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**Natural Organic Matter Characterization and Lead Speciation as Indicators of Potential  
Lead Bioavailability in Aquatic Environments**

by

Giselle Cimprich

Bachelor of Science, Honours Chemistry, Wilfrid Laurier University, 2013

THESIS

Submitted to the Department of Chemistry  
in partial fulfilment of the requirements for  
Degree of Master of Science in Chemistry

Wilfrid Laurier University

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## Abstract

Trace metals and natural organic matter (NOM) are ubiquitous in aquatic environments. Some trace metals are essential for aquatic life, while other non-essential metals (like lead) can be toxic if present in great enough concentrations. Natural waters contain a combination of inorganic, and organic ligands capable of binding metals. While the chemistry of inorganic Pb species is well understood, and National Institute of Standards and Technology (NIST) certified K values are readily available, the interactions between metals and organic matter is not so clearly defined. It is important to understand how NOM interacts with metals in the environment, as the properties of NOM vary with source. If these differences in NOM chemistry induce source-dependent Pb binding, the industrial and environmental implications would be significant. This study aimed to characterize a variety of NOM sources of diverse origin, measure Pb speciation in order to determine if Pb-NOM binding is indeed source-dependent, and to determine which property/ies would best explain Pb-NOM binding. NOM sources were characterized using: total organic carbon/dissolved organic carbon (TOC/DOC), fluorescence excitation-emission matrices (FEEM), parallel factor analysis (PARAFAC), fluorescence index (FI), specific absorption coefficient ( $SAC_{340}$ ), chromium-reducible sulfide (CRS), thiol, dissolved organic nitrogen (DON), and proton binding index (PBI). These methods allowed for a quantification of organic carbon; humic acid-, fulvic acid-, tyrosine-, and tryptophan-like components; origin; aromaticity; sulfide ligands; nitrogen ligands; and oxygen ligands.  $SAC_{340}$ , FI, %HA, %FA, %Trp, %Tyr, CRS, thiol, DON, and PBI values ranged from: 7.76 – 40.84, 1.04 – 1.84, 46.41 – 82.41%, 13.32 – 39.21%, 1.02 – 16.21%, 1.34 – 14.99%, 2.03 – 89.0 nmol/mgC, 71.8 – 186.5 nmol/mgC, 35.76 – 253.8  $\mu$ gN/mgC, and 0.33 – 1.72 respectively. No one parameter, or simple series of parameters was able to discriminate NOM source. However, CRS,

Trp, and Tyr may be able to discriminate saltwater from freshwater sources, while SAC<sub>340</sub>, CRS, thiol, DON, Trp, Tyr, and PBI may be able to discriminate between freshwater sources. Sources of terrestrial origin had significant SAC<sub>340</sub> and PBI, while sources of microbial origin had significant CRS, DON, Trp, and Tyr.

Free lead was then measured using flow-through titrations with a commercially available Pb ion-selective electrode (ISE) and an internal calibration method. To confirm that this method was applicable for trace-level analysis in NOM, ethylenediamine (EN) was used as a model ligand in both artificial freshwater (AFW) and artificial seawater (ASW), and the speciation modelled using certified logK values from NIST. In both AFW and ASW, the ISE accurately and reproducibly (within a factor of two) measured Pb<sup>2+</sup> speciation with EN as a model ligand. However, when this method was applied to speciation measurement in NOM, measured values did not agree well with WHAM. This indicates that the fundamental assumption (that Pb-NOM binding will not occur at low pH) made by the internal calibration method is not effective at predicting speciation in NOM, as WHAM predicts that Pb-NOM binding will occur at low pH. An alternate calibration method was tested – forcing measured values to agree with WHAM at low pH – and gave much better agreement. However, speciation measurements in NOM demonstrated reproducible variation with source (indicating source-dependent Pb-NOM binding) which was not described by WHAM within a factor of two, regardless of the calibration method. DON and SAC<sub>340</sub> for the titrated sources are significantly different, and may potentially explain differences in source-dependent Pb speciation in freshwater environments. This is of immense industrial and environmental importance, as current water quality guidelines (WQG) do not account for NOM, DON, or SAC<sub>340</sub>. Consequently, current guidelines could overestimate toxicity in highly aromatic sources, or underestimate toxicity in sources with high DON.



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## List of Abbreviations and Symbols

|                  |  |
|------------------|--|
| AFW              | Artificial Freshwater  |
| ANOVA            | Analysis of Variance   |
| ANZECC           | Australian and New Zealand Environment Conservation Council    |
| ASW              | Artificial Seawater  |
| AVS              | Acid Volatile Sulfide  |
| BLM              | Biotic Ligand Model  |
| CEC              | Commission for Environmental Cooperation                       |
| CCME             | Canadian Council of Ministers of the Environment               |
| CRS              | Chromium Reducible Sulfide                                     |
| CSIR             | Council of Scientific and Industrial Research                  |
| DIN              | Dissolved Inorganic Nitrogen                                   |
| DOC              | Dissolved Organic Carbon                                       |
| DOM              | Dissolved Organic Matter                                       |
| DON              | Dissolved Organic Nitrogen                                     |
| FEEM             | Fluorescence Excitation Emission Matrix                        |
| FI               | Fluorescence Index   |
| HIX              | Humification Index   |
| HTCO             | High Temperature Catalytic Oxidation                           |
| ISE              | Ion-Selective Electrode  |
| LA <sub>50</sub> | Lethal Accumulation Concentration                              |
| LC <sub>50</sub> | Median Lethal Concentration (required to induce 50% mortality) |

|         |  |
|---------|--|
| MDR     | Mixed Diamine Reagent                          |
| NIST    | National Institute of Standards and Technology |
| NOM     | Natural Organic Matter                         |
| PARAFAC | Parallel Factor Analysis                       |
| PBI     | Proton Binding Index                           |
| PLS     | Partial Least Squares Regression               |
| qBBr    | Monobromo(trimethylammonio)-biamine            |
| SAC     | Specific Absorption Coefficient                |
| TDN     | Total Dissolved Nitrogen                       |
| TOC     | Total Organic Carbon                           |
| TN      | Total Nitrogen                                 |
| USEPA   | United States Environmental Protection Agency  |
| WHAM    | Windermere Humic Aqueous Model                 |
| WQG     | Water Quality Guideline                        |
| XAS     | X-ray Absorption Spectroscopy                  |

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# Chapter 1 Introduction

## 1.1 Lead in the Environment

Lead is a relatively rare non-essential metal that can be found naturally within the Earth's crust in concentrations of 0.016 gPb/kg soil<sup>1</sup>. By comparison, the most abundant metal in the Earth's crust is aluminum, occurring at 82.3 gAl/kg soil<sup>2</sup>.

Lead is released into the environment through natural sources, such as the weathering of lead-containing rocks and minerals, volcanic activity, forest fires, and radioactive decay<sup>1,3</sup>. In marine waters, background concentrations of dissolved lead are reported to be in the range of 0.5 to 3 µg/L<sup>4</sup>. In surface waters, natural background concentrations of 1-23 ng/L have been reported<sup>3</sup>. However, as a result of human activity current concentrations can be much higher<sup>3</sup>. In areas exposed to anthropogenic input, lead concentrations can be as high as several hundred µg/L dissolved lead, or mg/L range for total lead<sup>3</sup>.

Lead has had an extensive history of anthropogenic input and toxicity<sup>5</sup>. It was used extensively by the Romans for a number of applications, including plumbing, cookware, and as a food additive<sup>5</sup>. In recent years (1923 - 2014), lead has been used as an antiknock additive in gasoline<sup>3,6</sup>. However, leaded fuels have been widely banned, making leaded fuels of lesser concern<sup>3</sup>.

Today, lead is primarily used for the production of lead-acid batteries, consuming almost 1.35 million tonnes of lead in the US in 2008 alone<sup>3</sup>. Major anthropogenic sources of lead include lead mining, smelting, and refining; domestic wastewater; and sewage sludge<sup>7</sup>. Comparatively, natural sources - like the weathering of minerals - contribute very little to the environment<sup>3</sup>. Anthropogenic sources of lead into aquatic ecosystems have been estimated to

contribute 138,000 metric tons annually, compared to natural sources, which contribute only 12,000 metric tons per year<sup>3,7</sup>.

Once released into the environment, lead can be introduced into aquatic environments through a number of natural processes<sup>8</sup>. However, deposition from the atmosphere is the primary route of entry for lead in aquatic systems<sup>7</sup>. Estimates suggest that as a result of atmospheric sources, lead deposition has skyrocketed, increasing 1000-fold since prehistoric times<sup>3</sup>.

## **1.2 Lead Toxicity in Freshwater and Saltwater**

Trace metals play an important role in aquatic environments. Some trace metals are essential for metabolic processes in aquatic organisms (e.g. copper) while others may be toxic (e.g. lead)<sup>5</sup>. Lead is one such metal and has a number of toxic effects on aquatic organisms.

In freshwater, fish may exhibit acute lead toxicity through a number of symptoms, including: an increase in mucus production, resulting in respiratory distress and iono-regulatory effects. Similarly, fish may exhibit chronic lead toxicity through hematological effects, stunted growth and developmental problems<sup>3</sup>.

In hard water, acute exposure to lead impacts calcium, sodium, and chloride regulation in rainbow trout<sup>3</sup>. Calcium uptake is inhibited as a result of competition with lead at calcium channels in the gills and kidney<sup>3</sup>. Lead also interferes with sodium and chloride regulation by rapid disruption of branchial carbonic anhydrase activity, and gradual disruption of sodium/potassium -ATPase activity<sup>3</sup>. However, in soft water recent evidence indicates that the primary mode of toxicity to fish is caused by a decrease in sodium levels in the blood<sup>3</sup>.

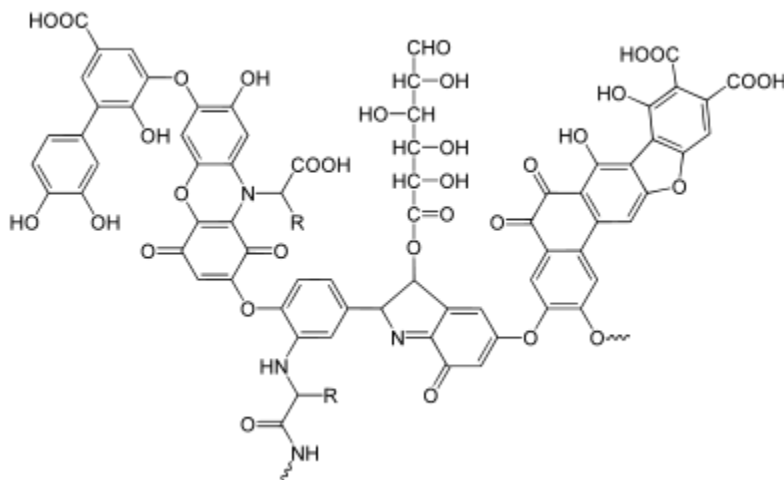
Unfortunately, there is far less literature on the toxicological effects of lead on saltwater species<sup>3</sup>. However, a study by Tellis *et al.* found that Pb toxicity to developing sea urchin embryos is primarily caused by a disruption in the osmotic balance of Ca within the organism<sup>9</sup>.



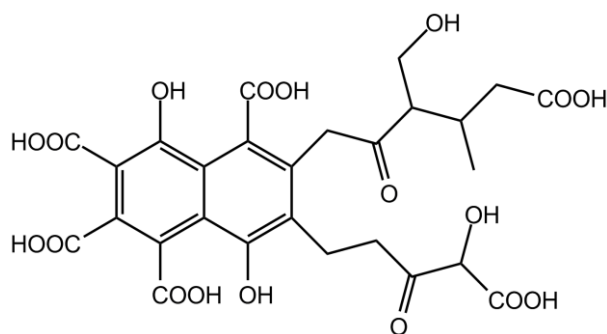
Calcium uptake, accumulation, and ATPase levels are most effected during the gastrulation stage of development, and in the event of continued exposure they may be able to recover<sup>9</sup>.

### 1.3 Natural Organic Matter

Natural organic matter (NOM) is a heterogeneous mixture of organic molecules that can be found universally in water, sediment and soil<sup>10</sup>. NOM found in natural waters can be either terrestrial (allochthonous) - the result of terrestrial plant decay - or microbial (autochthonous) - the by-product of bacteria, algae, and plant/animal matter decay - in origin<sup>10</sup>. Terrestrial NOM usually has greater humic and fulvic (hypothetical molecular structures can be seen in figures 1.31 and 1.32 below) content and is darker in colour (high aromatic and phenolic content), while autochthonous NOM typically contains more proteinaceous material, amino acids (such as tyrosine and tryptophan), and is lighter in colour (low aromatic and phenolic content)<sup>11,12</sup>. Theoretical structures of humic acid and fulvic acid are given below.



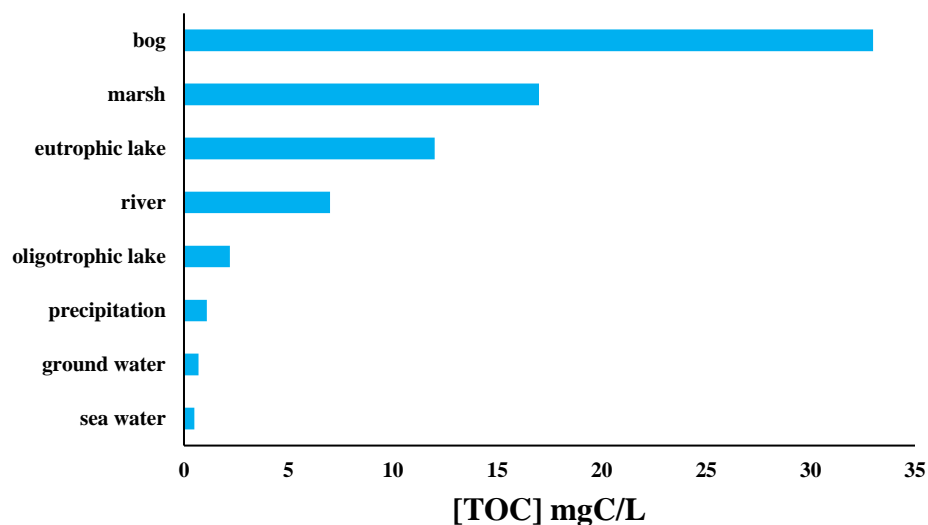
**Figure 1.31** Hypothetical molecular structure of humic acid<sup>13</sup>



**Figure 1.32** Hypothetical molecular structure of fulvic acid<sup>13</sup>

NOM has both hydrophobic and hydrophilic components. Typically, hydrophobic acids account for 50% of total organic carbon (TOC) in water, making it the largest fraction of NOM<sup>10</sup>. Among these hydrophobic acids are humic acids, fulvic acids, and humins<sup>10</sup>.

