2017

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Mona Muhawwis Alshammari
Wilfrid Laurier University, alsh1860@mylaurier.ca

Recommended Citation

Alshammari, Mona Muhawwis, "Determination of Silver Binding to Natural Organic Matter Using Ion Selective Electrode" (2017). *Theses and Dissertations (Comprehensive)*. 1945.
https://scholars.wlu.ca/etd/1945

Determination of Silver Binding to Natural Organic Matter

Using Ion Selective Electrode

by

Mona Muhawwis Alshammari

THESIS
Submitted to
The Department of Chemistry & Biochemistry
Faculty of Science
In partial fulfilment of requirements for the
Master of Science in Chemistry
Wilfrid Laurier University

Waterloo, Ontario, Canada

ABSTRACT

Natural organic matter (NOM) is considered an important factor in the protection of the aquatic environment; organic ligands can complex with metals, to decrease their toxicity and bioavailability. NOM is measured as dissolved organic carbon (DOC) in mg C/L. Silver metal can enter into the aquatic environment from natural and anthropogenic sources, and is found in different chemical forms. Free silver ion (Ag^+) is considered the most toxic form of the metal. However, it can form complexes with both inorganic and organic ligands, such as ammonia, chloride, hydroxyl, carbonato, phosphato, and thiol groups. Thiol is the most important ligand because it forms very strong complexes with Ag. To date, the behavior of silver in the aquatic environment, is still not well understood. The aim of this research is divided into three steps. First, the experimental objective of this study was to determine free silver ion binding to NOM in three different commercially available sources. Also, samples were tested under different pH (4.0, 6.0, 8.0 and 10.0) conditions. In the laboratory tests, the technique that was used was a flow through ion selective electrode (ISE) system utilizing an Ag_2S electrode. In the second step, Windermere Humic Aqueous Model (WHAM), which is a very important risk assessment tool, was validated by comparing WHAM and ISE silver speciation results. Silver binding to NOM depends on the specific ligands in each samples. For example, the sample with the most sulphur content was also strongest in binding to silver. Also, pH plays an important role in silver bioavailability. When pH increases, NOM has more sites available to bind to silver ions. In contrast, proton concentration increases at low pH and competes with free silver cations to bind with NOM. Finally, optical characterization of NOM was performed using absorbance (SAC_{340}) and fluorescence indices (FI) in order to distinguish among NOM samples. It was found that optical characteristics have similar value for all NOM samples.

ACKNOWLEDGEMENTS

I would like to start by thanking my supervisor Dr. Scott smith for accepting me and offering this wonderful opportunity and continued support me during my study. You have set an example of excellence as a researcher, mentor, instructor, and role model. I am also very grateful to him for providing exceptional guidance, for challenging me to utilize all my potentials and for teaching me countless things including many life lessons.

I also thank my advisory committee Dr. Masoud Jelokhani-Niaraki, Dr.Vladimir Kitaev, and Dr. Jim McGeer. I really appreciate their taking out the time from their busy schedules and providing me with their insight and expertise. Thank you for the time, advices, trust, encouragement and expertise you have given me that greatly improved my thesis manuscript.

To my lab-mates, thanks for the fun and support. Of all the people I have worked with in the lab many thanks for help and great company. I would like to thank my father muhawwis and my mother Bushra. My parents always pray and encouraged me, to continue my study. Thanks Dad and mom to allow me travel to the abroad to learning so many thing on my life. Thanks for your trust and teaching me that it is important to try to improve my personality to be success on my life. Thanks for all my sisters and brothers to for supporting me on study and my life in general.

My special appreciation goes also to my unbelievably supportive husband Satam. Words cannot express how much grateful I am for your support. Thank you for motivating me and helping me continue the research work. Thank you for everything that you have done for me. Special thanks for my lovely son, Abdullah, for your sweet smile which makes my day beautiful and your words and songs that makes me happy.

Last but not least, a special acknowledgment and thanks to His Majesty King Abdullah for giving us the scholarship, who passed away during the time I was studying. I would also like to thank my financial sponsors (Unilever and WCA environment Ltd) and to all the staff at Wilfrid Laurier University.

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List of Abbreviations and Symbols

Ag	Element Silver
BLM	Biotic Ligand Model
CCME	Canadian Council of Ministers of the Environment
CCRME	Canadian Council of Resource and Environment Ministers
CWQG	Canadian Water Quality Guideline
DOC	Dissolved Organic Carbon
FA	Fulvic Acid
FI	Fluorescence Index
HA	Humic Acid
ISE	Humic Acid
LC$_{50}$	The concentration which is lethal to 50% of the experimental biota.
logK	Equilibrium Constant
NOM	Natural Organic Matter
pH	Negative log of the hydrogen ion concentration, = -log[H+]
SAC	Specific absorbance coefficient at 340nm
SSD	Species Sensitivity Distribution

Chapter 1

Introduction

1.1 Environmental Characterization of Silver

Silver (Ag) is a soft and naturally occurring element, which is found in the earths crust as mineral ores in sulfide and chloride. Naturally occurring silver is associated with other metals such as gold, copper, lead, and zinc (The Silver Institute, 2017). Even though silver is considered a rare metal (Howe and Dobson, 2002), 60% of silver in the aquatic environment comes from natural sources (CCME, 2015) at a concentrated range of $0.001 - 0.01$ μg/L (CCME, 2015), although anthropogenic sources of silver have increased in recent years. Due to its unique characteristics, such as high electrical and thermal conductivity, Ag is used in many applications including conductive adhesives, electronic devices and photovoltaic devices (The Silver Institute, 2017). Furthermore, silver plays an important role in health and medicinal applications. Silver is used as an antimicrobial in personal care products (Edwards-Jones, 2009). Silver may be present in aquatic systems, as a result of mining waste and industrial discharges. Also, Ag may enter the environment from wastewater treatment facilities (Purcell and Peters, 1998). Silver in aquatic environments, whether coming from natural or anthropogenic sources, can cause toxicity to aquatic organisms (Purcell and Peters, 1998). Silver can exist in many different oxidation states in aquatic environments such as $+1$, $+2$, and $+3$. However, free silver ion is the most reactive and toxic. Studies have shown that silver toxicity depends mainly upon its bioavailability and bioaccumulation of free silver in aquatic organisms (Luoma *et al.*, 1995). Dissolved organic carbon (DOC) can reduce the acute toxicity of silver in fish according to studies done by VanGenderen *et al.* (2003). Natural organic matter contains inorganic and organic ligands such as carboxyl, phenolic, amine and sulfidic. These ligands can associate with free silver metal, thus, reducing the availability of free silver (Smith *et al.*, 2002), but there are various factors that can affect DOC binding to metal such as pH, hardness, salinity, and alkalinity (Al-Reasi *et al.*, 2016). Silver metals behaviour in aquatic environments is not well known and the goal of this research is to understand the interactions of silver in freshwater.

1.2 Silver Toxicity

In the past few years, there have been many studies illustrating the toxicity and bioavailability of silver in aquatic organisms; however, there is still a gap in understanding silvers environmental behaviour (Hogstrand and Wood, 1998). Moreover, silver does not prove to be an essential metal for aquatic organisms (CCME, 2015). Many studies have been conducted on aquatic organisms, such as fish, in order to understand acute and chronic silver toxicity in freshwater. The results of these studies show that silver toxicity seems to be caused by the free ion. Ionic silver (Ag^+) competes with Na^+ at the gills and causes disruption of ion balance, and inhibits the activity of sodium potassium ATPase. Thus, Na^+ and Cl^- uptake is reduced and adversely affects fish life in freshwater environments (McGeer and Wood, 1998). With regards to the environmental protection, free silver ion can form complexes with organic and inorganic ligands causing acute silver toxicity (Wood *et al.*, 1999). According to the studies done by McGeer and Wood (1998) the most important ligand is Cl^- , since Cl^- does not inhibit gill after strong binding with silver. Moreover, pH and alkalinity are inversely related to the silver toxicity (Erickson *et al.*, 1998). However, these factors with respect to chronic exposures show less protection (Davies *et al.*, 1998).

1.3 Toxicity Modifying Factors

Silver can negatively affect aquatic life. However, water chemistry factors can decrease silver bioavailability in the aquatic environment, and these factors should be considered when conducting toxicity tests. Gaining the understanding of how silver reacts in water is essential in explaining its environmental fate and potential toxicity.

