.6 mg L$^{-1}$, 0.42 % of the total lead, 6.4 % of the total copper, and 0.18 % of the total aluminum was released from the coal ash.
Figure 5.2. The fraction of lead, copper, and aluminum released from whole coal ash from the Kingston Fossil Plant as a function of the concentration of Everglades DOM isolate. The standard deviation the duplicate experiments are shown as the error bars. If error bar is not shown, it is smaller than the data point.
**Arsenic and Selenium**

The release behavior of the arsenic and selenium from the coal ash was different from that observed for lead, copper, and aluminum (Figure 5.3). In the absence of DOM, the background levels of the arsenic and selenium released were $3.7 \pm 0.17$ and $1.1 \pm 0.03 \mu g \text{ L}^{-1}$, respectively. As the DOM concentration added to the solution was increased from 0 to $36.6 \text{ mg L}^{-1}$, the concentration of arsenic released increased slightly to a maximum value of $6.0 \pm 0.13 \mu g \text{ L}^{-1}$ (8.4 % of the total) at a DOM concentration of $16.5 \text{ mg L}^{-1}$. The selenium concentration released from the coal ash did not show a significant increase as the DOC concentration increased. The concentration released remained low, but the fraction of the total selenium released from the coal ash is approximately 25-30 % across the range of DOC concentrations measured.
Figure 5.3. The fraction of arsenic and selenium released from whole coal ash from the Kingston Fossil Plant as the DOM concentration added in increased from 0-36.6 mg L\(^{-1}\) for the arsenic release experiments and from 0-16.5 mg L\(^{-1}\) for the selenium release experiments.
DOM Source

Release experiments using six different DOM isolates (Table 5.1), which cover a wide range of SUVA\textsubscript{254} values found in natural environments, were carried out in order to examine effects of DOM aromaticity on trace element release from the coal ash. The concentration of each metal and metalloid released from the coal ash was normalized to the DOM concentration added to each solution to account for any differences in concentration of the different stock solutions.

**Lead, Copper and Aluminum**

The release of lead, copper and aluminum from the whole coal ash by the six DOM isolates revealed a correlation between the dissolved concentrations of both metals and the SUVA\textsubscript{254} of the DOM (Figure 5.4). The correlation of copper release with SUVA\textsubscript{254} was stronger ($R^2 = 0.69$) than the correlation of aluminum release with SUVA\textsubscript{254} ($R^2 = 0.41$) or the lead release with SUVA\textsubscript{254} ($R^2 = 0.45$). The correlations for both aluminum and lead can be improved by removing the data point for the Penobscot River TPiA (SUVA\textsubscript{254} of 3.1 L (mg\textsubscript{C})\textsuperscript{-1} m\textsuperscript{-1}; $R^2 = 0.99$ for aluminum and $R^2 = 0.83$ for lead). The DOM isolate with the highest SUVA\textsubscript{254} value (Everglades HPoA; SUVA of 4.6 L (mg\textsubscript{C})\textsuperscript{-1} m\textsuperscript{-1}) released an approximately 3.5 times greater copper concentration and 1.8 times greater aluminum concentration than the lowest SUVA\textsubscript{254} isolate (Williams Lake TPiA; SUVA\textsubscript{254} = 1.4 L (mg\textsubscript{C})\textsuperscript{-1} m\textsuperscript{-1}).
Figure 5.4. Lead, copper and aluminum concentration released from the whole coal ash normalized to the DOM concentration as a function of SUVA$_{254}$ of the DOM isolates. The six DOM isolates used in this experiment are shown in Table 5.1 with their corresponding SUVA$_{254}$. 

- [Pb] (µg L$^{-1}$)/[DOM] (mg L$^{-1}$) vs. SUVA$_{254}$ (L (mg C$^{-1}$) m$^{-1}$) with $R^2 = 0.45$
- [Cu] (µg L$^{-1}$)/[DOC] (mg L$^{-1}$) vs. SUVA$_{254}$ (L (mg C$^{-1}$) m$^{-1}$) with $R^2 = 0.63$
- [Al] (µg L$^{-1}$)/[DOM] (mg L$^{-1}$) vs. SUVA$_{254}$ (L (mg C$^{-1}$) m$^{-1}$) with $R^2 = 0.32$
**Arsenic and Selenium**

Release of arsenic and selenium from the coal ash did not yield a significant correlation with SUVA$_{254}$ of the DOM isolates as observed for copper and aluminum (Figure 5.5). The correlation of arsenic concentrations with SUVA$_{254}$ was low ($R^2 = 0.13$). The correlation of selenium release with SUVA$_{254}$ was slightly greater ($R^2 = 0.23$), but still very low.
Figure 5.5. Arsenic and selenium concentration released from the coal ash normalized to the DOM concentration as a function of SUVA$_{254}$. The six DOM isolates used in this experiment are shown in Table 5.1 with their corresponding SUVA$_{254}$ values.
Effect of Calcium on Trace Metal Release