Humic, fulvic and humin components are defined in terms of their pH-dependent solubility. Fulvic acids are soluble at any pH, while humic acids are insoluble at pH<2, and humins are insoluble at all pH<sup>10,14</sup>. Dissolved organic carbon (DOC) is the fraction of TOC which may pass through a 0.45µm filter, and is used as an input in the US Environmental Protection Agency (USEPA) freshwater biotic ligand model (BLM) for Cu<sup>15</sup>. The following figures show the concentrations of organic matter present in various freshwater and saltwater environments.



**Figure 1.33** Approximate Concentrations of Total Organic Carbon in Various Aquatic Environments<sup>14</sup>

In general, the concentration of TOC and DOC increase as salinity decreases<sup>14</sup>. Seawater has some of the lowest DOC concentrations (0.5 mgC/L on average), estuaries have intermediate concentrations (ranging from 1-10 mgC/L), and freshwater environments have some of the highest concentrations (1-60 mgC/L)<sup>14</sup>. The quality of NOM is also variable depending on its origin<sup>14</sup>.

Larger water bodies have greater autochthonous and less allochthonous organic carbon content, while smaller water bodies have more allochthonous and less autochthonous organic carbon<sup>14</sup>. The open ocean tends to contain more autochthonous organic matter, with allochthonous carbon accounting for up to 50%<sup>14</sup>. However, this decreases with depth<sup>14</sup>. In shallow seawater of depth 0 – 300 m, DOC ranges from 0.3 – 2.0 mgC/L<sup>14</sup>. At depths greater than 300 m, DOC ranges from 0.2 – 0.8 mgC/L<sup>14</sup>. Freshwater environments contain comparatively large quantities of terrestrially derived organic matter<sup>14</sup>. In freshwater lakes allochthonous carbon can range from 0 – 70%<sup>14</sup>. These different types of organic matter contain

different types of functional groups, which make NOM capable of complexing metals and therefore impacting metal toxicity<sup>10,14</sup>. Much work has been done on characterizing NOM in an attempt to understand how it interacts with metals in water<sup>16</sup>. However, there is still much to be investigated, including: the relationship between dissolved organic matter (DOM) source/character and metal speciation, the relationship between DOM source/character and microbes, the interactions that occur within DOM components, and the DOM cycling process<sup>16</sup>.

#### **1.4 Lead Interactions in Natural Waters**

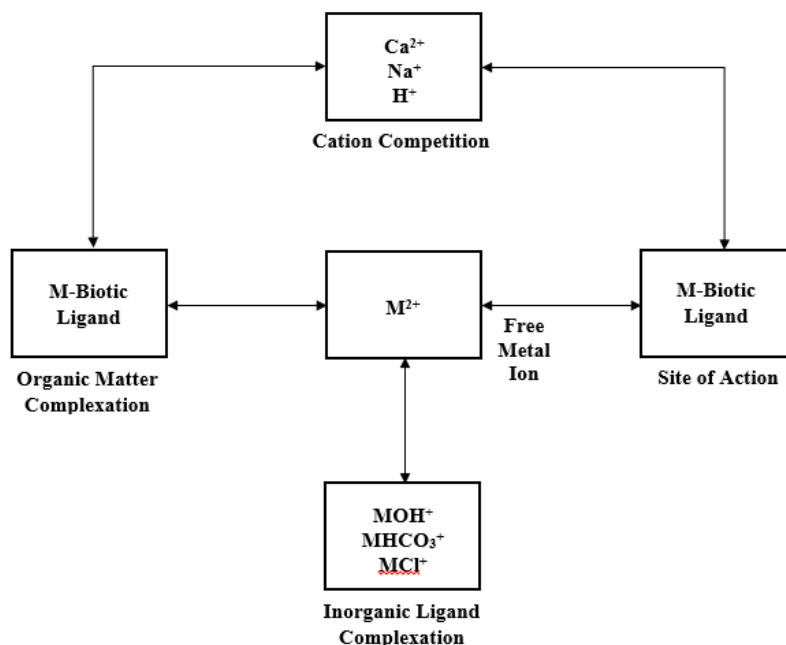
Lead can be found in a variety of different forms in aquatic environments. It can be attached to colloidal particles, organic complexes, inorganic complexes, or can occur as the free metal ion (which has high mobility and bioavailability)<sup>3,17</sup>.

Lead speciation in freshwater and seawater differs. In freshwater lead partially exists as  $Pb^{2+}$  at pH values lower than 7.5, but forms insoluble carbonate complexes ( $PbCO_3$ )<sub>(s)</sub> in basic conditions. The main inorganic ligands in seawater which bind lead are chloride, carbonate, sulfate, hydroxide, and fluoride. In seawater lead primarily exists as cationic lead chloride ( $PbCl^+$ ) and soluble lead carbonates ( $PbCO_3$  and  $Pb(CO_3)_2^{2-}$ )<sup>18</sup>. Lead is also able to form complexes with NOM at binding sites such as thiol, amino, carboxyl and phenolic functional groups<sup>10</sup>.

Lead's speciation in aquatic environments is controlled by a number of factors, including pH and salinity<sup>3</sup>. In acidic water lead is more soluble, and is typically found as the free metal ion,  $PbSO_4$ ,  $PbCl_4^{2-}$ ,  $Pb(OH)_2$ , and cationic forms of lead hydroxide (e.g.  $PbOH^+$ )<sup>3,19</sup>.

These different forms, or species, display different mechanisms and levels of toxicity<sup>3</sup>. This makes speciation and the factors that influence it important to consider when addressing lead toxicity, as water chemistry varies greatly with location<sup>20</sup>.

## 1.5 The Biotic Ligand Model



**Figure 1.51** Schematic diagram explaining the biotic ligand model<sup>20</sup>.

The BLM is based on the concept that free-metal binding to the biotic ligand (such as the gills of a fish) causes toxicity, which is directly proportional to accumulation<sup>20,21</sup>. The free metal ion is the most bioavailable and toxic form, and is therefore used in modelling<sup>20</sup>. Organic and inorganic species present in natural waters are able to mitigate free-metal toxicity, either by competition or complexation<sup>20,22</sup>. Non-toxic cations such as  $\text{Na}^+$  or  $\text{Ca}^{2+}$  compete with the free metal ion for binding at the biotic ligand, and thereby reduce toxicity<sup>3,20,22</sup>. Similarly inorganic and organic ligands are able to complex the free metal ion, rendering it less bioavailable<sup>20,23</sup>. When the metal reaches a certain concentration at the biotic ligand – such as the  $\text{LC}_{50}$  (lethal concentration required to induce 50% mortality) – toxicity occurs<sup>20,22</sup>.

Only a fraction of the Pb present will bind to the biotic ligand to produce Pb-Biotic Ligand complex<sup>20</sup>. If the amount of Pb bound to the biotic ligand can be predicted, Pb toxicity

can be assessed and used to implement site-specific water quality guidelines<sup>21</sup>. This makes the BLM an attractive means of implementing water quality guidelines (WQG's) (shown in table 1.61 below), as unlike the current guidelines it can account for quality parameters such as pH and [DOC]<sup>21</sup>.

## 1.6 Lead Regulations for the Protection of Aquatic Life

**Table 1.61** Some international Pb water quality guidelines and criteria for the protection of aquatic life.

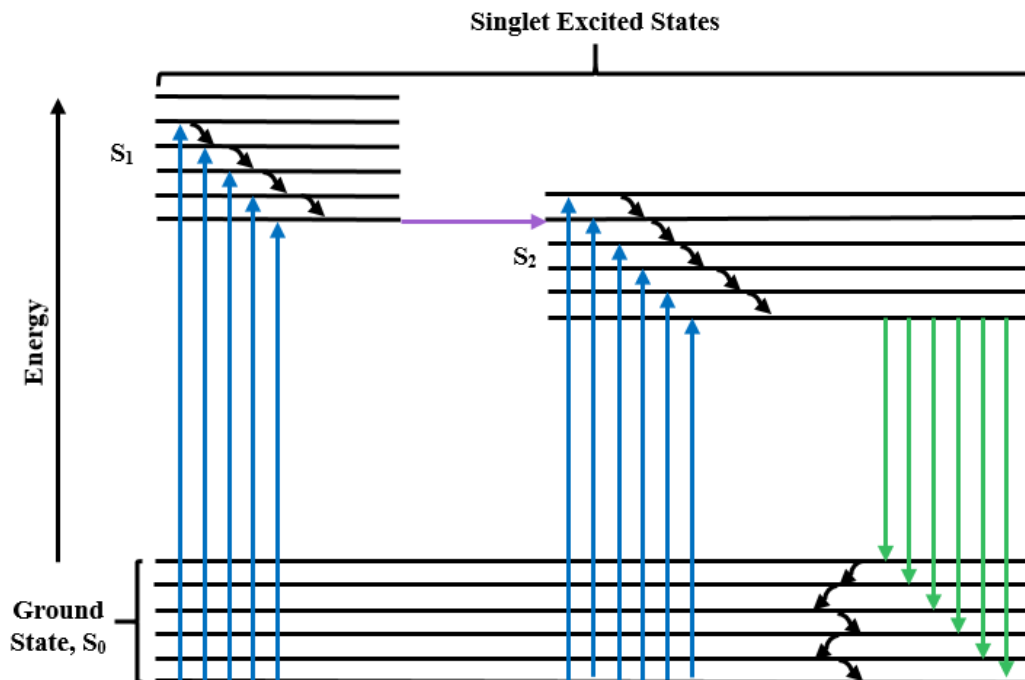
| Jurisdiction          | Reference                   | Acute<br>(µg/L) | Chronic<br>(µg/L) | Notes                                   |
|-----------------------|-----------------------------|-----------------|-------------------|---|
| Canada                | CCME (2008) <sup>24</sup>   |                 | 1                 | Hardness 0-60 mgCaCO <sub>3</sub> /L    |
|                       |                             |                 | 2                 | Hardness 60-120 mgCaCO <sub>3</sub> /L  |
|                       |                             |                 | 4                 | Hardness 120-180 mgCaCO <sub>3</sub> /L |
|                       |                             |                 | 7                 | Hardness > 180 mgCaCO <sub>3</sub> /L   |
| USA                   | USEPA (1985) <sup>25</sup>  | 10.8            | 0.4               | Hardness 20 mgCaCO <sub>3</sub> /L      |
|                       |                             | 30.1            | 1.2               | Hardness 50 mgCaCO <sub>3</sub> /L      |
|                       |                             | 136             | 5.3               | Hardness 200 mgCaCO <sub>3</sub> /L     |
|                       |                             | 210             | 8.1               | Saltwater                               |
| European Union        | CEC (2006) <sup>26</sup>    |                 | 7.2               | Annual Average for All Surface Waters   |
| Australia/New Zealand | ANZECC (2000) <sup>27</sup> |                 | 2                 | Hardness 20 mgCaCO <sub>3</sub> /L      |
|                       |                             |                 | 6.5               | Hardness 50 mgCaCO <sub>3</sub> /L      |
|                       |                             |                 | 37.8              | Hardness 200 mgCaCO <sub>3</sub> /L     |
|                       |                             |                 | 4.4               | Seawater                                |
| South Africa          | CSIR (1996) <sup>28</sup>   | 4               | 0.5               | Hardness < 60 mgCaCO <sub>3</sub> /L    |
|                       |                             | 7.0             | 1.0               | Hardness 60-119 mgCaCO <sub>3</sub> /L  |
|                       |                             | 13              | 2.0               | Hardness 120-180 mgCaCO <sub>3</sub> /L |
|                       |                             | 16              | 2.4               | Hardness > 180 mgCaCO <sub>3</sub> /L   |
|                       | CSIR (1995) <sup>4</sup>    |                 | 12                | Seawater                                |

As seen in table 1.61, international guidelines and criteria for lead are largely uniform across countries. With the exception of the European Union, freshwater guidelines account for some water chemistry – i.e. differences in hardness - but fail to account for pH and [DOC], which have been shown to significantly impact metal toxicity in freshwater<sup>23</sup>. Saltwater guidelines are far less widespread, and do not account for differences in water chemistry. There are currently no national WQG's for Pb in marine waters, however, British Columbia has implemented provincial marine guidelines for both acute and chronic exposure<sup>3</sup>. At present, no BLM has been implemented for Pb in both freshwater and seawater, however, BLMs for Pb in freshwater do exist<sup>23,29,30</sup>.