1.3.1 Reduced sulfur

Silver is a soft metal and is classified as a transition element metal from class B (Smith *et al.*, 2002). It contains the properties to bind strongly with soft ligands, such as reduced sulphur, under normal environmental conditions (The Silver Institute, 2017; Smith *et al.*, 2002). Silver can potentially bind with different ligands such as O-containing ligands, N- containing ligands, and S- containing ligands. See the list values of log K for silver complexes in Table 1.1 below. Silver complexes strongly bind with reduced sulfur for up to several orders of magnitude higher than the other ligands containing N or O (Smith *et al.*, 2002).

Silver complexes	**logK**
Inorganic sulfide[a]	14-21
Organic sulfids (thiols)[b]	12-15
N (NH3 and amines)[b]	3-6
Cl^{-b}	3
O[b]	<2

Table 1.1: **Formation constants of silver complexes with different ligands. [a](Cloke, 1963), and [b](Martell *et al.*)**

Silver complexes strongly bind with ligands such as "carboxyl (M–OOCR), amine ($[M–NH_2R]^+$, M–NHR), phenolic (M–OAr), metal sulfides (M–SM), and thiolatesulfide (M–SR)" (Smith *et al.*, 2002). Table 1.1 shows the formation constants of silver complexes with other ligands such as chloride, oxygen, and amine groups. All these have low constants of (K), that is, between 2 and 6. The highest stability constants (K) for Ag(I) are for the inorganic sulfide log K=14-21, and then for thiols log K=12-15. Sulphides play an important role in the behavior of silver, since they strongly bind with Ag, due to its high polarizability (Smith *et al.*, 2002) and the mechanism of this electron transfer usually occurs through ligand-exchange mechanism (Bell and Kramer, 1999); for example,

$$Ag - S_1 + S_2 = S_1 + Ag - S_2 \tag{1.1}$$

Where S_1 is the sulphide ligand, and S_2 is the biomolecule ligand. In Eq. 1.1 complexation of Ag is potentially dependent upon the sulfur ligand's affinity for Ag(I) and on their concentration (CCME, 2015).

1.3.2 Natural Organic Matter

Natural organic matter (NOM) is the organic material present in water, created due to the decomposition of plants and animals (GreenFacts, 2001). NOM is a heterogeneous substance. The amount of NOM present in natural water is often measured as dissolved organic carbon (DOC) in mg C L^{-1} (Smith *et al.*, 2002), because it contains approximately 50 % carbon by mass (Duarte *et al.*, 2015). NOM has variable characteristics depending upon the type of water and its geochemistry. These variable characteristics play an important role in reducing silver toxicity in water (VanGenderen *et al.*, 2003). In freshwater, NOM consists of two major components, humic acid (HA) and fulvic acid (FA) (Thurman, 1985) as given in Table 1.2. Humic and fulvic acids are used to describe the dissolved organic carbon (DOC) due to their difference in organic matter chemistry (Santore *et al.*, 2001).

	%C	%O	%H	%N	%S
HA and HN	50-60	30-35	4-6	2-4	0-2
FA	40-50	50-55	4+	1-3	0-2
Average	52	40	5.9	2.5	0.7

Table 1.2: **Elemental composition ranges for NOM (Thurman, 1985)**

Natural organic matter (NOM) or humic substances have different functional groups, including carboxyl (–OOCR), amine ($[–NH_2R]^+$, –NHR), phenolic (–OAr), metal sulfides

(–SM), and thiolatesulfide (–SR) groups that may be associated with metal ions to form complex compounds (Smith *et al.*, 2002). Free silver ions can form complexes with NOM and thus reduce bioavailability of metal ions, thus protecting aquatic organisms against Ag toxicity. Moreover, pH plays an important role in binding of NOM with metals because of the presence of carboxylic acids in water that can be found either in protonated or deprotonated form based on pH (Grabowski and Gurnell, 2016). When pH increases, NOM has more sites available to bind with silver ions. In contrast, protons will increase at low pH and there will be fewer sites available for free silver cation binding to NOM.

1.4 The biotic ligand model (BLM)

In recent years, the biotic ligand model (BLM), is a theoretical, potentially mechanistic approach, which has been used as a tool to quantitatively estimate toxicity modifying factors. These factors include water chemistry factors such as DOC, anions, sodium (Na^+ and Mg^+) , alkalinity, and pH levels (Paquin *et al.*, 2002). Metal accumulation is highly proportional to toxicity, which can be predicted by the BLM (Smith *et al.*, 2016). In order to predict toxicity for fish, the biotic ligand is assumed as the gill (Janes and Playle, 1995). In freshwater, the gills of a fish, or other species, bind with potentially toxic ions, and equilibration calculations of metal speciation in water are used to calculate toxicity (see Figure 1.1).

The scientific and regulatory communities have turned their attention to developing the

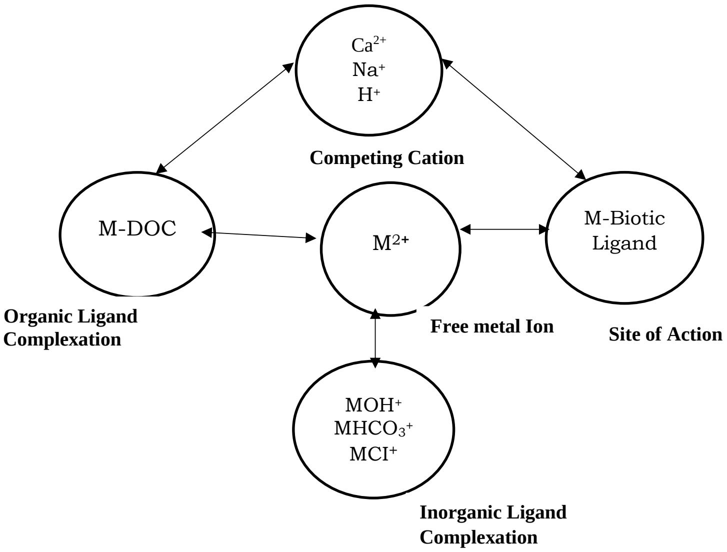

Figure 1.1: **Schematic diagram of the Biotic Ligand Model (Di Toro *et al.*, 2001)**

Biotic Ligand Model (BLM) approach because it is one of the most important tools used in developing water quality criteria (WQC) and in performing risk assessments for metals present in the aquatic environment (Paquin *et al.*, 2002). Specifically, BLM is designed to reflect the significant impact of site-specific water quality (Paquin *et al.*, 2002). Toxicity of organisms is also often determined by the LA50 parameter. In the aquatic systems the free ionic forms are often responsible for the toxicity (Smith *et al.*, 2016). In terms of acute toxicity prediction of free silver ions, there are several versions of the BLMs model that have been developed. The development of BLM is often based on the gradual development of the original gill Ag-binding study. The basis of BLM is dependent upon the metal accumulation at the gill, which is proportional to toxicity, and BLM can also be used to predict metal ions in the exposed water (Paquin *et al.*, 2002; Bielmyer *et al.*, 2007). McGeer *et al.* (2000) has determined the speciation parameters (log K’ and LT) for the biotic ligands to develop a model. They obtained physiologically-based conditional equilibrium constants to describe the effect of metal (silver) ionic interactions on the inhibition of gills Na$^+$ / K$^+$-ATPase in rainbow trout (McGeer *et al.*, 2000). However, silver speciation parameters with NOM are still not clear. Moreover, in their studies, (Smith *et al.*, 2016), used ion selective electrode to directly characterize copper and silver binding to rainbow trout gill cells. They also found that cultured gill epithelia provide useful approach to testing direct metal complexation with the biotic ligand in laboratory tests. A key aspect of each version of the BLMs model deals with the importance of DOC Ag binding prediction which is the most important protection factor in the aquatic environment (Al-Reasi *et al.*, 2012).