Lead, Copper and Aluminum

The concentration of lead, copper and aluminum released from the coal ash decreased as calcium was added to the coal ash suspension (Figure 5.6). As the calcium concentration was increased to $10^{-2.54}$ M from $10^{-3.55}$ M, the copper concentration in solution decreased. Increasing the concentration of calcium in solution by one order of magnitude resulted in a 44% decrease in the concentration of copper and a 70% decrease in the aluminum concentration released from the coal ash into solution. Lead decreased to the greatest extent with an 88% decrease in the concentration of lead after addition of the highest calcium concentration (Figure 5.6).
Figure 5.6. (a) Lead, (b) copper and aluminum released from the whole coal ash as the calcium concentration was increased from $10^{-3.55}$ to $10^{-2.54}$ M. The lowest calcium concentration was the amount of calcium released from the coal ash in the absence of any calcium addition.
Arsenic and Selenium

The release of the trace elements arsenic and selenium was not significantly inhibited by the presence of calcium. The average dissolved concentrations of arsenic decreased slightly as the calcium concentration was increased, but the experimental error of the replicates was greater than the observed decrease (Figure 5.7). The concentration of dissolved selenium decreased even more slightly than the arsenic and again, the experimental error of the replicates was larger than the decrease (Figure 5.7).
Figure 5.7. Arsenic and selenium released from the coal ash as the Ca$^{2+}$ concentration was increased from $10^{-3.55}$ to $10^{-2.54}$ M. The lowest Ca$^{2+}$ concentration was the calcium released from the coal ash in the absence of any calcium additions.
Discussion

Effect of DOM on the Release of Trace Elements from Coal Ash

*Lead, Copper, and Aluminum*

Metals observed in this study increased in ionic character from soft (lead) to intermediate (copper) to hard (aluminum); however, they all had similar release behavior from coal ash as the DOM concentration was increased. In the absence of DOM, the concentrations of all the metals released were low or below the analytical detection limit. As the DOM concentration was increased, similar release trends were observed for all three metals (Figure 5.2). What was significantly different between the metals was the fraction of the total metal concentration that was released. The fraction of lead released from the coal ash at the highest DOM concentration measured (36.6 mg L\(^{-1}\)) was 0.42 %, which was similar to the fraction of aluminum released at the same DOC concentration (0.18 %) and much lower than the fraction of copper released (6.4%). The fraction of copper released is on the same order as the release of mercury (5.4%), a soft metal which was previously studied (Chapter 4).

The binding sites for each of these three metals in DOM are known to be different at low metal:DOM ratios. Soft metals, such as mercury, bind very strongly to the reduced sulfur functional groups that are present in organic matter in low concentrations (e.g., Skyllberg et al., 2000; Haitzer et al., 2002; Gerbig et al., 2011). Lead is often classified as a soft metal because it is able to form strong complexes with sulfide, and may interact with reduced sulfide functional groups in DOM. Lead will also bind with oxygen-containing functional groups, which are present in DOM at much greater abundances than reduced sulfur functional groups. Copper may preferentially bind with nitrogen-containing functional groups in DOM (Croue et al., 2003; Frenkel et al., 2000; Chapter 2). On the other hand, aluminum is a hard metal, which prefers to
bind with oxygen-containing functional groups, such as carboxylic acids. Metal release behavior or the fraction of each metal released, does not change as the metal type changes from soft to hard, which may indicate that the metal hardness does not play a significant role in determining the release behavior.

If metal hardness does not play a role in the metal release behavior from coal ash, the location of the metal in the coal ash (surface versus interior) may be responsible for the differences seen in the release of each metal. The interior, or bulk, of the coal ash has been identified as being composed of an aluminum-silica matrix (Brami et al., 1999; Kutchko and Kim, 2006; Akinyemi et al., 2012) and the ash digestion and elemental analysis performed on the Kingston coal ash sample confirmed this composition (Table 5.2). Lead was observed to associate with oxide minerals that are more evenly distributed throughout the coal ash matrix, instead of only being concentrated on the coal ash surface (Silva et al., 2011). The results from this study indicate that the smallest coal ash particle size (< 53 µm) contained the highest concentrations of lead, copper, and aluminum (Table 5.3). This behavior is consistent with that observed by Wadge et al. (1986), who observed that lead concentration was greatly enriched on the smallest, most inorganic-rich coal ash particles. In contrast, mercury is more concentrated on the larger, more organic-rich coal ash particles. The largest size fraction of coal ash (> 106 µm) shows a positive correlation with both the organic matter content and the surface area (Schure et al., 1985; Hower et al., 2000; Lopez-Anton et al., 2007; Suarez-Ruiz and Parra, 2007; Hower et al., 2010). A surface area dependent DOM-facilitated dissolution mechanism would be consistent with the greater fraction of mercury released from the coal ash compared to the fraction of lead and aluminum that was released.
The positive correlation between the released lead, copper and aluminum concentrations and SUVA$_{254}$ (Figure 5.5) is similar to the release of mercury from coal ash (Chapter 4) as well with the release of mercury from cinnabar (Waples et al., 2005). The observed correlation with SUVA$_{254}$ does not identify a mechanism for DOM-promoted dissolution of coal ash, but previous studies have shown that adsorption of DOM to the coal ash surface is important for trace metal release. For example, the DOM isolate with a higher SUVA$_{254}$ value (F1 HPoA; SUVA = 4.6 L (mgC)$^{-1}$ m$^{-1}$) had greater adsorption to the coal ash surface than the DOM isolate with a lower SUVA$_{254}$ (WL HPoA; SUVA = 1.8 L (mgC)$^{-1}$ m$^{-1}$) (Chapter 4).