## **1.7 Spectroscopic Characterization of NOM**

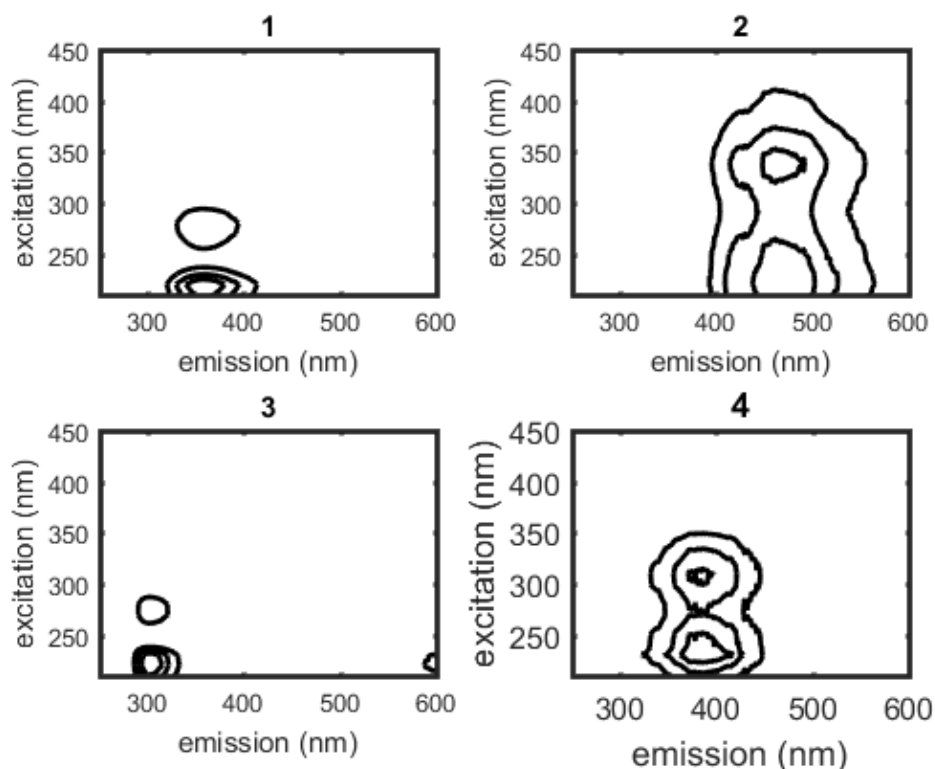
Fluorescence spectroscopy is an optical technique that has been used extensively in recent research for the characterization of NOM, partially due to its ease of use<sup>16,31,32</sup>. Its abundance of aromatic functional groups enables NOM to fluoresce, as they contain delocalized electrons that can easily become excited<sup>16,31</sup>. This process is best explained with a Jablonski diagram.





**Figure 1.71** Jablonski Energy Diagram. Absorption, vibrational relaxation, intersystem crossing, and fluorescence are represented by blue, black, purple, and green arrows respectively.

Fluorescence occurs with NOM when a delocalized electron absorbs a photon and becomes excited<sup>13,16,33</sup>. This excited electron is promoted from the ground state to a higher energy level (excited state)<sup>13,16,33</sup>. Once excited, it may then undergo internal conversion and/or vibrational relaxation before fluorescing, or it may fluoresce directly<sup>13,16,33</sup>. Each fluorophore potentially has a unique excitation and emission wavelength, making it possible to identify components based on their fluorescence excitation-emission (FEEM) spectra<sup>13,16,33</sup>. However, it is possible for a fluorophore to absorb at more than one excitation wavelength, which can be seen in some characteristic FEEMs of NOM<sup>33</sup>.



**Figure 1.72** Characteristic FEEMs for the Components of NOM: (1) tryptophan-like, (2) humic-like, (3) tyrosine-like, and (4) fulvic-like.

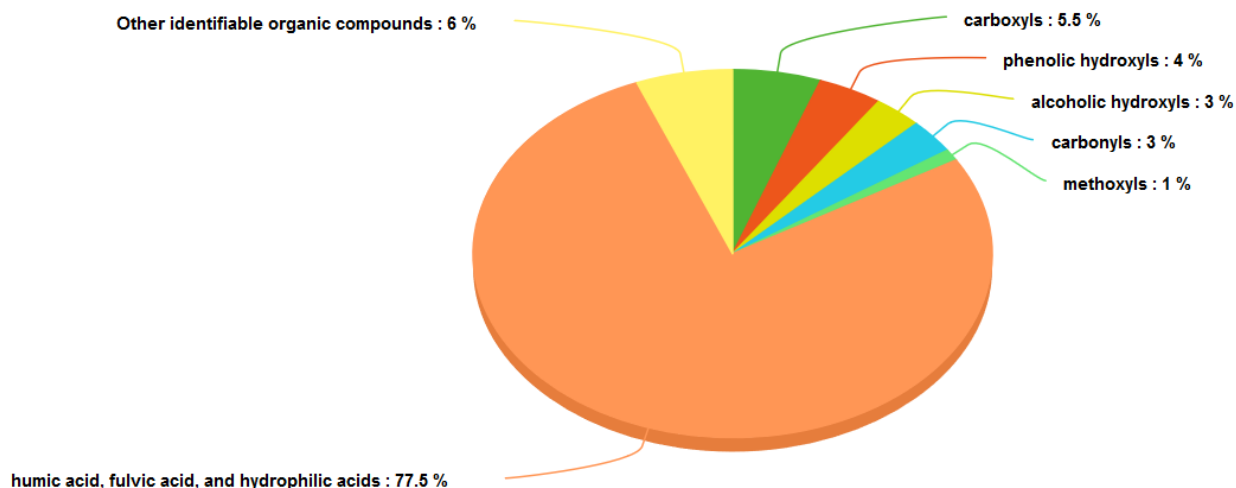
Figure 1.72 demonstrates the main components – humic and fulvic acids, tryptophan and tyrosine – resolved by FEEM analysis. Humic and fulvic-like moieties can be detected at Ex/Em wavelengths of 250-390 nm/460-520 nm, and 300-350 nm/400-450 nm respectively<sup>33</sup>. Similarly, tryptophan and tyrosine can be detected at Ex/Em wavelengths of 225-275 nm/350 nm, and 225-275 nm/300 nm respectively<sup>33</sup>. Some metals such as Cu are known to quench fluorescence in NOM, while others (like Fe) are optically colourful<sup>13,34</sup>. However, if removed via resonating with a cation exchange resin, metal quenching should cause negligible interference<sup>35</sup>.

Indices - including fluorescence index (FI), humification index (HIX), and specific absorbance coefficient (SAC) – and statistical techniques – analysis of variance (ANOVA), parallel factor analysis (PARAFAC), and partial least squares regression (PLS) – have been used

to analyze optical characterization data<sup>3,16,31</sup>. FI provides a measure of autochthonous to allochthonous DOC; HIX provides a measure of humic content; SAC at 340 nm (SAC<sub>340</sub>) can measure aromaticity; and ANOVA and PARAFAC can provide information on the origin and composition of the DOM<sup>11,36–38</sup>. SAC<sub>340</sub> has been directly correlated with Pb toxicity to freshwater rainbow trout by Schwarz *et al.*, with greater values indicating reduced toxicity<sup>39</sup>.

## 1.8 Acid-Base Titrations and Proton Binding Index

NOM contains a vast array of functional groups capable of binding metals, with an extensive range of potential pKa values<sup>40,41</sup>. Different NOM sources may have different pKa distributions and concentrations of the different types of functional groups<sup>41</sup>. Carboxylates and phenols are oxygen functional groups, and the most abundant in DOM<sup>38</sup>.



**Figure 1.81** Median Concentrations of the Various Components of DOC with an Emphasis on Oxygen-Containing Functional Groups

Oxygen-containing functional groups account for approximately 16.5% of DOC<sup>14,42</sup>. Of that 16.5%, carboxyls and phenols account for 2-9% and 2-6% respectively<sup>42</sup>. Smith *et al.*

reported a logK value of  $2.55 \pm 1.12$  for Pb binding to carboxyl groups<sup>42</sup>. Although they are not strong binding sites, their abundance allows them to compete for metals with other prospective binding sites<sup>38</sup>. These monodentate weak-binding sites are of particular importance at higher concentrations of metals, as stronger-binding tridentate sites are saturated<sup>40</sup>.

Acid-base titrations can be used as a measure of NOM chemical reactivity by describing its pKa distribution<sup>40</sup>. Three general binding sites are considered: acidic ( $pK_a \leq 5$ ), intermediate ( $5 < pK_a \leq 8.5$ ), and basic ( $pK_a > 8.5$ )<sup>40</sup>. NOM generally displays peaks in its pKa spectra at a pKa of approximately 3.5 (acidic region) and 10 (basic region)<sup>40</sup>. More variable peaks can be found in the intermediate region<sup>38</sup>. The acidic peaks are interpreted as carboxylic sites, basic peaks are interpreted as monodentate phenolic binding sites, and intermediate peaks are interpreted as variable types of functional groups<sup>38</sup>. Proton binding index (PBI) has been proposed by Al-reasi *et al.* as a means of relating the ratio of proton binding sites to the toxicity of metals<sup>38</sup>. PBI is described by equation 1.8.1 below, where acid, int, and base are the proton binding capacities of the acidic, intermediate, and basic binding sites respectively<sup>38</sup>.

$$PBI = \frac{int}{\left(\frac{acid+base}{2}\right)} \quad \text{equation 1.8.1}$$

High PBI values (values typically range from 0 – 1) have been linked to darker colour, greater SAC<sub>340</sub> values, and reduced Cu toxicity to freshwater *Daphnia magna*<sup>38</sup>.

## 1.9 Dissolved Organic Nitrogen

Nitrogen is a common element, and in its gaseous form, comprises 78% of the atmosphere<sup>43</sup>. Although N is essential for life, atmospheric N is inaccessible to most organisms, and must be converted to bioavailable forms through natural processes<sup>43</sup>. Inorganic ammonia and nitrate are produced through lightning, precipitation, and nitrogen-fixing organisms<sup>43</sup>. Organic N is introduced to soil through the decay of plant and animal matter<sup>43</sup>. Organic N species in aquatic

systems may be introduced via precipitation, infiltration, surface runoff, or swamps<sup>43,44</sup>. Anthropogenic inputs of organic N to aquatic environments have been increasing due to agricultural applications and wastewater effluents<sup>44-46</sup>.

In rivers and lakes DON typically accounts for 40-50% of TN (but may exceed 85%), while in estuaries, it contributes 20-90%<sup>44,46</sup>. DON that originates from agriculture or forested areas tends to contain more aromatic compounds, while municipal wastewater primarily consists of aliphatic compounds<sup>44</sup>. Waters exposed to greater anthropogenic input – be it agricultural runoff or wastewater effluent – have elevated concentrations of amino acids<sup>42</sup>. In freshwater, amino acid content ranges from 0.08-1  $\mu\text{M}$ , while in seawater it is typically 0.04  $\mu\text{M}$ <sup>42</sup>. Alanine, glycine, aspartic acid, and glutamic acid are all major amino acids present in aquatic humic substances<sup>42</sup>. These organic forms of N contain ligands capable of binding metals by donating electrons, and are partially responsible for NOM's ability to strongly bind metals<sup>45</sup>. An approximate logK value of  $4.53 \pm 1.12$  was reported by Smith *et al.* for Pb binding to amino ligands in NOM, suggesting it may be an intermediate binding site for Pb<sup>42</sup>. However, a study by Atalay *et al.* demonstrated that binding sites containing N groups are less strongly influenced by Ca and Mg competition than other functional groups present in NOM<sup>45</sup>. It is important to understand the structure and properties of DON in order to maintain the integrity of aquatic systems<sup>46</sup>.

There are currently no methods available for the direct detection of DON, however, it is practical to measure DON by subtracting the dissolved inorganic N fraction (DIN) from the total dissolved N (TDN)<sup>46,47</sup>. High-temperature catalytic oxidation (HTCO) and persulfate oxidation are commonly employed in the determination of TDN, although HTCO is more practical, as it

can simultaneously measure TOC and TN<sup>46</sup>. It is also common practice to determine ammonia with Hach kits, though they may also be used to determine both nitrate and nitrite<sup>46</sup>.

Unfortunately, this indirect means of DON determination results in cumulative analytical error and imprecise results, particularly in sources with a high DIN to DON ratio<sup>46-48</sup>. Consequently, negative DON values have been reported in literature<sup>46,48</sup>. Pretreatment methods have been successfully developed for DON analysis, however they impractical for wastewater treatment facilities, as they are either expensive or time consuming<sup>47</sup>. Fluorescence spectroscopy has shown promise as a new method for DON quantification, though further research is necessary to validate the method<sup>47</sup>.

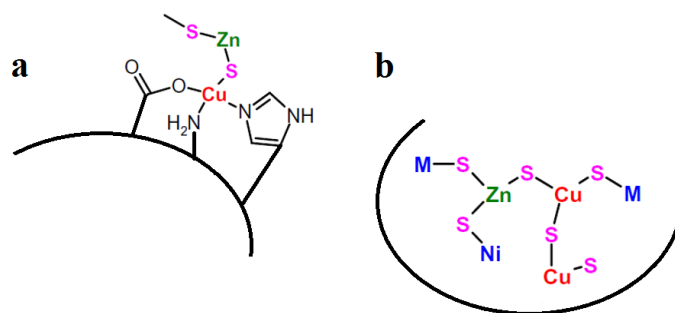
### **1.10 Chromium-Reducible Sulfide**

Elemental sulfur is present in a number of naturally occurring compounds, and can be introduced into aquatic environments through the weathering of minerals and volcanic activity<sup>49</sup>. It can also be introduced through anthropogenic sources, including the combustion of fossil fuels, surface runoff, and acid mine drainage, the latter of which is the primary source of pollution in mid-Atlantic surface waters<sup>49,50</sup>. Once it has been introduced into aquatic environments, sulfur species may undergo both biotic and abiotic processes, converting them to sulfide<sup>49</sup>.

In marine surface waters, transition metal salts catalyze the oxidation of dissolved SO<sub>2</sub> and H<sub>2</sub>S to sulfate<sup>49</sup>. However, in freshwater the reduced salt content prevents these species from readily oxidizing<sup>49</sup>. The resulting sulfate may then be reduced to sulphide by sulfur-reducing bacteria in both freshwater and deeper oceanic waters<sup>49,51</sup>.