1.5 Water Quality Guidelines and Criteria for Silver

Whether metals, including silver species, are naturally occurring or from anthropogenic sources, they may cause negative effects in the aquatic environment. Thus, Canadian Water Quality Guidelines (CWQG) seek to protect the environment from acute silver (Ag) toxicity through evaluating water quality to ensure the health of aquatic organisms. The Canadian Council of Ministers of Environment (CCME, 2007) revised the protocol for improving water quality guidelines. In the past, water quality criteria was established based on the hardness of water, and in the guidelines to protect aquatic life from metal toxicity, including silver, and it can be calculated using the following equation:

$$Max.TotalRecovAg(\mu g L^{-1}) = e^{(1.72[hardness]-6.52)} \tag{1.2}$$

	Long-term Exposure (μg Ag/L)	**Short-term Exposure (μg Ag/L)**
Freshwater	0.25	NRG
Marine	NRG	7.5

Table 1.3: **Canadian Water Quality Guidelines (CWQG) for the protection of aquatic organisms from the silver metal, were developed using the species sensitivity distribution (SSD). NRG, meaning no recommended guideline, it should also be pointed out that this guideline is not applicable for silver nanoparticles (CCME, 2015)**

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However, in recent years, studies have demonstrated the shortcomings of relying on water hardness for two reasons (Galvez and Wood, 1997). First, (Ca^{2+} and Mg^{2+}) are not effective elements in providing accurate studies for reducing Ag toxicity. Second, water quality parameters such as alkalinity, dissolved organic carbon (DOC), and chloride were not included in the hardness equation (Karen *et al.*, 1999). There are several factors in water chemistry that can impact silver toxicity in water systems, and these should be taken into account when applying risk assessment tools. At this time, a chronic Biotic Ligand Model BLM for silver is not available. Also, the use of BLM in the development of future guidelines is currently being investigated (CCME, 2015). The recently published CWQG uses Species Sensitivity Distribution (SSD) (See Table 1.3) for the protection of aquatic life from the silver metal. The long-term silver exposure limit in fresh waters is 0.25 μg/L and for short-term exposure in fresh waters the limit is 0.22 μg/L, which is almost equal. The results of the short-term are not as expected, and therefore, CWQG is not recommended for short-term exposure in freshwater (CCME, 2015).

1.6 Analytical Methods

1.6.1 Ion Selective Electrode

Ion selective electrodes are often utilized in order to determine binding equilibrium constant and the concentrations of free ions (Sikora and Stevenson, 1988). These electrodes have the ability to respond to free ion concentrations, which are proportional to the bioavailability of the metal (Tessier and Turner, 1995). The free metal ion is the most important species in the aquatic environment because it can permeate through biological membranes and potentially cause toxicity. However, these ions are present in very low concentrations and the presence of interferences make the determination of ion concentrations, such as silver ions, very difficult. Thus, there are very few methods available to measure free metal ion concentrations in waters (Batley *et al.*, 2004). The use of an Ion Selective Electrode (ISE, potentiometry) is a common electrochemical technique used to determine free metal ions, and the most sensitive electrodes are the solid state (Durst, 1969)electrodes which are available to measure cadmium, copper, lead and silver cations (Paquin *et al.*, 2002). Moreover, ISE has detection limit which is usually in between 10^{-8} - 10^{-6} (Batley *et al.*, 2004) but in buffered systems metal's low amounts can be estimated. In the case of silver metal, the membrane uses a silver sulfide (Ag_2S) electrode and, as Durst (1969) pointed out, the lowest limit of detection is based on the solubility product $K_{sp(Ag_2S)} = 10^{-51}$ and it potentially responds to both silver and sulfide free ions (Durst, 1969). Because the membrane contains sufficient silver sulfide, it allows the silver ion to pass through, and then works as a silver ion detector (Durst, 1969). Another advantage of using a silver sulfide electrode is that it can determine silver binding with ligand in natural water (Smith *et al.*, 2004). The measured potential of an ion selective electrode is determined according to the Nernst equation:

$$E = E^o + \frac{RT}{NF} ln(Ag^+) \tag{1.3}$$

E and E^o - electric potential, measured and reference, respectively

R - Gas constant (8.314 J/mol K)

F - Faraday constant (96487 C mol^{-1})

T - Temperature (assume room temperature of 298 K or 25 ^{o}C)

N - Number of electrons exchanged

(Ag^+)- Silver ion activity

The temperature is assumed to be at room temperature (25 ^{o}C). Furthermore, in order to convert from the natural logarithm to the base ten logarithm, it should be multiplied by 2.303 and low ionic strength for monovalent silver actual connections are small and activity can be assured equal to concentration. In the case of silver, the number of electrons exchanged will be 1, thus the equation can now be written as:

$$E = E^o + 0.0591 log[Ag^+] \tag{1.4}$$

The toxicologists use ion selective electrodes to measure ion toxicity because this technique is very sensitive and responsive to specific metal species. Since free silver ions are known as the most toxic silver species in aquatic environment, determining the presence of free silver is important to protect water system. The ion selective electrode Ag_2S ISE is used in this project.

1.6.2 The Windermere Humic Aqueous Model

Windermere Humic Aqueous Model (WHAM) is a computerized model that predicts free metal ion binding to humic substances (humic and fulvic acids). WHAM is used extensively as a risk assessment tool for cationic elements in freshwater. The model is dependent upon the quantity and quality of the input data (Lofts and Tipping, 2011). WHAM takes into account multiple input measurements; model parameters such as pH and dissolved organic carbon. It is then used to estimate the free ion for different metals (Lofts and Tipping, 2011) such as copper, lead, cadmium, and silver (DePalma, 2009). The WHAM concept that the proton and the metal cations are competing (Di Toro *et al.*, 2001), is similar to the Biotic Ligand Model (Playle, 1998) and therefore, both of these models will help to predict metal speciation and used as validation risk assessment tools. In terms of silver, the WHAM software program considers one of the current models which contains the impact of total and dissolved organic carbon (TOC and DOC) in calculating silver speciation. The WHAM model also considers functional groups such as carboxylic and phenolic (Dudal and Grard, 2004).

1.6.3 Optical Characterization

Natural Organic Matter (NOM), defined as dissolved organic carbon (DOC in mg C L^{-1}), is a complexing factor in natural waters, and an important factor in mitigating waterborne metal toxicity; however, different NOM sources, as well as similar DOC concentrations, are substantial (Al-Reasi *et al.*, 2011). Absorbance and fluorescence spectroscopy have been used to distinguish the various molecular properties of natural sources, as well as to distinguish between fulvic and humic material. Optical properties such as the Specific Absorbance Coefficient, SAC, estimated as 2.303 x absorbance at Abs (340nm) wavelength divided DOC concentration as shown below in Equation 2.1 (Curtis and Schindler, 1997). The DOC was also characterized using Fluorescence Indices (FI), which can determine the origin of the NOM, (McKnight *et al.*, 2001). It is shown that FI is the ratio of fluorescence intensities at 450 nm and 500 nm as shown below in Equation 2.2 for fixed excitation of emission 370 nm. In order to better understand the protective effects of these metals, the optical characterization of DOC is very important. The SAC_{340} and fluorescence indices (FI) were used to characterize all NOM investigated in this thesis.

1.7 Research Goals and Objective

The overall objective of this thesis is to improve the understanding of dissolved silver behavior in freshwater in order to protect aquatic environments. To achieve this objective several smaller tasks are addressed:

1. Experimental determination of silver binding isotherms for three sources of NOM at four pH values (4, 6, 8, and 10)
2. Evaluation of WHAM as a risk assessment tool by comparing measured and modeled silver binding
3. Assessing if any simple optical measurements of NOM quality can predict relative Ag binding properties.

Chapter 2

Materials and Methods

The following sections show sample preparation and experimental steps of ion-selective electrode method, which includes instrumental procedures.

2.1 Instrumentation

A Cole Parmer Silver/Sulfide ISE was applied to all experiments in the lab in cooperation with an Orion double junction Ag/AgCl reference electrode (Model 900200, Boston, MA, USA). All experiments were performed in a flow-through system represented in Figure 2.1. A cell contained the reference electrode, pH electrode and the sample. The flow cell contained the silver/sulfide electrode above the opening from where the sample passed through. In addition, the samples were adjusted to different pH values (4, 6, 8 and 10) for each experiment using HCl and NaOH, and this pH value was maintained throughout the experiment. The experiment also maintained fixed ionic strength (0.01) M KNO_3. Each electrode was connected to a potentiometer and a pump was used to deliver the sample through the system. In addition, the silver/sulfide electrode was polished daily using aluminum oxide before each experiment.