**Arsenic and Selenium**

The release of the metalloids arsenic and selenium was different than the release of the metals lead, copper, and aluminum from the coal ash. At a neutral pH, arsenic and selenium are released from the coal ash in the absence of DOM. There was not a significant increase in the released metalloid concentrations as the DOM concentration was increased compared to lead, copper, and aluminum (Figure 5.3). A slight increase in the concentration of arsenic released in the presence of low concentrations of DOM (1.7 mg L$^{-1}$) was observed, but the amount released does not increase as a function of increased DOM concentration. The release behavior of selenium is different than what was seen for arsenic; there was no significant increase of dissolved selenium seen at low DOM concentrations (Figure 5.3). Arsenic and selenium exist primarily as arsenate and selenite in coal ash (Huggins et al., 2007) and are released as oxyanions (Wang et al., 2009; Ruhl et al., 2009; Bednar et al., 2010), which may help to explain some of the differences in leaching behavior compared to the trace metals. The slight increase in the arsenic concentration released at low DOM concentrations may be due to surface competition with the DOM and desorption of arsenic. This has been observed to be an important mechanism
controlling the release of arsenate release from ferric oxide surfaces (Wang and Mulligan, 2006; Bauer and Blodau, 2006). In the absence of DOM, pH-dependent adsorption and precipitation reactions on the coal ash surface control the release of arsenic and selenium from coal ash. The maximum metalloid release for both surface adsorption and precipitation reactions occurs at near neutral pH values, which is consistent with the high concentrations of arsenic and selenium released at pH 6.8±0.5 in the absence of DOM (Goh et al., 2004; Jankowski et al., 2006; Gitari et al., 2009). At high pH, arsenic leaching may be reduced by the precipitation of arsenic in calcium minerals on the coal ash surface (van der Hoak et al., 1994; Gitari et al., 2009). Because the calcium released from the coal ash increased slightly as the DOM concentration increased, arsenic release due to calcium-phase dissolution could be another explanation of the observed arsenic behavior.

**Competition with Calcium**

*Lead, Copper and Aluminum*

Addition of calcium decreased the concentrations of lead, copper and aluminum released from the coal ash. This decrease may be due to $\text{Ca}^{2+}$ competing with the metals for DOM binding sites, which could reduce the aqueous metal concentration that forms complexes with the DOM. The release of aluminum, which binds with oxygen-containing functional groups, was decreased by 70% when the calcium concentration was increased to $10^{-2.54}$ M. Lead also significantly decreased after the addition of calcium (88 %). Calcium had a lesser effect on the release of copper; the dissolved copper concentration decreased by 44% when the calcium concentration was increased by the same amount. Calcium is a hard metal, which means that it prefers to bind with the oxygen-containing functional groups, similar to the behavior of aluminum and lead. If calcium is competing for the same binding sites as aluminum and lead,
this may explain why calcium inhibits the release of aluminum and lead to a greater extent than copper. Ravichandran et al. (1998) measured 5.45 mequiv g$^{-1}$ for carboxylic groups, and 0.77 mequiv g$^{-1}$ for hydroxyl groups present in a DOM sample collected from the sample location used in this study (F1 site in the Florida Everglades). If it is assumed that Ca$^{2+}$ forms a 1:1 complex with each functional group, then at the lowest calcium concentration present (10$^{-3.6}$ M), the dissolved calcium is in excess of the carboxyl and hydroxyl binding sites available in the DOM. This excess of calcium would strongly compete with any metals binding to oxygen-containing functional groups, and may displace the aluminum and lead from carboxyl and hydroxyl groups in the DOM.

Copper also binds with oxygen-containing functional groups at high Cu:DOM ratios, and may bind with functional groups that contain nitrogen at lower copper concentrations (Korshin et al., 1998; Frenkel et al., 2000; Croue et al., 2003). The copper concentrations (< 10 μg L$^{-1}$) released from coal ash in this study may result in copper binding to nitrogen functional groups that are less favorable for competition with calcium.