Reduced sulphide commonly occurs in nM concentrations in freshwater, seawater, and wastewater<sup>42</sup>. Studies have found that sulphide concentrations in oxic saltwater ranges from low picomolar to nanomolar concentrations<sup>52</sup>. There is less data available for oxic freshwaters<sup>52</sup>, however, concentrations greater than those of many toxic Group B metals (<1-100 or thousands of nM) have been reported<sup>52,53</sup>. In wastewaters and anoxic environments thiolate-sulphide is higher<sup>42</sup>.

Whether it has been introduced by atmospheric deposition or bacterial activity, sulfide is then meta-stabilized by binding to soft Group B metals, particularly Cu ( $-SCu$ )<sup>33,52,53</sup>. Copper-sulphide meta-stability was successfully demonstrated by Dehnen *et al.* in 1996<sup>33</sup>. These meta-stable metal-sulphide clusters can then either be occluded by NOM, or bound to its surface<sup>53</sup>.



**Figure 1.10.1** Potential structures of metal-sulphide clusters within NOM. Black curved lines represent NOM surfaces. Metal-sulphide clusters can be either (a) NOM bound, or (b) be occluded by NOM as colloidal clusters<sup>53</sup>.

As a soft ligand, sulphide has a strong affinity for Group B metals like Pb due to its high polarizability<sup>33,42,53</sup>. There are no definite logK values for  $-SM$  or  $-SR$  ligands present in NOM, though evidence suggests  $-SCu$  may be an important binding site for waters influenced by wastewater inputs<sup>42</sup>. An approximate logK value of  $10.2 \pm 2.2$  was reported by Smith *et al.* for Pb binding to sulfide ligands in NOM, suggesting it could be a strong binding site<sup>42</sup>. Toxicological studies have found that ZnS clusters reduce the toxicity of Ag(I) to freshwater *Daphnia*

*magna*<sup>42,53</sup>. This could have significant industrial implications, as current metal regulations do not consider the presence of sulphide ligands<sup>52</sup>.

The current methods for determining sulfide ligands either measure an acid-volatile sulphide (AVS) fraction, or total sulfide<sup>52</sup>. Total sulphide measurement is preferable to AVS as AVS incompletely detects some metal sulphide species, including Cu (the most abundant) and Pb sulfides, while total sulfide measurement does not<sup>52</sup>. Total sulfide is estimated using the chromium-reducible sulphide (CRS) method, which relies on a redox reaction between Cr(II) and metal-bound sulphides<sup>52</sup>. It has been well established for total sulphide determination in sediment, and neither thiols nor sulfates interfere<sup>52</sup>. However, mixed findings have been reported for oxic waters<sup>52,54</sup>. Sulfate was shown to interfere with sulfide measurement in oxic freshwaters by Mylon *et al.*, though Kramer *et al.* found that it did not interfere for either oxic freshwater and seawater sources<sup>52,54</sup>. CRS is known to detect metal disulfides, polysulfides, elemental sulfur, thiosulfate, and sulfites<sup>42</sup>. If sulfates were to cause an interference, it could pose a significant problem with sulfide detection<sup>54</sup>.

### 1.11 Thiol Assay Kits

Organic thiols are a type of reduced S-ligand present in NOM, and are produced naturally within organisms throughout their life cycle<sup>55–57</sup>. Thiol exists primarily as cysteine and glutathione within the organism, and is thought to provide a means of metal transport and mitigating metal toxicity<sup>57</sup>. Typical concentrations range from pM to  $\mu\text{M}$ ; concentrations of 0.70–3.60 nM were reported by Radwan *et al.* in the Western North Sea and English Channel coastal areas<sup>56,57</sup>. Radwan *et al.* also found that thiols in estuarine environments did not originate from freshwater sources, but rather from marine phytoplankton<sup>56</sup>.



Thiol groups in NOM are known to bind soft metals, and could significantly impact their speciation<sup>55-57</sup>. In NOM itself however, the forms of thiol and its' interactions are not well known, and methods for its quantification are limited<sup>55,57</sup>. This is in part due to the complications associated with its quantification, including its' low concentrations, susceptibility to air oxidation (which poses a problem for stored NOM samples), and analogous spectroscopic properties<sup>55,57,58</sup>. Current available methods include electrochemical, X-ray absorption (XAS), optical spectroscopic, and mass-spec analysis<sup>55,59</sup>. XAS has a high detection limit ( $\mu\text{M}$ ) and electrochemical methods have not been directly applied for NOM<sup>55</sup>. However, optical spectroscopic methods can provide low detection limits if chemical labelling is used<sup>55,59</sup>. Fluorescent chemical tags like monobromo(trimethylammonio)-biamine (qBBR) have been utilized in NOM with success<sup>55,59</sup>.

### **1.12 Ion-Selective Electrode**

Ion-selective electrodes (ISE's) function by responding to an ion of interest selectively<sup>60</sup>. This is made possible by a thin membrane containing a ligand that selectively binds only to the ion of interest<sup>60</sup>. In the case of solid-state ISE's the membrane is composed of an inorganic crystal<sup>60</sup>. In liquid-membrane ISEs, the membrane utilizes a hydrophobic organic polymer doped with an ion-exchanger<sup>61</sup>. The Pb ISEs used were examples of solid-state and liquid membrane ISEs.

The solid-state ISE used in this research utilized a membrane composed of  $\text{Ag}_2\text{S}$  doped with  $\text{PbS}$ <sup>60</sup>. The crystal can be cleaved to expose a layer of sulfide ions, which binds free lead in solution<sup>60</sup>. Lead ions can then travel through the membrane in the direction of lower Pb concentration, resulting in a difference in charge across the surface of the membrane<sup>60</sup>. This difference in charge can be measured as an electric potential, and is dependent on the

concentration of free lead in solution. The potential is measured against a reference electrode. The measured electric potential can then be converted to concentration using the Nernst equation:

$$E = E^o - \frac{RT}{nF} \ln(Q) \quad \text{Equation 1.12.1}$$

Where E is the measured potential,  $E^o$  is the reference potential, R is the ideal gas constant (in  $\text{J mol}^{-1} \text{K}^{-1}$ ), T is the temperature (in K), n is the charge of the ion of interest, F is Faraday's constant (in C/mol), and Q is the reaction quotient.

The solid-state ISE used here is reported to have a detection limit of 0.2 ppm total Pb at pH 7<sup>62</sup>. However, in well buffered systems, lower detection limits have been observed<sup>13</sup>. The basis for the Pb electrode is comparable to that of the Cu ISE used by Tait *et al.*, thus lower detection limits may be possible.

### 1.13 Research Goals and Objectives

NOM can be described both in terms of its quantity (concentration) and quality. The question posed by this study was whether NOM quantity accurately explains NOM reactivity with metals, or if there are differences between sources that cannot be explained by quantity alone. If indeed quantity does not completely describe reactivity of various NOM sources, then they can be said to be of different quality. This quality can be interpreted in terms of optical properties, functional group chemistry, and metal reactivity. In order to quantify the quality of NOM sources, the objectives of this research were to:

- I. Characterize NOM sources of varying composition and origin in terms of their functional group content using fluorescence and absorbance spectroscopy, acid-base titrations, HTCO/Hach kits, CRS, and thiol assay measurements.
- II. Validate the commercially available Pb ISE as a practical and effective method for determining trace level Pb speciation in freshwater and saltwater environments.
- III. Determine Pb speciation in various NOM sources using a commercially available Pb ISE, compare it to Pb speciation modelled by the Windermere Humic Aqueous Model (WHAM), determine if WHAM accurately predicts Pb speciation, and deduce which NOM characteristics best explain Pb-NOM binding.

The results for objectives I, II, and III can be found in sections 2.3 and 3.3.

## 1.14 Significance of Research

Trace metals play an important role in aquatic systems. While some trace metals are essential for aquatic life, other metals may cause toxicity to biota if present in high enough concentrations<sup>29</sup>. Lead is one such metal, and can be found as a variety of different species in aquatic environments<sup>3</sup>. These different species display different mechanisms and levels of toxicity<sup>3</sup>. This makes speciation and the factors that influence it important to address when developing and implementing regulations, as water chemistry varies greatly with source<sup>29</sup>. NOM is one parameter affecting speciation and therefore toxicity, however, there is currently a limited understanding of its interactions with metals<sup>42</sup>.

It is widely accepted that metal regulations should account for differences in water chemistry, including the quantity and quality of organic material<sup>30</sup>. However, current regulations do not consider the effects of important (and potentially strong-binding) functional groups present in NOM<sup>42</sup>. Effective methods for approximating the concentrations of these binding sites could have significant industrial implications, as these ligands have the potential to reduce Pb bioavailability and toxicity. If a relationship can be found between N, O, or S ligand concentration and Pb speciation, then the resulting data could be used to: validate or disprove WHAM predictions, or develop freshwater or saltwater BLMs that could better predict Pb bioavailability. This research will aid in the development and implementation of freshwater and saltwater BLM's for Pb in order to achieve site-specific water quality guidelines. This could have significant industrial implications, as current regulations do not account for specific binding sites such as N, O, and S functional groups<sup>52</sup>.

## 1.15 References

- (1) Pattee, Oliver H.; Pain, D. J. In *Handbook of Ecotoxicology*; Hoffman, David J.; Rattner, Barnett A.; Burton, Allen G. Jr.; Cairns, J. J., Ed.; Lewis Publishers, 2003; p 374.
- (2) Sarkar, B. *Heavy Metals in the Environment*; Marcel Dekker, 2002.
- (3) Mager, E. M. In *Fish Physiology - Homeostasis and Toxicology of Non-Essential Metals*; Wood, Chris M.; Farrell, Anthony P.; Brauner, C. J., Ed.; 2011; pp 186–225.
- (4) CSIR. *Water Quality Guidelines for Coastal Marine Waters Natural South African Water Quality Guidelines*; 1995; Vol. 1.
- (5) Lewis, A.G; Cave, W. . In *Oceanography and Marine Biology*; Barnes, H., Ed.; 1982; pp 471–695.
- (6) Kitman, J. . *The Nation* **2000**, 270 (11), 11.
- (7) Nriagu, Jerome O.; Pacyna, J. M. *Nature* **1988**, 333, 134–139.
- (8) Zirino, A. *Limnol. Oceanogr.* **1972**, 17 (5), 661–671.
- (9) Tellis, M. S.; Lauer, M. M.; Nadella, S.; Bianchini, A.; Wood, C. M. *Aquat. Toxicol.* **2014**, 146, 220–229.
- (10) Sillanpaa, M. E. T. *Natural Organic Matter in Water: Characterization and Treatment Methods*, 1st ed.; 2015.
- (11) McKnight, D. M.; Boyer, E. W.; Westerhoff, P. K.; Doran, P. T.; Kulbe, T.; Andersen, D. T.; E. W. Boyer; P. K. Westerhoff; P. T. Doran; T. Kulbe; Anderson, D. T. *Limnol. Oceanogr.* **2001**, 46 (1), 38–48.
- (12) Tipping, E.; Hilton, J.; Freshwater, B. J.; Association, B.; Tipping, E.; Hilton, J.; James, B. *Freshw. Biol.* **1988**, 19, 371–378.
- (13) Tait, T. N. Determination of Copper Speciation, Bioavailability and Toxicity in Saltwater

- Environments, Wilfrid Laurier University, 2013.
- (14) Thurman, E. M. *Oceanic Geochemistry of Natural Waters*; 1985.
  - (15) Reiley, M. C. *Aquat. Toxicol.* **2007**, 84, 292–298.
  - (16) Hudson, N.; Reynolds, D. *River Res. Appl.* **2007**, 23 (6), 631–649.
  - (17) Abbasse, G. A.; Uddane, B. O.; Ischer, J. C. F. *Anal. Sci.* **2003**, 19 (4), 529–535.
  - (18) UNEP. *Final review of scientific information on lead*; 2010.
  - (19) DePalma, S. G. S.; Arnold, W. R.; McGeer, J. C.; Dixon, D. G.; Smith, D. S. *Appl. Geochemistry* **2011**, 26 (3), 394–404.
  - (20) Di Toro, Dominic M; Allen, H. E.; Bergman, H. L.; Meyer, J. S.; Paquin, P. R.; Santore, R. C. *Environ. Toxicol. Chem* **2001**, 20 (10), 2383–2396.
  - (21) Paquin, P. R.; Santore, R. C.; Wu, K. B.; Kavvasdas, C. D.; Di Toro, D. M. *Environ. Sci. Policy* **2000**, 3 (Supplement 1), 175–182.
  - (22) Hatano, A.; Shoji, R. *Comp. Biochem. Physiol. Part C* **2010**, 151 (1), 25–32.
  - (23) Arche, Ghent University, K. and I. *Lead BLM SSD Normalization Tool*; Vol. Version 1.
  - (24) CCME. In *Canadian Water Quality Guidelines*; Sheffer, M. G., Chong Chu, B., Andrews, J., Windle, W., Eds.; CCME, 2008.
  - (25) USEPA. *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection Of Aquatic Organisms and Their Uses*; Washington, DC, 1985.
  - (26) CEC. *Proposal for a Directive of the European Parliament and of the Council on Environmental Quality Standards in the Field of Water Policy and Amending Directive 2000/60/EC*; Brussels, 2006.
  - (27) ANZECC. *Australian and New Zealand Guidelines for Fresh and Marine Water Quality*; Canberra, 2000.