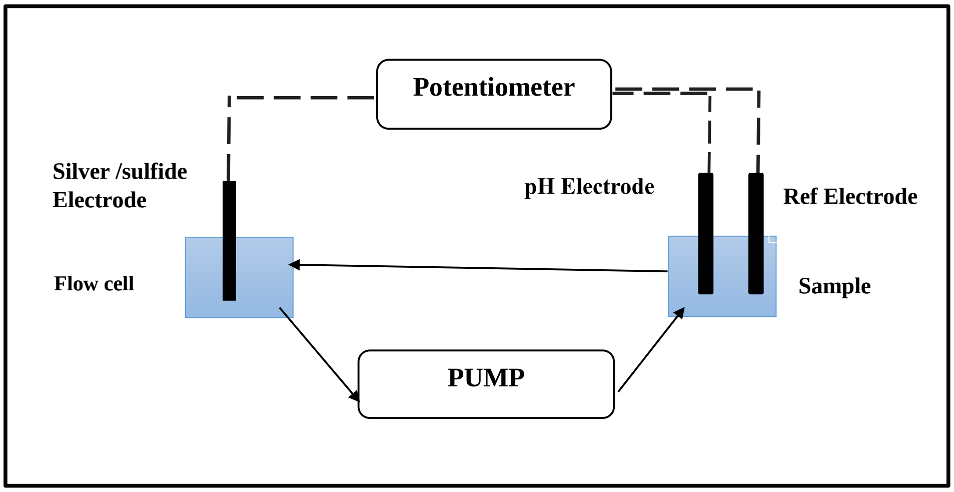

Figure 2.1: **Schematic of the measurement of the free silver ion through selective electrode flow through system**

2.2 Sample Source

The following section describes all of the commercially available samples used in this study. Samples were purchased from IHSS (International Humic Substance Society) in powder form and were stored according to the recommendations of the manufacturer. Three freshwater samples were tested to determine free silver ion levels at different conditions of pH. The titrations were done by using approximately 100 mg C L^{-1} from each sample, and pH was adjusted individually for each sample using NaOH and HNO_3 at 4.0, 6.0, 8.0 and 10.0 pH before titration. The list of DOC sources can be found in Table 2.1. Ultrapure water (18.2 M Ω, Milli Q) was used to prepare the samples and dilute the stock solution for all NOM sources. Knowing the sources of the samples is potentially very important in understanding the free silver ion binding with NOM. Each sample had different chemical

Code	Description	DOC concentration
NR	Nordic Reservoir (IHSS)	100 mg C $L^{-}1$
SW	Suwannee River (IHSS)	100 mg C $L^{-}1$
UM	Upper Mississippi River (IHSS)	100 mg C $L^{-}1$

Table 2.1: **Samples utilized to determine silver ion concentrations.**

characteristics based on (IHSS) (see Table 2.2). Ion selective electrodes (ISE) were used to directly characterize silver ion binding with NOM, which is able to bind with free silver due to the chemical elements it contains, as shown in Table 2.2. The ion selective electrode is the most accurate and sensitive method that has been used to determine free silver ion (Durst, 1969). This method can be applied to both freshwater and marine water (Smith *et al.*, 2016).

All data results were performed in MATLAB code, and then the data was transferred to the WHAM program. In addition, all DOC sources were characterized between fulvic and humic acids from the same source using absorbance SAC_{340}. Also, SAC_{340} has been referenced as a measure of NOM quality and it presented good correlation with metal toxicity in freshwater based on the equation (Schwartz *et al.*, 2004).

$$SAC_{340} = 2.303 \times \frac{Abs_{340}}{DOC} \tag{2.1}$$

Sample	**Cat. No.**	**H_2O%**	**Ash%**	**C%**	**H%**	**O%**	**N%**	**S%**
SR	2R101N	5.69	4.01	50.70	3.97	41.48	1.27	1.78
NR	1R108N	nd	41.4	53.17	5.67	nd	1.10	nd
UM	1R110N	8.55	8.05	49.98	4.61	41.4	2.36	2.62

Table 2.2: **Characteristics of samples used for silver titrations based on (IHSS).** *(nd: not determined)*

In addition, the fluorescence index was used to distinguish NOM sources and composition such as an indicator of the origin of DOC (McKnight *et al.*, 2001), as shown in the following equation:

$$\mathrm{FI}_{\mathrm{ex370}} = \frac{\mathrm{em}_{450}}{\mathrm{em}_{500}} \tag{2.2}$$

Fluorescence at 370nm excitation wavelength and, 450 and 500 nm emission wavelengths was measured using a spectrofluorimeter. For all fluorescence and absorbance spectra measurements, 1 cm quartz cuvettes were utilized.

Chapter 3

Results and Discussion

The ideal Nernstian response is 59 per decade of concentration in case of silver metal refer equation. All the experiments reported, have achieved the slope of approximately 59 ± value at 25^o C.

3.1 Effect of pH on silver binding with NOM

It is important to understand the speciation of silver in terms of pH, which can potentially affect the degree of toxicity in the aquatic environment. Silver binding parameters with the various NOM sources, listed in Table 2.1, were determined using an ion selective electrode under different condition of pH. The silver measurement results from the titration of the three freshwater samples over different pH values of 4.0, 6.0, 8.0, and 10.0 are shown in Figure 3.1. The data demonstrates a significant change in potential silver availability depending on the value of pH. At low pH, silver ions bind weakly with different NOM and as a result, free silver ions are more available at pH 4. On the other hand, when the value of pH was increased, silver ions bind strongly with diverse NOM sources. Specifically at pH 10, there are fewer free silver ions available due to the strongest binding with all NOM samples. In addition, it can be seen clearly in all NOM samples that when the pH tends to be average, there is intermediate binding strength between Ag^+ and NOM (See Figure 3.1 at pH 6). In fact, all NOM samples have similar reaction with a different values of pH as seen in Figure 3.1. To sum up, silver ions are considered the most toxic form of silver that can affect aquatic organisms. pH plays an important role in binding silver ions with NOM; and as the results of this study proves, as pH increases the potential bioavailability of the silver ion decreases.

There are two reasons for such results in terms of the effects of pH on metal toxicity. First, metal ion toxicity can be decreased by the complexation of hydroxide at increased pH (Hamelink *et al.*, 1994; Pagenkopf, 1983). Another reason is that toxicity can be increased by competition of hydrogen with toxic ion metals for binding to NOM (Pagenkopf, 1983). In the case of non-ionic silver metal even at the highest pH, no significant complexation is expected even by hydroxide (Brown and Allison, 1987). pH can influence the toxicity of silver through interactions with the natural waters NOM. In other words, at low pH, NOM samples have fewer binding sites open to metal ions because there are more protons associated with the binding sites, and these protons compete with free silver ions available to interact with NOM, as shown in Figure 3.1. As demonstrated in previous research (Janes and Playle, 1995), in rainbow trout *(Oncorhynchus mykiss)*, protons do not compete with silver ions in gill sites of organisms but they do compete for interaction with NOM. In contrast, when pH is increased, there are more sites available for binding to NOM because of less proton competition (Erickson *et al.*, 1998) resulting in less silver bioavailability.