**Arsenic and Selenium**

Calcium does not inhibit the release of arsenic and selenium to the same extent as it did for the trace metals. This difference in release behavior may be due to differences in the metalloids interactions with DOM compared to trace metals. It has been suggested that metal bridging by cations, such as calcium, magnesium, iron, aluminum, and manganese, may be important in forming soluble DOM-arsenic complexes (Redman et al., 2002; Lin et al., 2004; Wang and Mulligan, 2006). A Ca$^{2+}$ bridging mechanism would cause the additions of Ca$^{2+}$ to increase the arsenic concentration released from the coal ash. In contrast, other work has shown that Al$^{3+}$ concentrations in excess of arsenic were able to compete with arsenic for the strongest
binding sites in the DOM (Buschmann et al., 2006), and Ca\textsuperscript{2+} behaves similarly to Al\textsuperscript{3+}. High concentrations of Ca\textsuperscript{2+} were shown to inhibit the leaching of arsenic and selenium from coal ash under alkaline conditions (Wang et al., 2009). This effect is believed to be caused by the formation of calcium-oxyanion precipitates at high pH, but was not observed at neutral pH values. These contrasting mechanisms for the enhancement and inhibition of arsenic release in the presence of calcium make it difficult to understand what was observed in this study.

**Conclusions and Environmental Implications**

This study reveals that the effect of DOM on the release of trace elements from coal ash varies between element types (metal vs. metalloid) and may vary based upon the element’s location within the coal ash (surface vs. bulk). The results from this study indicate that DOM has a greater effect on the release of trace metals, such as lead, copper, and aluminum, compared to the metalloids arsenic and selenium. Arsenic and selenium are readily leached from the coal ash in the absence of DOM, while the metals lead, copper, and aluminum were released in low concentrations that were near or below the detection limit. The release of arsenic and selenium from coal ash is a concern because even though the release of these elements is not significantly increased in the presence of DOM, the concentrations released in the absence of DOM may still have an impact on the aquatic environment.

Trace metals that are concentrated on the surfaces of the coal ash, such as mercury, have the greatest potential to be released from the coal ash in the presence of DOM compared with other trace metals, such as lead and aluminum which are incorporated into the coal ash matrix. Again, the behavior of selenium contrasts with that of the trace metals because even though the concentrations of selenium are highest on the coal ash particles with the greatest surface area, the release of selenium was not affected by the presence of DOM.
References


30. Weishaar, J. L.; Aiken, G. R.; Bergamaschi, B. A.; Fram, M. S.; Fujii, R.; Mopper, K., Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and


Chapter 6

Conclusions

Summary of Work

In this study, we used a competitive ligand exchange-solid phase extraction (CLE-SPE) method to determine conditional stability constants for Cu\(^{2+}\)-DOM binding at near neutral pH and 0.01 M ionic strength over a range of Cu:DOM ratios that bridge the detection windows of copper-ion selective electrode (Cu-ISE) and voltammetry measurements. As the Cu:DOM ratio increased from 0.0005 to 0.1 mg copper (mg DOM\(^{-1}\)), the measured conditional binding constant \((K_{CuDOM})\) decreased from \(10^{11.5}\) to \(10^{5.6}\) M\(^{-1}\). A comparison of the binding constants we measured using CLE-SPE with literature values measured by copper ion-selective electrode and voltammetry demonstrates that the Cu:DOM ratio is an important factor controlling Cu\(^{2+}\)-DOM binding strength even for DOM isolates of different types and different sources and for whole waters samples.

When the site-specific copper binding constants of DOM in streams draining the Pebble deposit in southwestern Alaska were measured, the same dependence of the binding constant on the Cu:DOM ratio was observed. A combination of Cu-ISE titration, CLE-SPE, and modeling approaches were used to estimate copper binding affinities and capacities of natural DOM. The Cu\(^{2+}\)-DOM binding constants varied as a function of Cu:DOM ratio, with higher conditional binding constants associated with lower Cu:DOM ratios. This behavior is consistent with the presence of higher-affinity but less abundant copper binding sites in the DOM. When these higher-affinity sites get filled, Cu\(^{2+}\) then fills the more abundant, lower-affinity binding sites.

In order to understand the effect that DOM has on trace element leaching from coal ash, a series of release experiments were performed using coal ash generated from the Kingston Fossil Plant in Harriman, Tennessee as a function of DOM concentration, aromaticity, and calcium
concentration. Results indicate that DOM plays an important role in the leaching of the trace elements mercury and copper from the coal ash, while less of an effect was seen with the release of lead, aluminum, arsenic and selenium. DOM isolates with higher SUVA\text{254} values, which is a property that has been correlated to the aromatic content, were found to release higher concentrations of mercury, copper and aluminum from the coal ash. The release the metalloids arsenic and selenium, whose primarily species in the coal ash are the oxyanionds arsenate and selenite, did not show the same correlation with the SUVA\text{254}. It was determined that the presence of calcium inhibits the release of mercury, copper and aluminum from the coal ash. Calcium inhibited the release of aluminum to the greater extent, and the inhibitory effect of calcium decreased as the trace metal decreased in hardness. Calcium was not observed to inhibit the release of arsenic and selenium from the coal ash.