- (28) CSIR. *South African Water Quality Guidelines*, 1st ed.; Department of Water Affairs and Forestry: Pretoria, 1996; Vol. 7.
- (29) Nys, C.; Janssen, C. R.; Mager, E. M.; Esbaugh, A. J.; Brix, K. V.; Grosell, M.; Stubblefield, W. A.; Holtze, K.; De Schamphelaere, K. A. C. *Environ. Toxicol. Chem.* **2014**, 33 (2), 394–403.
- (30) De Schamphelaere, K. A. C.; Nys, C.; Janssen, C. R. *Aquat. Toxicol.* **2014**, 155, 348–359.
- (31) Korak, J. A.; Dotson, A. D.; Summers, R. S.; Rosario-ortiz, F. L. *Water Res.* **2013**, 49, 327–338.
- (32) Chen, W.; Westerhoff, P.; Leenheer, J. A.; Booksh, K. *Environ. Sci. Technol.* **2003**, 37, 5701–5710.
- (33) Depalma, S. G. S. Characterization of Dissolved Organic Matter and Reduced Sulfur in Coastal Marine and Estuarine Environments : Implications for Protective Effects on Acute Copper Toxicity by, University of Waterloo, 2009.
- (34) Kritzberg, E. S.; Ekstrom, S. M. *Biogeosciences* **2012**, 9, 1465–1478.
- (35) Al-Enezi, G.; Hamoda, M.; Fawzi, N. *J Env. Sci Heal. A Tox Hazard Subst Env. Eng* **2004**, 39 (2), 455–464.
- (36) Ohno, T. *Environ. Sci. Technol.* **2002**, 36 (4), 742–746.
- (37) Wood, C. M.; Al-Reasi, H. A.; Smith, D. S. *Aquat. Toxicol.* **2011**, 105 (3–4 SUPPL.), 3–8.
- (38) Al-reasi, H. A.; Wood, C. M.; Smith, D. S. *Environ. Int.* **2013**, 59, 201–207.
- (39) Schwartz, M. L.; Curtis, J. P.; Playle, R. C. *Environ. Toxicol. Chem.* **2004**, 23 (12), 2889–2899.
- (40) Al-reasi, H. A.; Wood, C. M.; Smith, D. S. .
- (41) Smith, D. S.; Kramer, J. R. *Environ. Int.* **1999**, 25 (2–3), 307–314.

- (42) Smith, D. S.; Bell, R. A.; Kramer, J. R. *Comp. Biochem. Physiol. Part C* **2002**, 133, 65–74.
- (43) Scholars, P.; Biology, C.; Board, E.; Society, E.; Society, E.; Av-, M. **2010**.
- (44) Czerwionka, K. *Oceanologia* **2016**, 58 (1), 39–45.
- (45) Atalay, Y. B.; Di Toro, D. M.; Carbonaro, R. F. *Geochim. Cosmochim. Acta* **2013**, 122, 464–477.
- (46) Wang, Q.; Hua, B.; Yang, J.; Liu, F.; Zhu, G.; Deng, B. *J. Chem.* **2015**, 2015.
- (47) Hu, H.; Ren, H. *Chemosphere* **2016**, 164, 299–303.
- (48) Chen, B.; Westerhoff, P.; Zhang, L.; Zhu, A.; Yang, X. I. N.; Wang, C. **2015**, 249–276.
- (49) Kellogg, A. W. W.; Cadle, R. D.; Allen, E. R.; Lazrus, A. L.; Martell, E. A. *Science* (80-). **2016**, 175 (4022), 587–596.
- (50) USEPA. Abandoned Mine Drainage.
- (51) Huisman, J. L.; Schouten, G.; Schultz, C. *Hydrometallurgy* **2006**, 83 (1–4), 106–113.
- (52) Bowles, K. C.; Ernste, M. J.; Kramer, J. R. *Anal. Chem. Acta* **2003**, 477 (October 2002), 113–124.
- (53) Kramer, J. R.; Bell, R. A.; Smith, D. S. *Appl. Geochemistry* **2007**, 22 (8 SPEC. ISS.), 1606–1611.
- (54) Mylon, S. E.; Hu, H.; Benoit, G. *Anal. Chem.* **2002**, 74 (3), 661–663.
- (55) Rao, B.; Simpson, C.; Lin, H.; Liang, L.; Gu, B. *Talanta* **2014**, 119, 240–247.
- (56) Al Farawati, R.; Van Den Berg, C. M. G. *Am. Chem. Soc.* **2001**, 35 (10).
- (57) Joe-wong, C.; Shoenfelt, E.; Hauser, E. J.; Crompton, N.; Myneni, S. C. B. *Environ. Sci. Technol.* **2012**, 46 (18), 9854–9861.
- (58) Appathurai, V.; Mopper, K. *Anal. Chem. Acta* **1990**, 236, 363–370.



- (59) Mangal, V.; Guéguen, C. *Anal Bioanal Chem* **2015**, 407, 4305–4313.
- (60) Harris, D. C. *Quantitative Chemical Analysis*, 7th ed.; W.H. Freeman and Company: New York, 2007.
- (61) Ross, J. W. In *Ion-Selective Electrodes*; Durst, R. A., Ed.; (WA): Department of Commerce National Bureau of Standards: Washington, DC, 1969; pp 57–88.
- (62) Thermo Scientific. *User Guide Lead Ion Selective Electrode*; Thermo Fischer Scientific Inc., 2008.

## **Chapter 2 – Characterizing NOM by its Optical and Chemical Properties**

## 2.0 Abstract

Natural organic matter (NOM) is ubiquitous in aquatic environments. Different sources of organic matter with varying origin have been shown to possess different optical and chemical properties. These chemical differences are of great industrial and environmental significance, as they are able to govern metal speciation, and consequently also their bioavailability. This study aimed to characterize a variety of NOM sources of differing origin using optical properties that have been well established in the literature (fluorescence and absorbance spectroscopy), as well as the less extensively studied functional group chemistry. Organic matter sources were characterized using: total organic carbon/dissolved organic carbon (TOC/DOC), fluorescence excitation-emission matrices (FEEM), parallel factor analysis (PARAFAC), fluorescence index (FI), specific absorption coefficient ( $SAC_{340}$ ), chromium-reducible sulfide (CRS), thiol, dissolved organic nitrogen (DON), and proton binding index (PBI). These methods allowed for a quantification of organic carbon; humic acid-, fulvic acid-, tyrosine-, and tryptophan-like components; origin; aromaticity; sulfide ligands; nitrogen ligands; and oxygen ligands.  $SAC_{340}$ , FI, %HA, %FA, %Trp, %Tyr, CRS, thiol, DON, and PBI values ranged from: 7.76 – 40.84, 1.04 – 1.84, 46.41 – 82.41%, 13.32 – 39.21%, 1.02 – 16.21%, 1.34 – 14.99%, 2.03 – 89.0 nmol/mgC, 71.8 – 186.5 nmol/mgC, 35.76 – 253.8  $\mu$ gN/mgC, and 0.33 – 1.72 respectively. No one parameter, or simple series of parameters was able to discriminate NOM sources. However, CRS, Trp, and Tyr were all present in significant quantities (outside a factor of four) in saltwater sources, and are capable of discriminating saltwater from freshwater sources.  $SAC_{340}$ , CRS, thiol, DON, Trp, Tyr, and PBI are all essential for discriminating between freshwater NOM sources. Sources of terrestrial origin had significant  $SAC_{340}$  and PBI, while sources of microbial origin had significant CRS, DON, Trp, and Tyr. Although significant variability (not explained

by a factor of four) in NOM quality was demonstrated, metal speciation data is needed in order to determine if this variation is of environmental importance.

## **2.1 Introduction**

Natural organic matter (NOM) is ubiquitous in aquatic environments and plays an important role in the fate and speciation of metals<sup>1,2</sup>. It can have organic matter that is terrestrially (allochthonous) or microbially (autochthonous) derived, and has different chemistry depending on its origin<sup>1,2</sup>. Terrestrially derived NOM originates from plant matter decay, typically contains more humic and fulvic acids, and is darker in colour due to its greater aromatic and phenolic content<sup>1-4</sup>. Microbially derived NOM is the by-product of bacteria, algae, and aquatic plants/animal matter decay within the water column<sup>1</sup>. It tends to have greater proteinaceous content, amino acid content, be lighter in colour, and have lower aromatic and phenolic content<sup>2-4</sup>. Total organic carbon (TOC) has been used as a means of quantifying NOM, and can range from as low as 0.5 mgC/L in the open ocean, to 60 mgC/L in freshwater swamps, marshes, and bogs<sup>2</sup>. However, the quality of this organic matter can also vary depending on its source<sup>2</sup>.

In general, larger water bodies contain NOM that is more microbial in origin, while smaller water bodies contain more terrestrial NOM<sup>2</sup>. When comparing freshwater to saltwater environments, the open ocean contains more microbial NOM (which decreases with depth), smaller freshwater bodies contain more terrestrially derived NOM, and estuaries possess intermediate qualities<sup>2</sup>. These different sources of NOM contain different types and concentrations of functional groups which are capable of binding metals and reducing their bioavailability<sup>1,2</sup>. Much work has been done in an attempt to describe NOM, however, the available data on DOM functional properties is not representative of DOM or its components<sup>5,6</sup>.

There is no one property that adequately describes the variation in NOM sources, thus a method for systematically characterizing the variability in NOM functional chemistry would be valuable<sup>55</sup>.

The “NOM Typing Project” was one of the most extensive studies on the properties of NOM, however, it was intended to compare properties relevant to water treatment<sup>6,7</sup>. The investigated sources were all allocthonous and of limited geographical range, from the same climate, and ecological region<sup>6,7</sup>. Only freshwater sources without industrial or agricultural input were chosen<sup>7</sup>. Thacker *et al.* described a series of functional assays that could be used to characterize NOM, but saltwater and sewage sources were not included, and specific NOM-binding sites (such as N and O functional groups) were not quantified<sup>6</sup>. They found that the results for three of the eleven assays could be explained by analytical error, and that some of DOM’s functional properties can be predicted using simpler optical methods<sup>6</sup>.

Fluorescence and absorbance spectroscopy have received widespread attention for being an efficient and effective means of characterizing NOM. The fluorophores present in NOM have potentially unique excitation and emission wavelengths, and can be used to identify the components present based on their fluorescence excitation-emission (FEEM) spectra<sup>8,5,9</sup>. Humic- and fulvic-like components can be detected at Ex/Em wavelengths of 250-390 nm/460-520 nm, and 300-350 nm/400-450 nm respectively<sup>9</sup>. Similarly, tryptophan- and tyrosine-like components can be detected at Ex/Em wavelengths of 225-275 nm/350 nm, and 225-275 nm/300 nm respectively<sup>9</sup>. Parallel factor analysis (PARAFAC) can then be used in order to determine the presence and concentrations of the various components of NOM<sup>10</sup>. Other indices and statistical methods including: fluorescence index (FI), humification index (HIX), and specific absorbance coefficient (SAC), analysis of variance (ANOVA), and partial least squares regression (PLS),

have also been used to describe the origin, humic acid content, aromaticity, and composition of NOM<sup>11,5,12,33–35</sup>. The specific absorbance coefficient at 340 nm (SAC<sub>340</sub>) has been directly correlated with Pb toxicity to freshwater rainbow trout by Schwarz *et al.*, with greater values indicating reduced toxicity<sup>13</sup>. Though valuable, optical properties like SAC and FI may not provide a universal and definitive explanation of all NOM and its interactions<sup>5,12,14</sup>. For example, in natural waters, silver primarily binds to S(-II) ligands, which are not quantified by previously described optical properties<sup>15,16</sup>. Not all components of NOM are coloured and/or fluorescent<sup>17</sup>. It could therefore be of great value to utilize robust methods for the detection of functional groups present in NOM, so that source-dependent chemistry may be better described and used to explain source-dependent NOM-binding.

Once thought to be of little significance due to their poor stability in oxic environments, reduced sulfide species are now known to be meta-stabilized as metal-sulfide complexes, and are present at concentrations comparable to those of many toxic Group B metals (<1-100 or thousands of nM)<sup>18</sup>. Due to its high polarizability, sulfide has a strong affinity for Group B metals like Pb, and may be important to include in speciation modelling<sup>9,16,18</sup>. Total sulfide can be approximated via the chromium reducible sulfide (CRS) method, which has been well established for use in sediment, though mixed results have been reported for oxic waters<sup>19,20</sup>.

Organic reduced S-ligands are also present in NOM in the form of thiols. Their concentrations range from pM to  $\mu$ M. Concentrations ranging 0.70 – 3.60 nM were reported by Radwan *et al.* in the Western North Sea and English Channel coastal areas<sup>21,22</sup>. Currently there is a limited understanding of the forms and interactions of thiol, and quantification methods are limited<sup>22,23</sup>. However, optical spectroscopic methods that utilize chemical labels (such as qBBr) can provide low detection limits and have been successfully used in NOM<sup>23,24</sup>.

Although they are weaker binding sites (Smith *et al.* reported a logK value of  $2.55 \pm 1.12$  for Pb binding to carboxyl groups) for Group B metals, oxygen-containing functional groups are the most abundant in DOM and may be of great significance<sup>2,10,16</sup>. Oxygen functional groups account for ~16.5% of DOC<sup>16</sup>. Carboxyls and phenols are the most abundant of the O– functional groups, accounting for 2-9% and 2-6% respectively<sup>16</sup>. These weaker-binding sites become particularly important at higher concentrations of metals, when stronger-binding sites are saturated<sup>25</sup>. Potentiometric acid-base titrations are able to quantify these O– functional groups by relating the pKa distribution of NOM to its binding sites<sup>25</sup>. Three general binding sites are considered: acidic ( $pK_a \leq 5$ ), intermediate ( $5 < pK_a \leq 8.5$ ), and basic ( $pK_a > 8.5$ )<sup>25</sup>. Peaks generally appear in the pKa spectra at a pKa of ~3.5 (acidic region) and ~10 (basic region)<sup>25</sup>. More variable peaks can be found in the intermediate region<sup>10</sup>. Acidic peaks are interpreted as carboxylic sites, basic peaks are interpreted as phenolic sites, and intermediate peaks are interpreted as variable types of functional groups<sup>10</sup>. As proposed by Al-reasi *et al.*, proton binding index (PBI) can then be used to describe the ratio of stronger-binding intermediate sites, to weaker binding sites<sup>10</sup>. PBI values range from 0 – 1, and has been correlated to  $SAC_{340}$  values, Cu toxicity to freshwater *Daphnia Magna*<sup>10</sup>. The equation for PBI is given below, where acid, int, and base are the proton binding capacities of the acidic, intermediate, and basic binding sites respectively<sup>10</sup>.

$$PBI = \frac{int}{\left(\frac{acid+base}{2}\right)} \quad \text{equation 2.1.1}$$

Like S– and O– ligands, N– containing functional groups also have strong affinities for hard metals<sup>16,26</sup>. In the case of N-containing functional groups, Ca and Mg competition for NOM binding are less significant, increasing the likelihood of dissolved organic nitrogen (DON) content impacting metal speciation in natural waters<sup>26</sup>. In freshwater rivers and lakes DON may

exceed 85% of total N (TN), but typically contributes 40-50%<sup>27,28</sup>. In estuaries, DON accounts for 20-90% of TN<sup>27,28</sup>. However, due to agriculture and wastewater effluents, anthropogenic input of organic N has been increasing<sup>26-28</sup>.