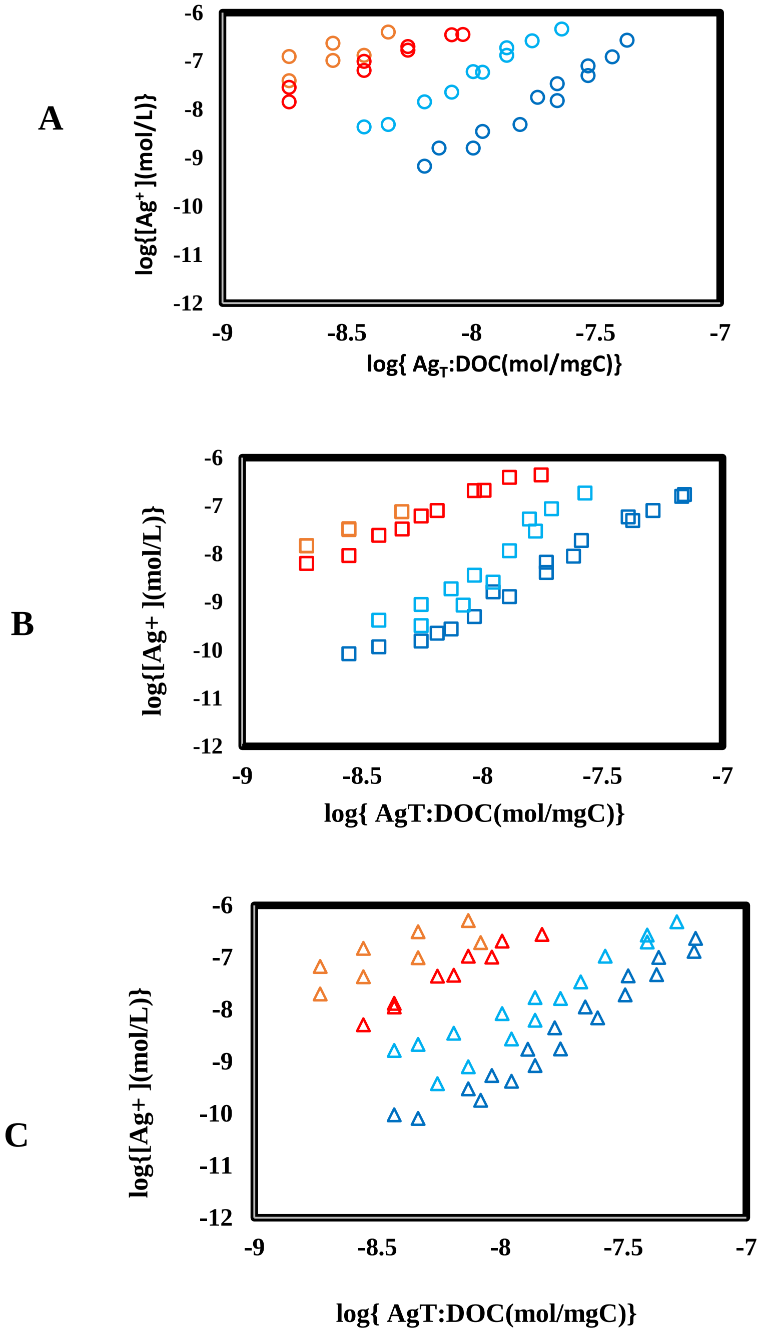

Figure 3.1: **Silver binding with DOM samples A=NR, B=UM, C=SW and each sample was tested at different values of pH: 4.0=orange, 6.0=red, 8.0= blue, and 10= dark blue in 0.01 M ionic strength (KNO_3)**

Copper showed similar binding behavior to that of the silver (Smith *et al.*, 2016). Wang *et al.* (2016) have done studies to show the relationship between pH and toxicities of ten metals with respect to freshwater organisms. They found that 42% of metals were influenced by pH, as shown in Figure 3.1. Silver showed decreased toxicity with the increase in pH; thus, there is less free silver bioavailability at high pH. Four other elements (copper, lead, selenium and silver) have showed similar response to pH. To sum up, pH plays a very important role in relation with metals, which explains the behavior of silver in the aquatic environment and can, thus, safely be used to improve the protection models against toxicity (Wang *et al.*, 2016; Pagenkopf, 1983; Erickson *et al.*, 1998).

3.2 Influence of dissolved organic matter sources on silver toxicity

Natural organic matter is expected to bind to metal ions because it has a number of functional groups, such as carboxyl, sulfide and amino groups (Tipping *et al.*, 2011). The toxicity of silver to aquatic life in natural water depends on silver bioavailability. Silver metal toxicity experiments usually use, $AgNO_3$ because NO_3^- binds weakly with Ag^+ ion, (Karen *et al.*, 1999). Natural organic matter (NOM; measured as DOC) can decrease free silver availability when NOM forms complexes with silver (Ratte, 1999). One important key to this research is to understand silvers behavior and how the NOM has a noteworthy protective impact against silver toxicity. In addition, there is a need to determine silver binding with natural organic matter through ion selective electrode method. Various NOM sources of freshwater were purchased from IHSS. Comparisons of all NOM samples binding with free silver under different pH conditions can be observed in Figure 3.2. Of all the conditions of pH it was found that the Upper Mississippi River (UM) source showed the strongest binding for Ag^+ and the lowest values for free silver ion availability. In contrast, Nordic Reservoir (NR) showed weak binding with more free silver ions available under different conditions of pH. In addition, Suwannee River (SR) exhibited less binding at pH 4; however, it exhibited more strong binding with free silver when the pH was increased. These results confirm that NOM can reduce free silver ions in a NOM source dependent manner and DOC is more protective at higher pH. Similar results were also observed by Wang *et al.* (2016) and Ratte (1999). It can be seen in Figure 3.2 that NOM sources in fresh water can reduce the bioavailability of free silver ions. Bioavailability and toxicity of silver depends on the complexion with ligands in aquatic environments that can be affected by pH levels (Chen *et al.*, 2017). In chapter 2, the pH dependent binding to NOM was explained, in the following part, NOM Source will be addressed. In order to discuss differences of source response, it is important to characterize the properties of each NOM sample. Table 2.2 in the previous section shows the elemental analysis of our sources as defined by IHSS. Upper Mississippi River and Suwannee River contain sulfur which could be in the form of sulfide ligands, which strongly bind to silver due to the fact that sulfide ligands have a high affinity for soft metals, (such as silver and copper). However, silver binds stronger with sulfide ligands as opposed to copper (Smith *et al.*, 2002) and therefore, silver was chosen to determine the reduced sulfur in natural water (Smith *et al.*, 2004). The binding constant and logK of silver sulfide complex is approximately 11.3 (Kramer *et al.*, 2007):

$$K_{Ag-SL} = \frac{[Ag - SL]}{[Ag^{+}][SL]} \tag{3.1}$$

In addition, there are different sulfide groups in NOM which could be inorganic or organic sulfide ligands, such as metal sulfides (MSM), and thiolatesulfide (MSR) (Smith *et al.*, 2002). Ag(I) and S(II-) binding format is dependent upon the S(II-) concentration. For example, at low concentrations of S(Il-), mononuclear complexes with silver are formed as (AgL$_n$), and in above micromolar concentrations of S(II-) binding occurs as polynuclear complexes (Ag$_m$L$_n$) (Bell and Kramer, 1999). Sulfide play an important role in silver behavior and can decrease soluble ionic silver resulting in protection against silver toxicity in the environment of the Ministers of Environment. Silver complexes such as silver thiosulfate are 17,500 times less toxic than free silver ions (Leblanc *et al.*, 1984). It can be seen in Figure 3.2 that there is less silver binding in Nordic Reservoir than in other NOM sources possibly because it may contain less sulfur ligands that bind strongly with silver. However, Nordic Reservoir source has other weaker binding ligands to bind with silver, such as N and O.