**Environmental Implications**

The results found here can be used to more accurately predict the role of DOM in controlling copper speciation and toxicity. The Cu\textsuperscript{2+}-DOM binding constant results have implications for predicting the potential impact of increased copper concentrations in site-waters draining the Pebble deposit if copper concentrations were to increase because of mining activies. Under current pre-mining conditions, the low concentration of ambient copper is able to bind to the strongest sites in the DOM. If copper concentrations to increase as a result of mining the Pebble deposit, dissolved copper would progressively fill weaker binding sites in the DOM and is thus likely to be less strongly complexed with DOM. The biotic ligand model (BLM) was used to estimate concentrations of Cu\textsuperscript{2+} in site-waters as a function of the total copper concentration. Visual MINTEQ was also used to predict Cu\textsuperscript{2+} concentrations in site-waters using experimentally measured binding constant values from Cu-ISE titration and CLE-SPE.
experiments. At high Cu:DOM ratios, the predicted Cu\(^{2+}\) concentrations in site-waters were considerably lower using the BLM than MINTEQ. This suggests that at most environmentally relevant total copper concentrations, the BLM over-predicts the amount of organically bound copper and could possibly under-estimate copper toxicity to aquatic biota in the Pebble watersheds, especially under mining scenarios. Our results indicate that the site-waters draining the Pebble deposit have a very low assimilative capacity for copper without adversely affecting aquatic biota.

Releases of coal ash to rivers and streams, such as occurred from the Tennessee Valley Authority’s Kingston Fossil Plant into the Emory and Clinch Rivers in December, 2008, may be of great concern because the high concentrations of toxic trace elements present in the ash. The conditions most favorable for mercury release from coal ash into natural surface waters are in environments where the water contains high concentrations of aromatic organic matter and low dissolved calcium concentrations. This work suggests that there is a low risk for the release of the trace metals mercury, copper, lead from coal ash into the surface waters surrounding the Kingston Fossil Plant, due to the low concentrations of DOM and low SUVA\(_{254}\) values. Coal ash exposed to waters containing higher concentrations of DOM or more aromatic DOM would be at a higher risk for trace metal release into the surface waters. The DOM concentration is not as important for the release of the metalloids arsenic and selenium from coal ash. The release of these elements may be a concern for waters containing low DOM concentrations, because both arsenic and selenium were released from the coal ash in the absence of DOM.

**Future Work**

**Copper Binding to DOM and Toxicity**

In Chapters 2 and 3, conditional binding constants were measured at a fixed pH and ionic strength. While it was determined that the Cu:DOM ratio has a very significant effect of the
measured Cu\(^{2+}\)-DOM binding constant, the pH and ionic strength will also affect the strength of Cu\(^{2+}\)-DOM binding (Cabaniss and Shuman, 1988; Lu and Allen, 2002). This makes the results from this study conditional to the pH and ionic strength at which the binding constants were measured. In order to determine the effect of the pH and ionic strength, Cu\(^{2+}\)-DOM binding constants must be measured over a range of pH and ionic strength. By measuring the Cu\(^{2+}\)-DOM binding constants values at a fixed Cu:DOM ratio and varying the pH and ionic strength, we can determine how much these variables affect Cu\(^{2+}\)-DOM binding. This will be very important when it comes to accurately modeling copper speciation and toxicity across a range of pH and ionic strength values.

Copper toxicity experiments are another area where future research can be done. In order to more completely understand how the Cu\(^{2+}\)-DOM binding constants affect the toxicity of copper, specifically in the waters draining the Pebble deposit, toxicity tests using site waters can be paired with Cu\(^{2+}\)-DOM binding measurements. This will allow us to evaluate how closely the experimental toxicity values compare with the toxicity predicted the BLM and the Cu\(^{2+}\) concentrations determined by modeling using the experimentally determined Cu\(^{2+}\)-DOM binding constants. Actual toxicity measurements will also be important in evaluating the potential risk of increased copper concentrations due to mining activities, since the modeling comparison done here was only able to compare the Cu\(^{2+}\) concentrations, and other copper species, such as CuOH\(^+\) and CuCO\(_3\) (aq) may be contribute to copper toxicity (Di Toro et al., 2001; Paquin et al., 2002).