There are currently no direct methods for the determination of DON – it is measured by subtracting the dissolved inorganic N fraction (DIN) from the total dissolved N (TDN)<sup>28,29</sup>. As a result of cumulative analytical error results are imprecise (with negative values having been reported), especially in the case of a high DIN/DON ratio<sup>28-30</sup>.

This study has characterized different NOM sources by measuring both optical and chemical properties of NOM. More specifically, optical methods include fluorescence and absorbance spectroscopy, with the following data reported: SAC<sub>340</sub>, FI, FEEM, and PARAFAC. Chemical methods include chromium-reducible sulfide (CRS), thiol (via commercially available thiol assay kits), dissolved organic nitrogen (DON), and proton binding index (PBI).

## **2.2 Methods**

### *2.2.1 Sample Collection*

Sample collection details can be seen in table 2.2.1 below. Four seawater samples from Barbara Tufts Playground (Rhode Island), 80 Elm Street (Groton, CT), and the Audubon Coastal Center (Milford Point, CT) at high tide and low tide, were collected by grab sampling in July 2015. Samples were pumped through a 1µm filter (String Wound Catridge filter, Hamburg, NY, USA) and into 2 L Nalgene bottles. The Nalgene bottles were acid washed prior to collection by soaking in 10% trace metal grade nitric acid for 24 h (Sigma Aldrich, Oakville, ON). Samples were then transported to Wilfrid Laurier University in a cooler, where they were transferred to 4 L polyethylene bottles, stored at 4°C under Ar, and kept in the dark until further analysis.



Freshwater samples were collected from the Amazon Rio Negro<sup>31</sup>, Luther Marsh<sup>31,32</sup> (Grand Valley, ON), Bannister Lake<sup>31,32</sup> (Ayr, ON), Preston sewage effluent<sup>32</sup> (Preston, ON), Lake Ontario<sup>32</sup> (Burlington, ON), and the Grand River (Kitchener, ON). Samples were collected using a method previously described in the literature<sup>31,32</sup>. Samples were pumped through a 1µm filter (String Wound Catridge filter, Hamburg, NY, USA) and into 20L Reliance Aqua Pak Water Containers (which were pre-rinsed with DI water and sample three times). The pre-filtered samples were concentrated by reverse osmosis (RO) (Sun *et al.*, 1995), resonated with sulfuric acid and cation exchange resin (AG50W-X8, H<sup>+</sup> form, Biorad, Richmond, CA, USA), and stored in polypropylene bottles at 4°C. Two commercially available aquatic NOM sources – Suwannee River and Nordic Reservoir (International Humic Substances Society, St. Paul, MN, USA) – were included as reference sources and used without treatment.

**Table 2.2.1** Locations and Origin of NOM Sources used in Characterization Analysis.

| Source                           | Abbreviation | Location                                   | Type                             |
|----------------------------------|--------------|--|----------------------------------|
| Suwannee River                   | SR           | –  | Terrestrial                      |
| Nordic Reservoir                 | NR           | –  | Terrestrial                      |
| Amazon Rio Negro                 | AM           | 3°5'41.5"S 60°21'19.6"W                    | Terrestrial                      |
| Luther Marsh                     | LM           | 43°37'N 80°26'W                            | Terrestrial                      |
| Bannister Lake*                  | BL*          | 43°30'N 80°38'W                            | Microbial                        |
| Preston Sewage Effluent*         | PR*          | 43°39'N 80°35'W                            | Sewage input                     |
| Lake Ontario                     | LO*          | 43°29'N 79°79'W                            | Microbial                        |
| Grand River*                     | GR*          | <b>43°25'20.0"N</b><br><b>80°24'43.9"W</b> | Terrestrial and sewage inputs    |
| Barbara Tufts Playground*        | BTP*         | 41°39'30.9"N 71°26'51.7"W                  | Sewage input                     |
| Elm St.                          | ELM          | 41°19'46.4"N 71°59'26.8"W                  | Microbial                        |
| Audubon Coastal Center Low Tide  | CCLT         | 41°10'34.7"N 73°06'4.2"W                   | Terrestrial input                |
| Audubon Coastal Center High Tide | CCHT         | 41°10'34.7"N 73°06'4.2"W                   | Terrestrial and microbial inputs |

### 2.2.2 Fluorescence and Absorbance Characterization

Prior to optical analysis, all sources were filtered through a 0.45µm polyethersulfone syringe filter (Whatman, Germany), diluted to approximately 5-10 mgC/L, and the pH was adjusted to circumneutral (pH 6 – 8 ± 0.1). Fluorescence and absorbance of the samples were measured in a 1 cm pathlength quartz cuvette using a Varian Cary Eclipse Fluorescence Spectrophotometer (Varian, Mississauga, ON) and Varian Cary 50 UV/VIS Spectrophotometer (Varian, Mississauga, ON) respectively.

For fluorescence analysis, the excitation wavelengths were set to 200-450 nm in increments of 10 nm, and the monochromator slit widths for the excitation and emission were both set to 5 nm. The photomultiplier tube was set to high sensitivity (800 V), and the resulting emission wavelengths were measured from 250 – 600 nm in 1 nm intervals.

In order to correct for inner-filtering effects, and calculate SAC<sub>340</sub> values, the absorbance of samples was measured from 250 – 600 nm. SAC<sub>340</sub> was calculated using equation 2.2.21<sup>13</sup>.

$$SAC_{340} = \frac{2.303 Abs_{340}}{[DOC]} \quad \text{equation 2.2.21}$$

Where Abs<sub>340</sub> is the sample absorbance at 340 nm, and [DOC] is the sample DOC concentration in mgC/L. If the absorbance at 254 nm was greater than 0.3, inner-filtering corrections were necessary for fluorescence spectra, and corrected values were calculated using equation 2.2.22<sup>33</sup>.

$$F = F_0(10^{-b(A_{Ex}+A_{Em})}) \quad \text{equation 2.2.22}$$

Where F is the corrected fluorescence intensity, F<sub>0</sub> is the measured fluorescence intensity, b is the pathlength (1 cm), A<sub>Ex</sub> and A<sub>Em</sub> are the absorbance values at the excitation and emission wavelengths respectively.

Fluorescence index was calculated using equation 2.2.23 in order to determine the origin of the various organic matter sources<sup>4</sup>.

$$FI_{Ex370} = \frac{Em_{450}}{Em_{500}} \quad \text{equation 2.2.23}$$

Where Ex370 is the fluorescence index at an excitation wavelength of 370 nm, Em<sub>450</sub> is the emission intensity at an emission wavelength of 450 nm, and Em<sub>500</sub> is the emission intensity at an emission wavelength of 500 nm.

MATLAB<sup>TM</sup> was used to generate FEEMs and remove Rayleigh peaks when Em=Ex and Em=2Ex for each source, which prevents optical interferences in subsequent PARAFAC analysis. FEEM data was resolved to four components using an in-house MATLAB<sup>TM</sup> PARAFAC code and compared to humic and fulvic acids, tryptophan, and tyrosine standard spectra in order to determine the concentrations of each component in the various sources<sup>8,34</sup>.

### 2.2.3 Chromium-Reducible Sulfide Characterization

The CRS method used was adapted from the method described by Bowles *et al*<sup>19</sup>. Degassed Milli-Q® water was prepared by purging Ar gas through an air stone for > 30 min, and was used for all sample preparation throughout the procedure. A 0.0312 M Na<sub>2</sub>S stock solution was prepared under Ar by diluting 1.873 g of Na<sub>2</sub>S · 9H<sub>2</sub>O and 5 mL of 10 M NaOH to 250 mL. This stock solution was stored in the dark and under Ar to prevent light or air oxidation.

Mixed diamine reagent (MDR) was prepared by mixing two parts, A and B, in a 1:1 ratio. Part A was prepared by dissolving N,N-dimethyl-*p*-phenylenediamine oxalate (Baker) in 50% v/v HCl to obtain a concentration of 8 mM. Part B was prepared by dissolving FeCl<sub>3</sub> (BDH) in v/v HCl to obtain a concentration of 8 mM.

A silver sulfide electrode was polished and preconditioned by gently swirling the membrane in an aluminum oxide (< 10 micron, 99.7%, Sigma Aldrich, St. Louis, MO) paste until the membrane surface was reflective, then storing it in Na<sub>2</sub>S stock solution. It was

subsequently used to standardize the sulfide stock solution by titrating 25mL of stock with  $\text{Cu}^{2+}$  standard (Assurance grade, SPEXCertiPrep, New Jersey). The standardized stock was then used to prepare a series of dilutions: 10, 100, 500, and 1000 nM  $\text{Na}_2\text{S}$ . These standards were treated in the same manner as NOM samples using a purge and trap system.

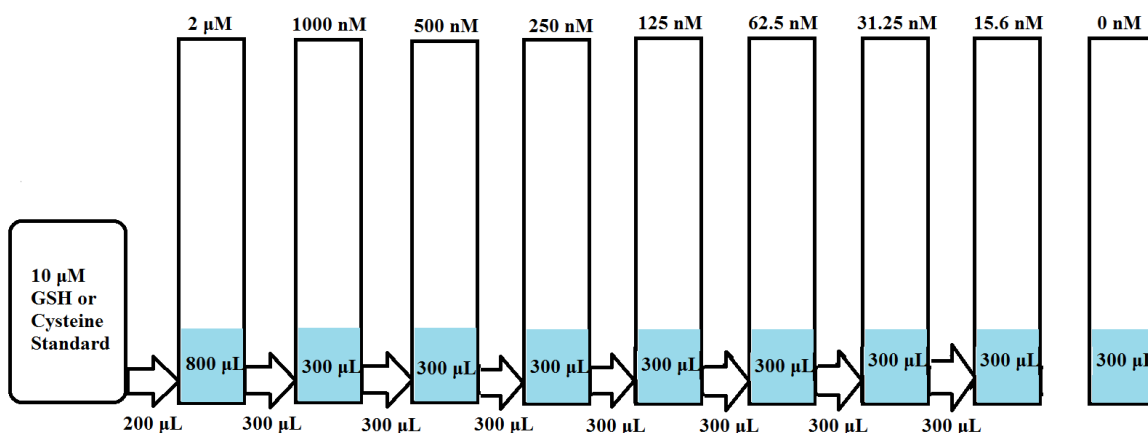
Under an Ar atmosphere, ~0.62 g of  $\text{CrCl}_2$  (99.99%, Sigma Aldrich, St. Louis, MO) was added to the reaction tube. 30mL of sample (NOM sources were diluted to ~50-100 mgC/L) was added and the system was purged with Ar at a flow rate of 65 mL/min for 5 min. 5mL of 50% v/v HCl was injected into the septum in order to commence the reaction. After a 30 min reaction period, the trapping solution was transferred to a vial and combined with 5mL of Milli-Q (used to rinse trapping tube). Reagent blanks were prepared by diluting 10mL of trapping solution with 5 mL of Milli-Q. 0.5 mL of MDR was added to the samples and reagent blanks, which were then allowed to develop in the dark for ~3 hours before measurement. After colour development, the absorbance was measured at 610.1 nm in a 10 cm pathlength quartz cuvette and compared to the reagent blank. To calibrate, CRS concentrations for the standards were plotted against values measured by ISE. The equation of the line could then be used to determine sulfide concentrations in the NOM sources.

#### *2.2.4 Thiol Characterization via Assay Kits*

Prior to optical analysis, all sources were filtered through a 0.45 $\mu\text{m}$  polyethersulfone syringe filter (Whatman, Germany). Freshwater concentrates were diluted to both ~10mgC/L and ~25mgC/L, while saltwater grab samples were measured undiluted. Thiol analysis for sources: SR, NR, LM, AM, and BL\* was conducted using a Sigma® fluorometric thiol assay kit. Analysis for the saltwater sources: BTP\*, ELM, CCLT, and CCHT was conducted with a

Cayman Chemical® thiol detection assay kit. Prior to analysis, thiol assay kits were stored in the dark at -20°C.

An assay buffer was prepared by diluting 5mL of assay buffer concentrate to 50mL with Milli-Q® water. Fluorometric detector was prepared by diluting 50µL of provided detector to 5mL in Milli-Q® water. Glutathione standard was prepared by dissolving 1 mg of glutathione in 3.25mL of prepared assay buffer, and further diluting this to 10µM by diluting 10 µL of standard to 1 mL with assay buffer. Calibration standards were prepared by serial dilution as seen in figure 2.2.41.



**Figure 2.2.41** Serial dilution of glutathione calibration standards for fluorometric thiol detection. Volumes of assay buffer used to dilute standards are indicated by blue.

50 µL of fluorometric detector and 50 µL of calibration standards or sample were measured in a 96-well clear-bottomed microplate. The plate was incubated in the dark at room temperature for 5 minutes, before measuring the fluorescence at an excitation of 385 nm and emission of 515 nm.