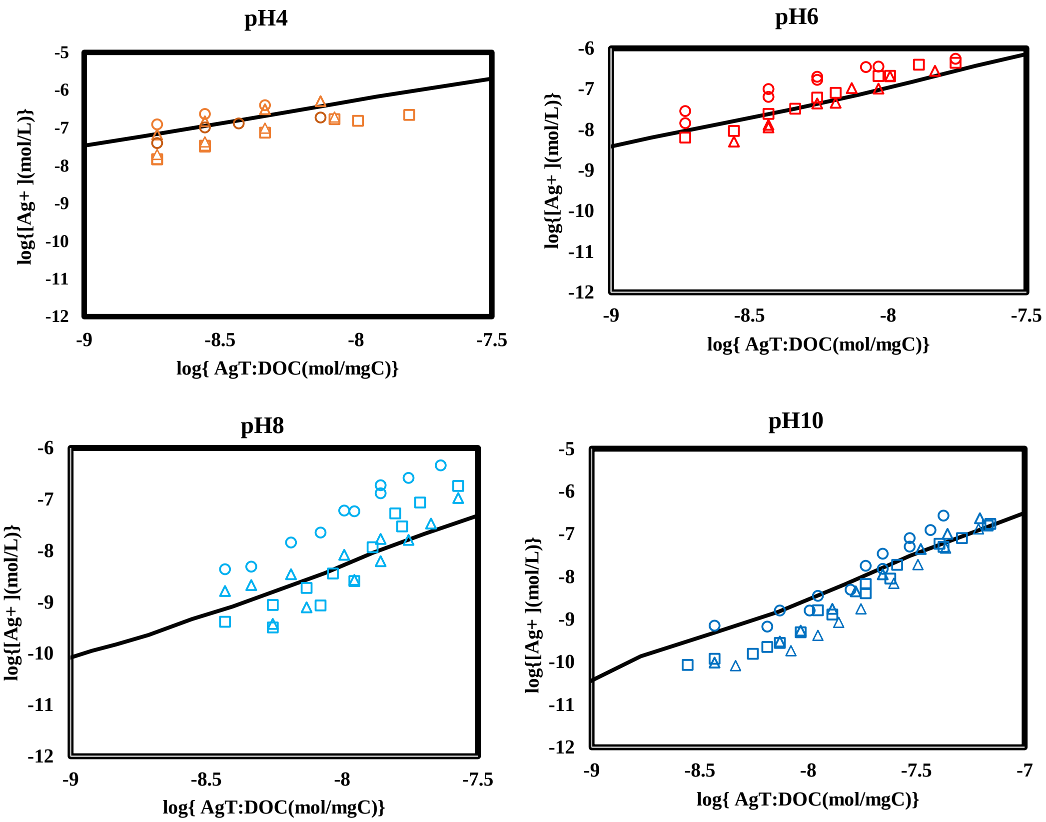

Figure 3.2: **Comparison of DOC samples binding with free silver over the range of various pH values with 0.01 M ionic strength (KNO_3). Circles=NR triangles=SW, squares=UM, and the solid black lines are WHAM predictions of silver binding to the DOM sample for each pH value: 4.0=orange, 6.0=red, 8.0= blue, and 10= dark blue**

On the other hand, protons can increase silver bioavailability, which is competitive with Ag^+ (Al-Reasi *et al.*, 2011). Therefore, it can be seen that NOM sources have potentially different levels of silver toxicity and different response to silver binding, depending on the content of ligands and pH conditions (Erickson *et al.*, 1998; Smith *et al.*, 2002). NOM sources has been of interest to environmental scientists (VanGenderen *et al.*, 2003), because NOM can control the transference, distribution, and toxicity of silver (Bury *et al.*, 1999).

Also, the diversity in NOM sources can help in understanding the metals behavior and help in predicting the aquatic environment toxicity (Tipping *et al.*, 2011). For example, in marine water, DOC content show that silver ions are less bioavailable because of the silver complex with Cl^-. These waters are then less toxic. Also, it has been shown that the silver species, ($AgCl_{aq}$), does not show a toxic physiological response (McGeer and Wood, 1998). In conclusion, various measurements of freshwater samples in Figure 3.2 confirm that silver is able to bind with different NOM and this binding to NOM is dependent upon ion metals, pH conditions, and NOM functional groups.

3.3 Comparison of ISE measurement to WHAM predictions at various pH

WHAM is used as a risk assessment tool to predict free metal ions in surface waters. Another goal of this researcher is to validate WHAM as a risk assessment tool specifically for silver. In order to validate the WHAM program, we experimentally determined silver binding isotherms for three sources of NOM under four pH values (4, 6, 8, and 10), and then comparisons were made between the laboratory results for SW samples and WHAM predictions. The WHAM modeling was done for all pH values, and the results are shown in Figure 3.3. For results of UM and NR samples please see the Appendix. The black solid lines in Figure 3.3 are WHAM predictions and the dashed lines show the lower and upper confidence limits of WHAM predictions. The WHAM model provides different predictions based on pH values. Figure 3.3 shows WHAM predictions for the Suwannee River source at different pH values (4, 6, 8, and 10) and demonstrates that WHAM and laboratory results agree within the confidence envelope. The Appendix summarizes parameters specific to the NOM samples with WHAM predictions. For all NOM samples, the results indicate that WHAM can predict silver speciation within the confidence limits of the model.

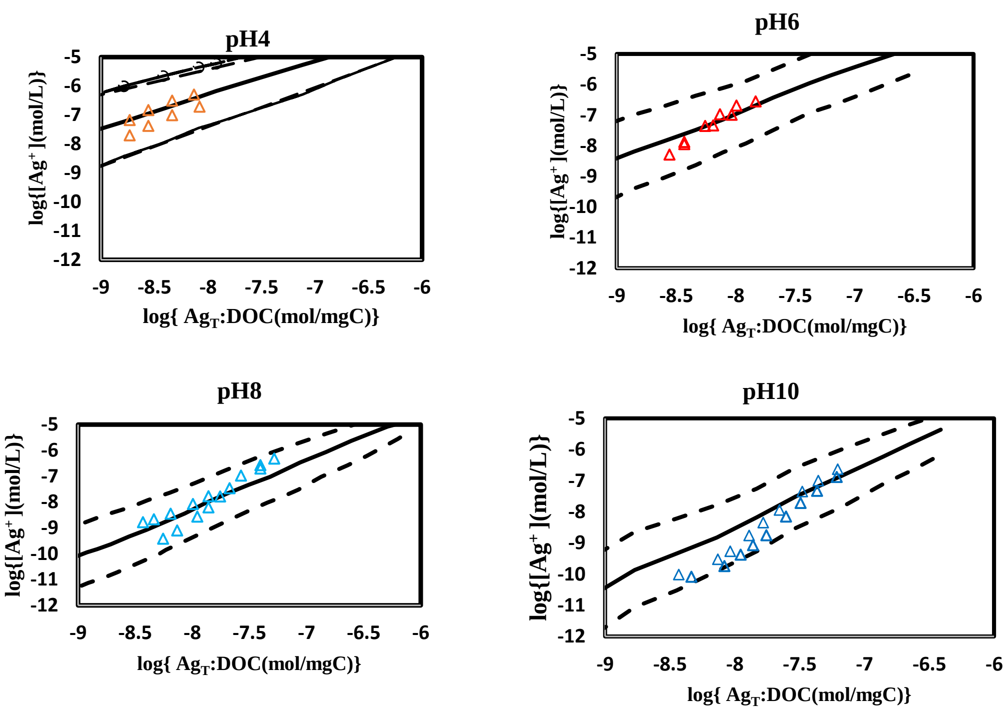

Figure 3.3: **WHAM predictions of silver binding for the Suwannee River data at different pH value: 4.0=orange, 6.0=red, 8.0= blue, and 10= dark blue. The black solid lines are WHAM predictions and the dashed lines show predictions with lower and upper WHAM predictions.**

3.4 Optical Characteristics

Figure 3.3 demonstrates that for Suwannee River, WHAM can predict silver speciation within the confidence limits of the model. In addition, all NOM have similar results (please refer to the Appendix). Because NOM plays an important role in binding silver, the optical characterization of NOM was done through SAC_{340} and fluorescence indices (FI) as summarized in Table 3.1. The SAC and FI values were used to test a range of samples in a DOC concentration 10 mg C L^{-1}, under the pH value around value of 7.0 ± 0.1. FI values tend to be close to 1.0, and also SAC_{340} have similar value for all NOM samples. These results can be used to validate the WHAM and ISE results which confirms the similarity that was noted in the results. Samples of similar quality bind silver in a similar measure.