**Release of Trace Elements from Coal Ash**

In order to more completely understand the mechanism responsible for the release of trace elements from coal ash, the speciation of these elements before and after release from the ash must be studied. In order to determine if colloidal release from the coal ash is an important
mechanism to consider, both dissolved and total fractions of the leachate must be monitored. The
release experiments done here only looked the fraction that was able to pass through a 0.45-µm
pore size filter. While this cutoff has been commonly used at the operational definition for
“dissolved”, it is known that colloids can exist at sizes smaller than 0.45-µm and pass through
the filter, making it difficult to distinguish between truly dissolved and colloidal species.

In order to determine if any of the trace elements are being released from the coal ash as
colloids, sequential filtrations can be performed on the coal ash suspensions after leaching. In
this study, all of the samples were filtered through a 0.45-µm filter opening in order to separate
the solution from the solid coal ash. Passing the 0.45-µm filtered solution sequentially through
progressively smaller filter sizes (0.2- and 0.1-µm pore openings) can be done to determine if
there is any distribution of any trace elements between the filter sizes. The sequential filtration
procedure could be a first step in order to determine if any elements are being released from the
coal ash as colloids or associated with colloids. If colloids are found, additional methods can be
used for characterization of the colloids and the associated trace metals. Scanning electron
microscopy (SEM) and energy dispersive x-ray (EDX) analysis can be used to determine the
size, shape, and elemental composition of the colloids. Field flow fractionation could also be
used to separate colloids by size and ICP-MS can be used determine the elemental composition
of the colloidal size fractions.

A limited attempt was made to understand the mercury speciation in the solid coal ash
before leaching. Sequential extractions were done for mercury, but only limited information
regarding the speciation was obtained due to the uncertainty of the mercury species present in
each extraction solution. X-ray absorption fine structure (EXAFS) spectroscopy is a useful
technique that can be used to examine specific binding environments for trace elements in the
coal ash. EXAFS analysis was not able to be done for mercury due to the solid concentrations being below the detection limit. For other elements with higher concentrations in the coal ash, EXAFS may be a useful technique to obtain specific binding site information. Another area that needs future research is studying how the release of trace elements from different coal ash samples (different coal source, different combustion method, etc.) is affected by DOM. The coal ash sample used in all of the release experiments was a freshly-produced sample from the Kingston Fossil Plant that contained high concentrations of calcium which was readily released from the ash. Other types of coal ash, such as those containing lower calcium and higher iron concentrations, or weathered coal ash samples, may have different elemental release behavior in the presence of DOM. Identical release experiments can be done using different coal ash sources in order to determine the characteristics or properties of coal ash that lead to an increased concentration of trace elements being released.
References


References


Martell, A.E.; Smith, R.M.; Motekaitis, R.J., \textbf{2004}. Database 46: NIST critically selected stability constants of metal complexes, Version 8.0, Texas A&M University, College Station, Texas.


Detailed Explanation of Equation 3 Calculations

\[
K_{CuBz2} = \frac{K_{CuL_{DOM}}[Bz^-]^2}{[Cu(Bz)_2^0][L_{DOM}]} \tag{3}
\]

\(K_{CuBz2}\) of \(10^{12.1}\) M\(^{-2}\) is the conditional binding constant for the \(\text{Cu}(Bz)_2^0\) complex adjusted for a pH of 6.6 and an ionic strength of 0.01 M.

\([\text{CuL}_{DOM}]\) is the concentration of copper that passes through the \(C_{18}\) resin bound to hydrophilic DOM (M). It is experimentally measured either using GF-AAS or ICP-MS.

\([Bz^-]\) is the concentration of the deprotonated form of benzoylacetonate (M), which was calculated for pH 6.6 from the total concentration and the pKa of benzoylacetonate. Since benzoylacetonate has a pKa of 8.4, the fraction of deprotonated benzoylacetonate at pH 6.6 (\(\alpha_{Bz}\)) can be calculated.

The free benzoylacetonate concentration \([Bz^-]\) added to the copper-DOM solution must be in excess of the bound benzoylacetonate concentration \([\text{Cu}(Bz)_2^0]\) according to the equation below:

\([Bz^-] = [Bz^-]_T - [\text{Cu}(Bz)_2^0]\)

\([\text{Cu}(Bz)_2^0]\) is the concentration of hydrophobic copper-benzoylacetonate complex (M). This is found by subtracting the copper concentration that passes through the \(C_{18}\) resin from the total copper concentration (added copper). This value is reported as a range because there is uncertainty about what the copper originally bound to hydrophobic DOM to bound to after the benzoylacetonate is added. The maximum \([\text{Cu}(Bz)_2^0]\) assumes that all of the copper originally bound to hydrophobic DOM is now bound to benzoylacetonate (\(\text{Cu}(Bz)_2^0\)). The minimum \([\text{Cu}(Bz)_2^0]\) assumes that the copper originally bound to hydrophobic DOM was unchanged after the benzoylacetonate addition, and it is still bound to hydrophobic DOM.