### 2.2.5 DON Characterization via HTCO and Hach Tests

Nitrogen analysis was performed on dissolved samples, filtered through a 0.45 µm polyethersulfone syringe filter (Whatman, Germany). Total dissolved nitrogen (DN) was

measured on the Shimadzu TOC-LCPH Total Organic Carbon and Nitrogen Analyzer. Nitrate and nitrite were measured with Hach Nitraver5 powder pillows, while ammonia was measured using ammonia cyanurate reagent powder pillows and ammonia salicylate reagent powder pillows. A Hach Pocket Colorimeter II was used for colorimetric analysis in order to determine inorganic N. The inorganic fraction was then subtracted from the total N as described in equation 2.2.51.

$$DON = DN - DIN \quad \text{equation 2.2.51}$$

#### *2.2.6 Oxygen Functional Group Characterization via Acid-Base Titrations*

~0.1 M carbonate-free NaOH titrant was prepared by boiling 1 L of Milli-Q water for half an hour, cooling it to room temperature under Ar, and using it to dilute 10 M NaOH (also stored under Ar) to 0.1 M. The titrant was transferred to a titrant vessel, where it was stored under Ar and a CO<sub>2</sub> scrubber (Ascarite II) prevented CO<sub>2</sub> exposure. The titrant was standardized against ~0.1 g of KHP which had been dried in an oven at 120°C for 2 hours.

Prior to analysis, a known volume of all samples was diluted to ~120-150 mgC/L and brought to a pH of ~2 via the addition of 1M HCl. The ionic strength was adjusted to 0.01M using 1M KNO<sub>3</sub> (Sigma Aldrich). Using an autotitrator (848 Titrino Plus attached to 801 magnetic stirrer with attached retort stand, Metrohm Canada) and pH electrode (6.0232.100 Internal Reference pH Electrode, Metrohm Canada), the samples were titrated under purge of N<sub>2</sub> gas in ~0.1 pH intervals via the addition of standardized ~0.1M NaOH titrant to a final pH of ~12. A fully optimized continuous model (FOCUS) was used to estimate proton-binding constants and site density using an in-house MATLAB code, as proposed by Smith and Ferris<sup>35</sup>.

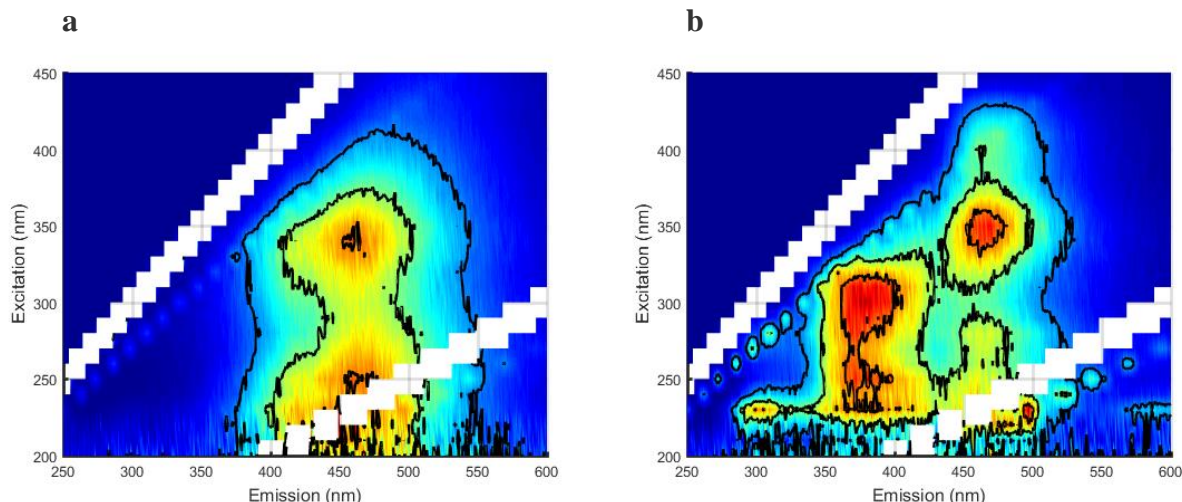
#### *2.2.7 Statistical Analysis: Hierarchical Cluster Analysis and Normalizing to the Lowest Point*

Hierarchical clustering is a statistical method that can be used to discriminate NOM sources based on their properties or parameters. Each parameter represents a dimension, can be compared to other parameters of different dimensions, and the distance between them calculated as Euclidean distances. These distances can then be plotted as a dendrogram, and used to discriminate between sources. The greater the distance the more different they are. The two sources that are the most similar (i.e. have the smallest distance) are grouped together and compared to other sources in order to determine the next most similar source and its level of difference. This process is repeated until the distances between all sources have been calculated. An in-house MATLAB code was used to generate the dendrograms in section 2.3 by using the *pdist* and *linkage* functions.

It is common practice in the literature to compare experimental data to a factor of four for the purposes of developing a BLM<sup>36</sup>. A factor of four plot was used in order to determine which parameters could be statistically significant. Values for each parameter – SAC<sub>340</sub>, FI, %HA, %FA, %Trp, %Tyr, CRS, thiol, DON, and PBI) – were divided by the lowest observed value for that parameter in order to normalize the data. This normalized data was then plotted and compared to a factor of four in order to determine which parameters were of significance for which sources.

## **2.3 Results and Discussion**

Two sample FEEMs for the Amazon Rio Negro and Preston sewage effluent can be seen below in figure 2.3.1 below. FEEMs for all sources can be found in appendix A. The four components – humic and fulvic acids, typtophan and tyrosine – can be seen at Ex/Em wavelengths of: 250-390 nm/460-520 nm, 300-350 nm/400-450 nm, 225-275 nm/350 nm, and 225-275 nm/300 nm respectively.



**Figure 2.3.1** FEEMs of tropical allochthonous, and temperate with sewage input freshwater sources: AM (a), PR\*(b).

Humic and fulvic acids were found to be present in all of the NOM sources. Freshwater sources with known sewage or microbial inputs displayed peaks indicating the presence of tryptophan, however, of the freshwater sources, only Preston sewage effluent shows a strong peak indicating the presence of tyrosine. All of the saltwater sources contained tryptophan, humic and fulvic acids, however, CCLT and CCHT contained much more humic and fulvic acids than BTP and ELM (which were more proteinaceous in nature). NOM from ELM had the greatest abundance of tryptophan.

PARAFAC was then used to calculate the relative abundance of these four fluorescent components as a percentage of the total fluorescence. The relative abundance of these components, as well as a graphical representation can be seen in table 2.3.1 and figure 2.3.2 respectively.

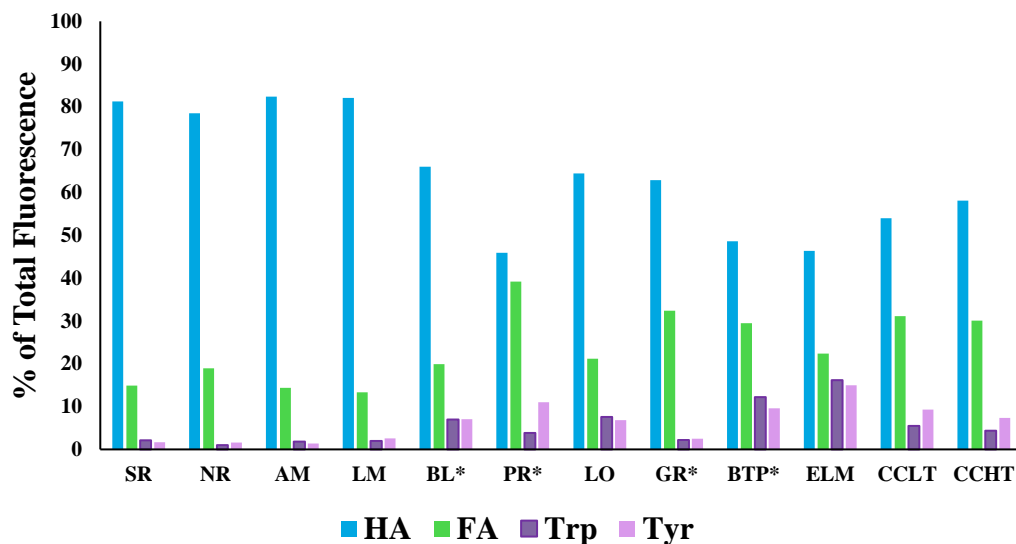
**Table 2.3.1** The relative abundance of the components of NOM (humic and fulvic acids, tryptophan and tyrosine) as resolved by PARAFAC analysis. Light blue indicates freshwater concentrates, dark blue indicates saltwater grab samples, and (\*) indicates sewage input.

| Source | HA (%) | FA (%) | Trp (%) | Tyr (%) |
|--------|--------|--------|---------|---------|
|--------|--------|--------|---------|---------|



|      |       |       |       |       |
|------|-------|-------|-------|-------|
| SR   | 81.29 | 14.87 | 2.13  | 1.71  |
| NR   | 78.50 | 18.91 | 1.02  | 1.57  |
| AM   | 82.41 | 14.39 | 1.86  | 1.34  |
| LM   | 82.11 | 13.32 | 2.00  | 2.58  |
| BL*  | 66.04 | 19.90 | 7.00  | 7.07  |
| PR*  | 45.91 | 39.21 | 3.86  | 11.02 |
| LO   | 64.44 | 21.16 | 7.56  | 6.84  |
| GR*  | 62.88 | 32.41 | 2.22  | 2.48  |
| BTP* | 48.65 | 29.49 | 12.24 | 9.62  |
| ELM  | 46.41 | 22.39 | 16.21 | 14.99 |
| CCLT | 54.02 | 31.14 | 5.52  | 9.32  |
| CCHT | 58.14 | 30.11 | 4.40  | 7.35  |

Freshwater sources of terrestrial origin (SR, NR, AM, LM) generally have much greater humic acid content (ranging from 78.50 – 82.41%); and less fulvic acid, Trp, and Tyr content (ranging from 13.32 – 18.91%, 1.02 – 2.13%, and 1.34 – 2.58% respectively). By contrast, saltwater sources and sources with microbial inputs (BL, PR, LO, GR, BTP, ELM, CCLT, and CCHT) tend to have less humic acid, and a greater percentage of fulvic acid, Trp, and Tyr. These sources have concentrations of humic acid, fulvic acid, Trp, and Tyr that range from 45.91 – 66.04%, 19.90 – 39.21%, 2.22 – 16.21%, and 2.48 – 14.99% respectively. However, PR appears unique when compared to the other sources, as it has nearly equal proportions of humic (45.91%) and fulvic (39.21%) acids.

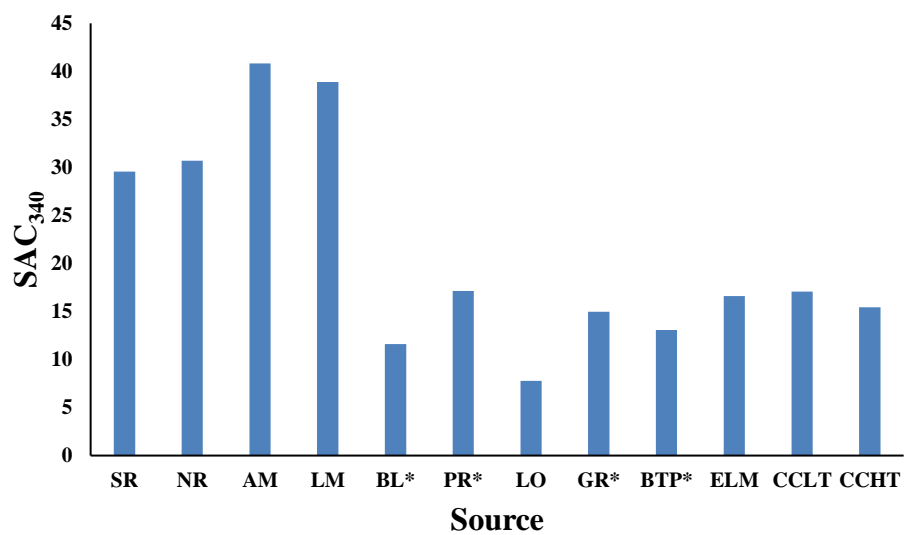


**Figure 2.3.2** The relative abundance of the components of NOM, as resolved by PARAFAC. Sources with sewage inputs are indicated by (\*).

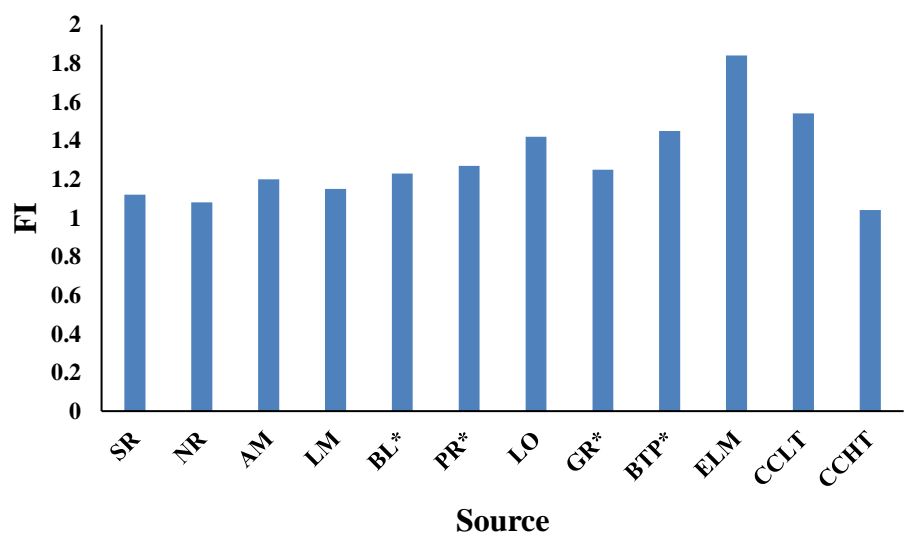
SAC<sub>340</sub> and FI values were also calculated for each source – values can be seen in table 2.3.2, figure 2.3.3 (SAC<sub>340</sub>), and figure 2.3.4 (FI).