Sample	**SAC_{340}**	**FI**
Upper Mississippi River	26	1.1
Suwannee River	37	1.0
Nordic Reservoir	34	1.0

Table 3.1: **Characteristics of samples used which was used for silver titrations**

Chapter 4

Conclusions and Future Studies

Free silver ion is the most reactive and toxic silver species in aquatic environments and silver toxicity depends on bioavailability and bioaccumulation of free silver in aquatic organisms (Fisher & Wang, 1998). Ionic silver (Ag^+) competes with Na^+ at the gills, causing disruption of ionic balance across cell membranes. It inhibits the activity of sodium/potassium-adenosine triphosphatase (Na/K-ATPase) that results in reduction of Na^+ and Cl^- uptake which adversely affects aquatic organisms (McGeer and Wood, 1998). In freshwater environments, water chemistry can cause protection against acute silver toxicity (Wood *et al.*, 1999). Natural organic matter (NOM) is considered an important factor in the protection of the aquatic environments. NOM contains different functional groups, such as, carboxyl (-OOC), phenolic (-OH), amino ($–NH_2R)^+$, metal sulfides (–SM) and thiolatesulfide (–SR) groups (Smith et al., 2002). Interaction and binding of silver to these groups decreases the bioavailability of free silver in aquatic environments. Since silver is a soft metal, it binds strongest to soft ligands such as reduced sulphur (Smith *et al.*, 2002). In Figure 3.2, it can be seen that silver binds more with NOM in samples which contains sulphur-containing ligands such as Upper Mississippi River source. Moreover, pH can affect metals in either a negative or a positive manner. In the former, the toxicity of silver ions is increased by competition of proton with toxic metals (Pagenkopf, 1983) as shown in Figure 3.1; at low pH there are more protons available to compete with Ag^+ ions in interaction with NOM (Janes and Playle, 1995). In contrast, when pH is increased, more sites are available for metal binding on NOM, because of less proton competition (Erickson *et al.*, 1998) and more silver ions can bind, resulting in less silver bioavailablility/toxicity. In this study, the flow through an ion selective electrode (ISE) system utilizing an Ag_2S electrode was thoroughly used to determine metal ion (silver ions) concentrations. This technique was utilized due to its high sensitivity and response to silver ions (Durst, 1969). After assessing silver ion concentration in different freshwater sources, the Windermere Humic Aqueous Model (WHAM) was used as a risk assessment (Lofts and Tipping, 2011) tool. The method was validated by comparing the WHAM and ISE results. All NOM sampling and analysis results were seen to be in agreement with WHAM within modeling confidence limits.

Future work related to this aspect of this study is to apply diverse samples and selection including different IHSS samples, real samples including wastewater and autochthonous sources. In order to test for more general applicability of WHAM which has very wide confidence limits from our modelling. Perhaps those limits can be decreased by including a “quality factor”. In addition, the studies should continue measuring optical indices include FI and SAC_{340} and also measuring chemical properties such as reduced sulphur and organic nitrogen content.

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Appendices A

A.1 Comparison of ISE measurement to WHAM predictions at various pH

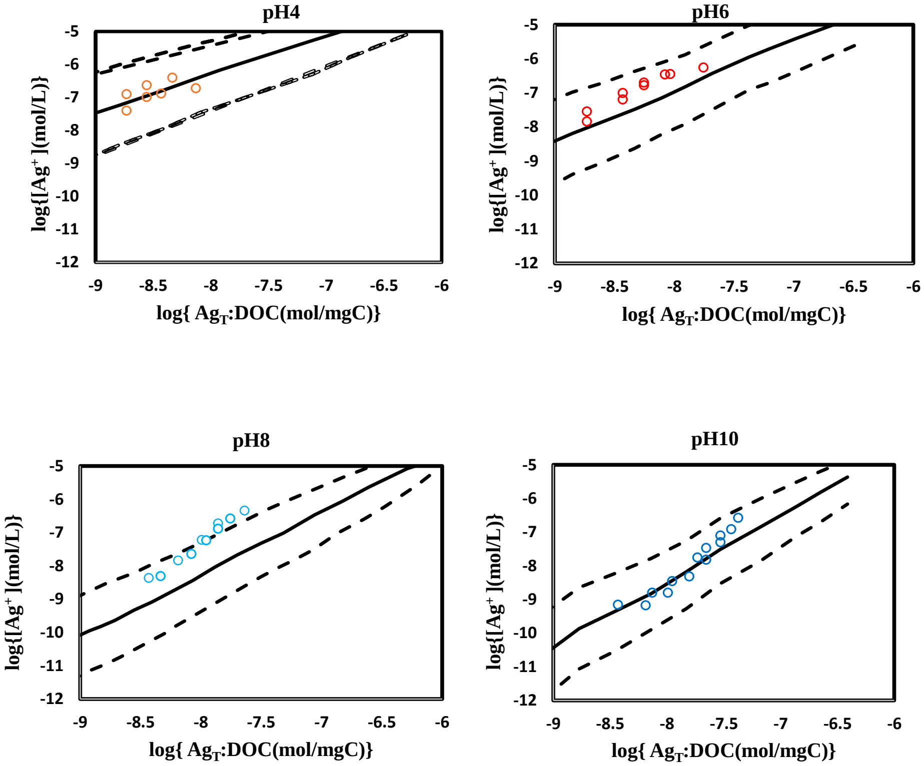

Figure A.1: **WHAM predictions of silver binding for the Nordic Reservoir data at different pH. The black solid lines are WHAM predictions and the dashed lines show predictions with lower and upper WHAM predictions**

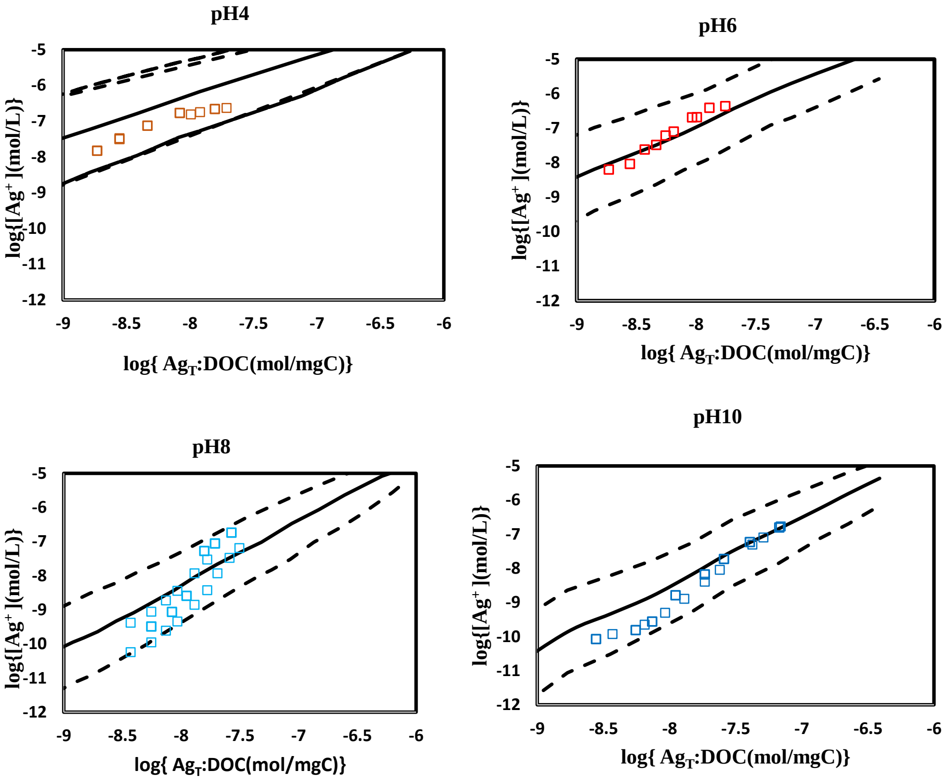

Figure A.2: **WHAM predictions of silver binding for the Upper Mississippi River data at different pH. The black solid lines are WHAM predictions and the dashed lines show predictions with lower and upper WHAM predictions**

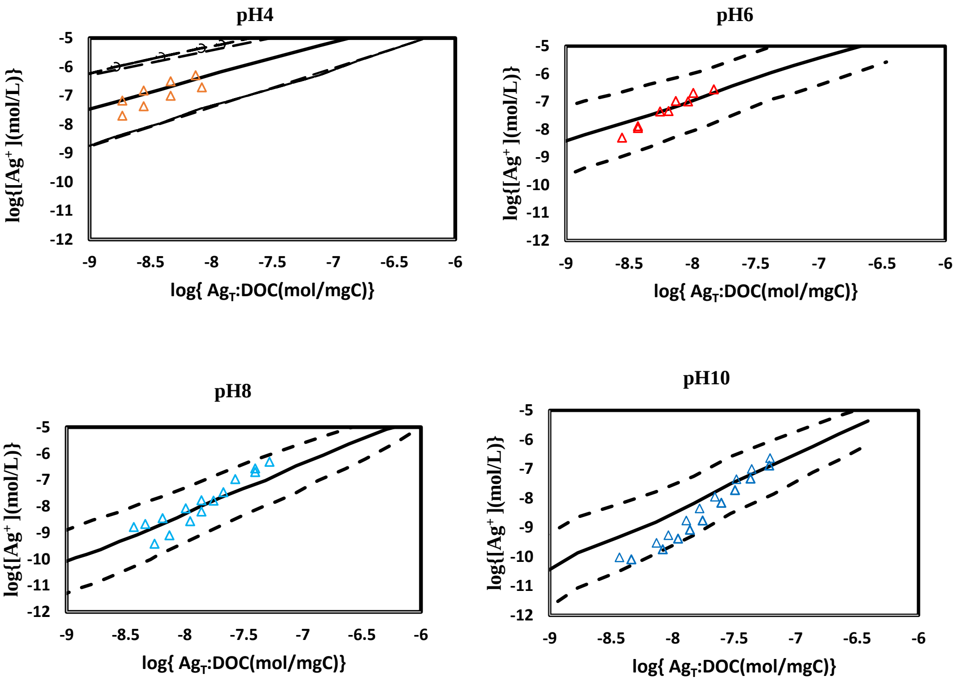

Figure A.3: **WHAM predictions of silver binding for the Suwannee River data at different pH. The black solid lines are WHAM predictions and the dashed line show predictions with lower and upper WHAM predictions**