\([L_{DOM}]\) is calculated by the difference in copper concentration passing through the resin before and after benzoylacetonate addition, assuming that the concentrations of \(\text{Cu}^{2+}\) and inorganic copper complexes are negligible. For example, if 50 nM copper passes through the resin in the absence of benzoylacetonate and 20 nM copper passed through the resin in the presence of benzoylacetonate, the \([L_{DOM}]\) would equal 30 nM. This value for \([L_{DOM}]\) is a minimum value and relies on the ligands being saturated with copper. A maximum concentration of \([L_{DOM}]\) could be estimated if more information about binding site capacity was known. Since this information is difficult to estimate and would be expected to change over different Cu:DOM ratios, only the minimum \([L_{DOM}]\) was used in the calculations.
Table A1. Water quality measurements for the whole water samples collected from the F1 site in the Florida Everglades.

<table>
<thead>
<tr>
<th></th>
<th>F1 site, Florida Everglades</th>
<th>Williams Lake, MN</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.5</td>
<td>7.9</td>
</tr>
<tr>
<td>DOC (mg C L⁻¹)</td>
<td>25.0</td>
<td>3.5</td>
</tr>
<tr>
<td>SUVA₂₅₄ (L mg of C⁻¹ m⁻¹)</td>
<td>3.6</td>
<td>1.2</td>
</tr>
<tr>
<td>Ca²⁺ (mg L⁻¹)</td>
<td>7.8</td>
<td>14.6</td>
</tr>
<tr>
<td>Mg²⁺ (mg L⁻¹)</td>
<td>2.4</td>
<td>5.1</td>
</tr>
<tr>
<td>Na⁺ (mg L⁻¹)</td>
<td>22.2</td>
<td>1.8</td>
</tr>
<tr>
<td>K⁺ (mg L⁻¹)</td>
<td>0.8</td>
<td>0.5</td>
</tr>
<tr>
<td>Cl⁻ (mg L⁻¹)</td>
<td>24.7</td>
<td>1.8</td>
</tr>
<tr>
<td>NO₃⁻ (mg L⁻¹)</td>
<td>BDL</td>
<td>1.3</td>
</tr>
<tr>
<td>SO₄²⁻ (mg L⁻¹)</td>
<td>4.2</td>
<td>1.3</td>
</tr>
<tr>
<td>Alkalinity (mg L⁻¹ as CaCO₃)</td>
<td>29.8</td>
<td>59.3</td>
</tr>
<tr>
<td>Hardness (mg L⁻¹ as CaCO₃)</td>
<td>29.3</td>
<td>57.4</td>
</tr>
<tr>
<td>Dissolved Copper (µg L⁻¹)</td>
<td>2.0</td>
<td>BDL</td>
</tr>
</tbody>
</table>

BDL= below detection limit

a measured by ion chromatography at the U.S. Geological Survey in Boulder, CO
b measured by acid titration at the U.S. Geological Survey in Boulder, CO
Table A2. Dissolved organic carbon data used to estimate the dissolved organic matter concentrations ([DOM]) for binding constant data obtained from references where [DOC] or [DOM] was not reported.

<table>
<thead>
<tr>
<th>reference</th>
<th>water source</th>
<th>[DOC] (mg C L$^{-1}$) from similar water sources</th>
<th>[DOM]$^a$ (mg L$^{-1}$) range</th>
<th>[DOM]$^b$ (mg L$^{-1}$) estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bruland et al. (2000)</td>
<td>Seawater sample from the lower Narragansett Bay estuary</td>
<td>1.34-1.92 (Krka River estuary; Louis et al., 2009)</td>
<td>1.0 - 6.38</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.50-3.19 (Gulf of Mexico seawater samples; Harvey, 1983)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.6-1.04 (seawater samples from Halifax, Novia Scotia; Zhou and Wangersky; 1989)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jin and Gogan (2000)</td>
<td>Freshwater from the Bonavista Peninsula area of Newfoundland</td>
<td>3.6 (Judy Reservoir, WA; Korshin et al., 1998)</td>
<td>5.2 - 12</td>
<td>10.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.6 (South Platte River, CO; Croue et al., 2003)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3-6 (fresh, Canadian coastal waters; Retamal et al., 2007)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sunda and Hansen (1987)</td>
<td>Seawater sample from the lower Narragansett Bay</td>
<td>1.34-1.92 (Krka River estuary; Louis et al., 2009)</td>
<td>1.0 - 6.38</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.50-3.19 (Gulf of Mexico seawater samples; Harvey, 1983)</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>0.6-1.04 (seawater samples from Halifax, Novia Scotia; Zhou and Wangersky; 1989)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Assumed that carbon comprises half of the total DOM.