Appendices B

B.1 Fluorescence intensities from excitation-emission matrices for the NOM samples

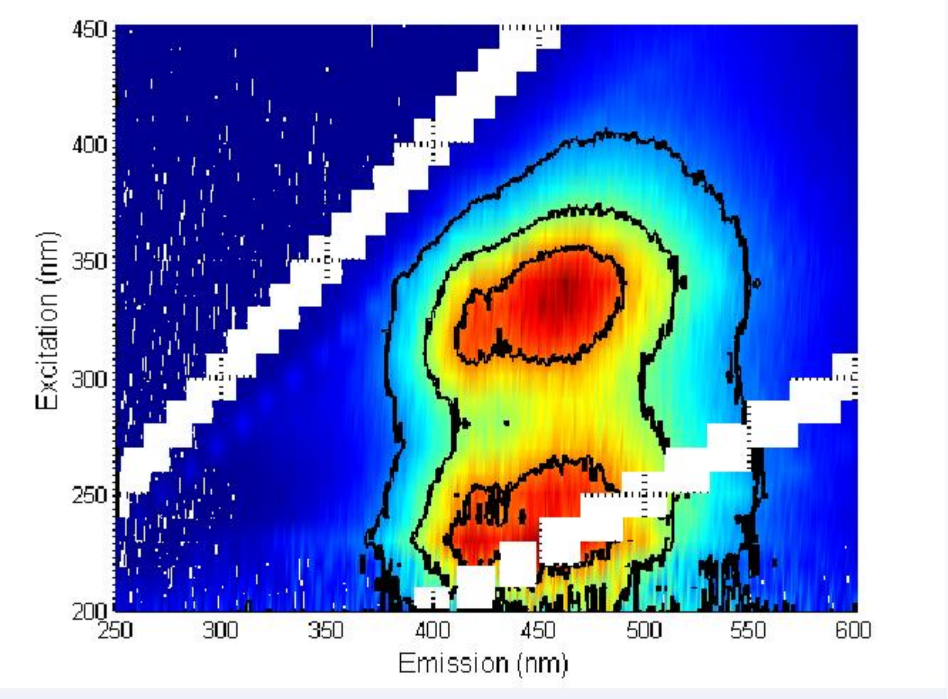

Figure B.1: **fluorescence intensities from excitation-emission matrices for the Nordic Reservoir**

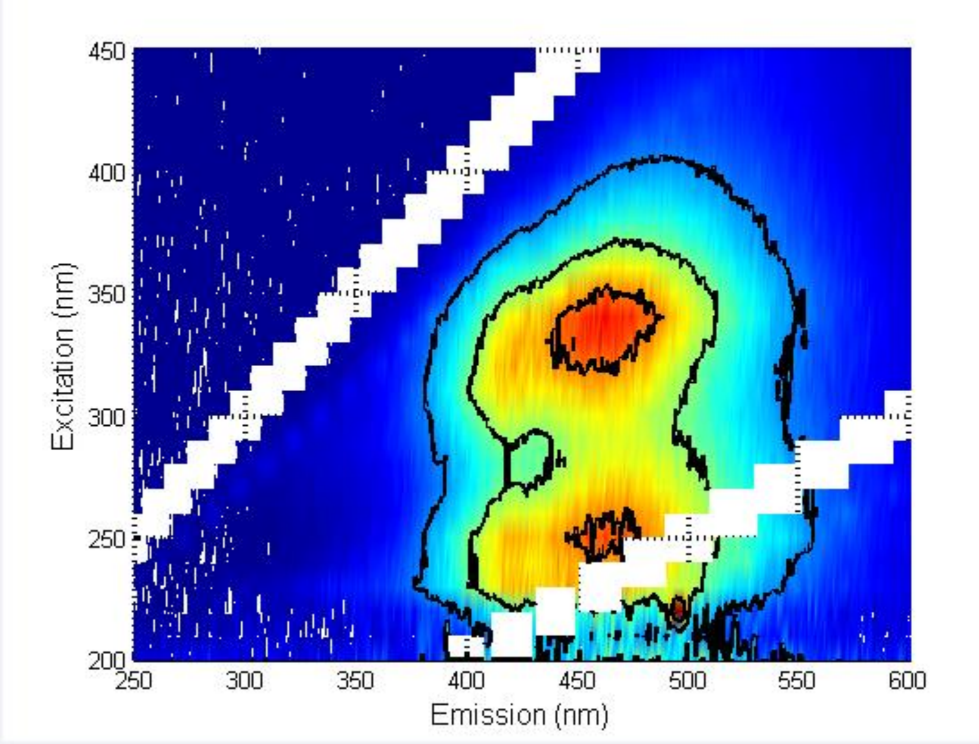

Figure B.2: **fluorescence intensities from excitation-emission matrices for the Suwannee River**

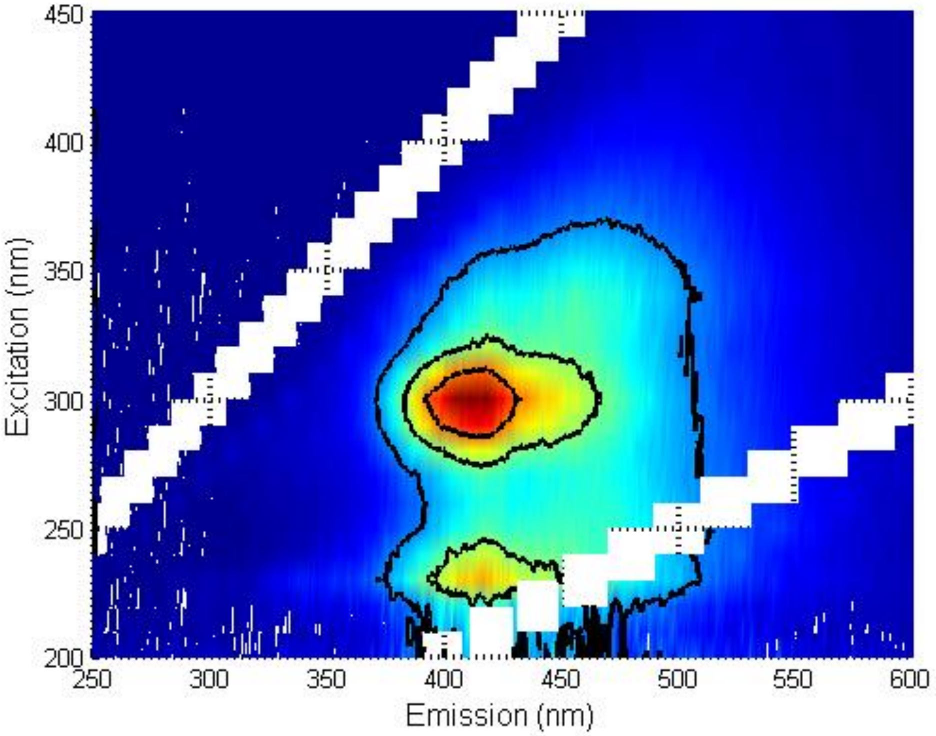

Figure B.3: **fluorescence intensities from excitation-emission matrices for the Upper Mississippi River**