$^b$ Value used to determine the Cu:DOM ratio.
Table A3. Inputs to the BLM and Visual MINTEQ for model calculations

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value (range used in modeling)</th>
<th>Input used for BLM or MINTEQ?</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.5</td>
<td>Both</td>
</tr>
<tr>
<td>Cu</td>
<td>$8 \times 10^{-9} - 3 \times 10^{-5}$ M (0.5–2,000 µg L$^{-1}$)</td>
<td>Both</td>
</tr>
<tr>
<td>DOC</td>
<td>25 mg C L$^{-1}$</td>
<td>BLM</td>
</tr>
<tr>
<td>$L_{\text{DOM}}$ (&quot;DOM ligand&quot;)</td>
<td>$8 \times 10^{-9} - 3 \times 10^{-5}$ M $^a$</td>
<td>MINTEQ</td>
</tr>
<tr>
<td>log $K_{\text{CuL}}$ $^b$</td>
<td>Cu total (µg L$^{-1}$) log K (M$^{-1}$)</td>
<td>MINTEQ</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>17.2</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>16.1</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>14.4</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>12.2</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>10.8</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>9.6</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>8.6</td>
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<td></td>
<td>1,000</td>
<td>5.8</td>
</tr>
<tr>
<td></td>
<td>2,000</td>
<td>5.4</td>
</tr>
<tr>
<td>Humic acid (%) $^c$</td>
<td>10</td>
<td>BLM</td>
</tr>
<tr>
<td>Calcium</td>
<td>7.8 mg L$^{-1}$</td>
<td>Both</td>
</tr>
<tr>
<td>Magnesium</td>
<td>2.4 mg L$^{-1}$</td>
<td>Both</td>
</tr>
<tr>
<td>Hardness</td>
<td>29.4 mg L$^{-1}$ as CaCO$_3$</td>
<td>BLM</td>
</tr>
<tr>
<td>Sodium</td>
<td>22.2 mg L$^{-1}$</td>
<td>Both</td>
</tr>
<tr>
<td>Sulfate</td>
<td>4.2 mg L$^{-1}$</td>
<td>Both</td>
</tr>
<tr>
<td>Chloride</td>
<td>24.7 mg L$^{-1}$</td>
<td>Both</td>
</tr>
<tr>
<td>Alkalinity</td>
<td>29.8 mg L$^{-1}$ as CaCO$_3$</td>
<td>Both</td>
</tr>
</tbody>
</table>

a. For “titration” in MINTEQ.
b. The log K was entered as a function of the total dissolved Cu concentration (Cu:DOM ratio).
c. A default input of 10% humic acid is recommended for the BLM when actual fraction is not known.
Table A4. Inorganic species formation reactions and equilibrium binding constants for BLM and MINTEQ model

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Species</th>
<th>log K</th>
<th>BLM</th>
<th>MINTEQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{Cu}^{2+} + \text{OH}^-$</td>
<td>$\text{CuOH}^+$</td>
<td>6.48</td>
<td>6.50</td>
<td></td>
</tr>
<tr>
<td>$\text{Cu}^{2+} + 2\cdot\text{OH}^-$</td>
<td>$\text{Cu(OH)}_2$ (aq)</td>
<td>11.78</td>
<td>11.77</td>
<td></td>
</tr>
<tr>
<td>$\text{Cu}^{2+} + \text{SO}_4^{2-}$</td>
<td>$\text{CuSO}_4$ (aq)</td>
<td>2.36</td>
<td>2.36</td>
<td></td>
</tr>
<tr>
<td>$\text{Cu}^{2+} + \text{CO}_3^{2-}$</td>
<td>$\text{CuCO}_3$ (aq)</td>
<td>6.55</td>
<td>6.77</td>
<td></td>
</tr>
<tr>
<td>$\text{Cu}^{2+} + 2\cdot\text{CO}_3^{2-}$</td>
<td>$\text{Cu}($CO$_3$)$_2^{2-}$</td>
<td>9.92</td>
<td>10.2</td>
<td></td>
</tr>
<tr>
<td>$\text{Cu}^{2+} + \text{Cl}^-$</td>
<td>$\text{CuCl}^+$</td>
<td>0.4</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>$\text{Cu}^{2+} + \text{H}^+ + \text{CO}_3^{2-}$</td>
<td>$\text{CuHCO}_3^+$</td>
<td>14.32</td>
<td>12.13</td>
<td></td>
</tr>
</tbody>
</table>
Figure A1. Copper-DOM binding constant as a function of the nitrogen-to-carbon (N/C) molar ratio of the DOM fractions. The binding constants are shown as averages with error bars representing the uncertainty in the constants due to assumptions associated with the CLE-SPE calculations.
Figure A2. Complete summary of copper-DOM binding constants from the literature as a function of the Cu:DOM ratio. This figure includes results that were considered for the discussion, but were not included in the manuscript to make the figure easier to read and interpret. The regression for all of the data points shown is $R^2 = 0.903$. 

$R^2 = 0.903$

$K_{CuDOM}$ (M$^{-1}$) vs. Cu:DOM (mg Cu (mg DOM$^{-1}$))