**Table 2.3.2** SAC<sub>340</sub> and FI values for NOM sources of differing origin. Light blue indicates freshwater concentrates, dark blue indicates saltwater grab samples, and (\*) indicates sewage input. Values reported by Al-Reasi *et al.* are indicated by (a) for comparison<sup>32</sup>.

| Source | SAC <sub>340</sub> | SAC <sub>340</sub> <sup>(a)</sup> | FI   | FI <sup>(a)</sup> |
|--------|--------------------|-----------------------------------|------|-------------------|
| SR     | 27.72              |                                   | 1.12 |                   |
| NR     | 28.94              | 28.76                             | 1.08 | 1.21              |
| AM     | 40.84              |                                   | 1.20 |                   |
| LM     | 36.03              | 39.30                             | 1.15 | 1.19              |
| BL*    | 14.56              | 14.16                             | 1.23 | 1.51              |
| PR*    | 17.14              | 14.77                             | 1.27 | 1.94              |
| LO     | 7.76               | 4.85                              | 1.42 | 2.54              |
| GR*    | 14.96              |                                   | 1.25 |                   |
| BTP*   | 13.08              |                                   | 1.45 |                   |
| ELM    | 16.59              |                                   | 1.84 |                   |
| CCLT   | 17.06              |                                   | 1.54 |                   |
| CCHT   | 15.44              |                                   | 1.04 |                   |



**Figure 2.3.3** SAC<sub>340</sub> values for all NOM sources. Sources with sewage inputs are indicated by (\*).



**Figure 2.3.4** FI values for all NOM sources. Sources with sewage inputs are indicated by (\*).

In previous research, SAC<sub>340</sub> has been used as a measure of aromaticity, and directly linked to reduced Pb toxicity to freshwater rainbow trout<sup>10,13</sup>. Sources of terrestrial origin (freshwater) are associated with greater SAC<sub>340</sub> values, sources of mixed origin (estuaries and wastewater) have intermediate SAC<sub>340</sub> values, and sources of microbial origin (seawater) have the lowest SAC<sub>340</sub> values<sup>2,13,32</sup>.

SAC<sub>340</sub> values for all of the sources ranged from ~7 – 41. The lowest values were observed in the saltwater sources and sources of known microbial origin (BL, PR, LO, GR, BTP, ELM, CCLT, and CCHT). Freshwater sources of terrestrial origin had SAC<sub>340</sub> values ranging from 27.72 – 40.84. By comparison, saltwater sources and sources of microbial origin had SAC<sub>340</sub> values ranging from 7.76 – 17.14. Lower SAC<sub>340</sub> values indicate low levels of aromaticity and humic/fulvic acid content<sup>4</sup>. The Amazon and Lake Ontario concentrates had the highest and lowest SAC<sub>340</sub> values, 7.76 and 40.84 respectively, which implies they could be the most/least protective source against Pb toxicity respectively<sup>13,34</sup>.

Some of the same sources (NR, LM, BL, PR, and LO) have been collected and measured by Al-Reasi *et al.*. They found these sources had SAC<sub>340</sub> values of 28.76, 39.30, 14.16, 14.77, and 4.85 for NR, LM, BL, PR, and LO respectively. The values reported in this study are quite comparable: 28.94, 36.03, 14.56, 17.14, and 7.76 for NR, LM, BL, PR, and LO respectively. However, in the study by Al-Reasi *et al.*, LM was more aromatic, while PR and LO were less aromatic. This suggests that aromaticity changes over time, as has been reported in previous literature<sup>6</sup>.

FI is another optical measure that has been widely used to describe the origin of NOM<sup>4</sup>. FI values range from 1-2, with sources of terrestrial origin having lower FI, and sources of microbial origin having higher FI<sup>4</sup>. McKnight *et al.* found that sources that were predominantly

terrestrial in origin had FI values of 1.4 – 1.5, while microbial sources had values of 1.7 – 2.0<sup>4</sup>. Greater FI values are generally associated with lower aromaticity and SAC<sub>340</sub> values<sup>4</sup>.

In this study, FI values ranged from 1.04 – 1.84, suggesting the presence of terrestrial, microbial, and mixed NOM sources<sup>4</sup>. The majority of the sources appear to be terrestrially derived, with FI values ranging from ~1 – 1.5 (SR, NR, AM, LM, BL, PR, LO, GR, BTP, and CCHT). CCLT contains a mix of sources, with an intermediate FI of 1.54; and ELM is predominantly microbial, with a FI of 1.84.

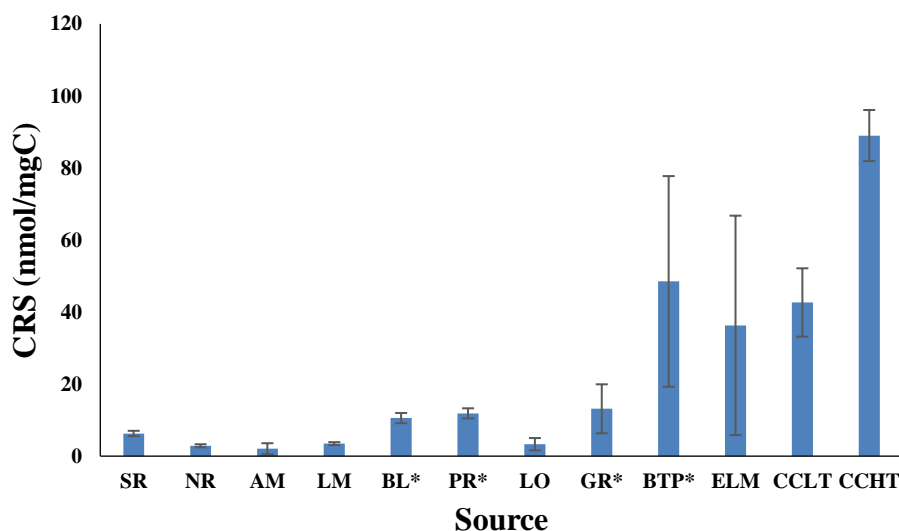
Al-Reasi *et al.* also measured FI for some of the same sources used in this study. Al-Reasi *et al.* reported FI values of 1.21, 1.19, 1.51, 1.94, and 2.54 for NR, LM, BL, PR, and LO respectively. In this study, FI values of 1.08, 1.15, 1.23, 1.27, and 1.42 were obtained for the same sources. While FI values for LM in this study are quite comparable, there are differences between these reported values for BL, PR, and LO. These three samples collected by Al-Reasi *et al.* are more autochthonous in origin than the samples collected for this study. In this study, LO was found to be nearly twice as allochthonous in origin as previously reported values suggest<sup>32</sup>. However, the properties of NOM are known to change over time<sup>6</sup>.

In general, freshwater sources of known terrestrial origin are less fluorescent than the saltwater sources, and sources of known microbial origin<sup>34</sup>. CCHT, a high-tide saltwater source is an exception to this trend, and has one of the lowest FI values – 1.04 (indicating terrestrial inputs). By comparison, the same source at low tide, CCLT, has a significantly higher FI value. This could provide insight into the potential protectivity of these sites, as terrestrially derived sources are generally assumed to have a greater impacts on metal bioavailability<sup>34</sup>.

CRS measurements ranged from ~2 – 90 nmol/mgC, and can be found in table 2.3.3 and figure 2.3.5.

**Table 2.3.3** CRS, Thiol, and DON concentrations for NOM sources of differing origin. Light blue indicates freshwater concentrates, dark blue indicates saltwater grab samples, (\*) indicates sewage input, and (x) indicates values were below detection.

| Source | CRS (nmol/mgC)   | Thiol (nmol/mgC) | DON ( $\mu\text{gN/mgC}$ ) | PBI             |
|--------|------------------|------------------|----------------------------|-----------------|
| SR     | 6.29 $\pm$ 0.743 | 120.2            | 1.705 $\pm$ 2.411          | 1.47 $\pm$ 0.69 |
| NR     | 2.87 $\pm$ 0.430 | 71.8             | 11.82 $\pm$ 10.48          | 1.72 $\pm$ 0.12 |
| AM     | 2.03 $\pm$ 1.56  | 186.5            | 3.715 $\pm$ 4.351          | 0.67 $\pm$ 0.10 |
| LM     | 3.47 $\pm$ 0.414 | 117.4            | 18.50 $\pm$ 26.16          | 0.72 $\pm$ 0.02 |
| BL*    | 10.6 $\pm$ 1.42  | 108.8            | 15.84 $\pm$ 19.13          | 0.33 $\pm$ 0.29 |
| PR*    | 11.9 $\pm$ 1.40  | Not measured     | 253.8 $\pm$ 8.811          | Not measured    |
| LO     | 3.31 $\pm$ 1.72  | Not measured     | x                          | Not measured    |
| GR*    | 13.1 $\pm$ 6.80  | Not measured     | 35.76 $\pm$ 14.75          | Not measured    |
| BTP*   | 48.5 $\pm$ 29.3  | x                | x                          | Not measured    |
| ELM    | 36.3 $\pm$ 30.5  | x                | x                          | Not measured    |
| CCLT   | 42.7 $\pm$ 9.50  | x                | x                          | Not measured    |
| CCHT   | 89.0 $\pm$ 7.10  | x                | N/A                        | Not measured    |



**Figure 2.3.5** CRS values for all NOM sources. Sources with sewage inputs are indicated by (\*).

The highest concentrations (which ranged from ~36 – 89 nmol/mgC) were found in saltwater grab samples (BTP, ELM, CCLT and CCHT), though they generally had much greater error. Of these saltwater sources, BTP (a source with sewage input) and CCHT had the highest CRS concentrations.

Freshwater concentrates had much lower CRS values (which ranged from ~2 – 13 nmol/mgC). Sources that were not impacted by sewage inputs (SR, NR, AM, LM, and LO) had some of the lowest CRS concentrations (~2 – 6 nmol/mgC). Sources that did contain sewage inputs had significantly higher CRS values, as their standard deviations do not overlap. CRS values ranged from ~11 – 13 nmol/mgC.

Similar findings were observed by De Palma *et al.* and Bowles *et al.* – CRS concentrations in saltwater sources (0.0125 – 1540.56 nmol/mgC)<sup>9</sup> were higher than those reported for freshwater (4.24 – 15.59 nmol/mgC)<sup>19</sup>. Mud flat sediments (like those present in CCLT) have also been found to contain high levels of reduced sulfide, and are released into the water column along with organic matter and other nutrients during high tides (like CCHT)<sup>37–41</sup>. CRS has also been reported to be elevated in wastewater effluents<sup>9</sup>. This data could indicate that saltwater and sewage sources may reduce soft-metal bioavailability more so than terrestrial freshwater sources as CRS provides strong binding sites for such metals, and that tide significantly impacts CRS levels in salt marshes.

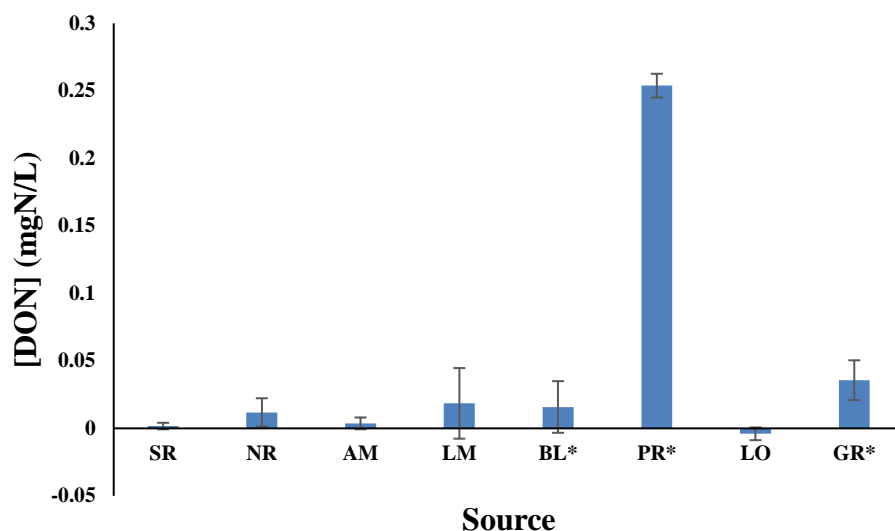
Thiol was measured for all sources with the exception of PR, LO and GR. Thiol concentrations for the various sources can be found in table 2.3.3. A Sigma MAK151 thiol assay kit was used to measure thiol content in SR, NR, AM, LM, and BL. The Cayman Chemical assay kit was used to measure thiol in the saltwater sources BTP, ELM, CCLT and CCHT. Saltwater sources formed precipitates with assay reagents and values were found to be below detection, as indicated by (\*). Thiol was not measured for PR, LO and GR. The lowest thiol concentration (71.8 nmol/mgC) was observed in NR (a drinking water reservoir), and the highest (186.5 nmol/mgC) was observed in the Amazon. Literature data on total thiol concentrations for comparison is limited, as DOC is not consistently reported<sup>31</sup>. Thiol concentrations of 0.90 – 5.00

nmol/mgC have been reported for freshwater lakes, rivers, and wetlands<sup>42</sup>. These values are up to three orders of magnitude lower than have been reported in this study. However, the sources tested by Bouchet *et al.* are all from a small boreal area in Sweden, and are not as geographically diverse as those examined in this study.

DON was measured for all sources with the exception of CCHT, as there was not enough sample remaining for analysis. DON values can be seen in table 2.3.3 above. Negative values were obtained for Lake Ontario and the saltwater sources, as indicated by (x). This is most likely due to a high DIN/DON ratio and has been observed in former studies<sup>28,30</sup>. Precipitates were also observed during Hach measurements with saltwater sources.

Positive values were obtained for the freshwater sources, although they are generally associated with large experimental error, and are below detection. The only sources with DON measureable by HTCO and Hach tests were PR (a sewage source) and GR (terrestrial freshwater with sewage input). These findings are consistent with those observed in literature, as wastewater treatment plants remove mostly inorganic nitrogen species, with little to no effect on the organic fraction<sup>43,44</sup>. Negative values have also been previously reported in the literature<sup>28–30</sup>. Figure 2.3.6 shows a visual representation of the DON data and can be seen below.

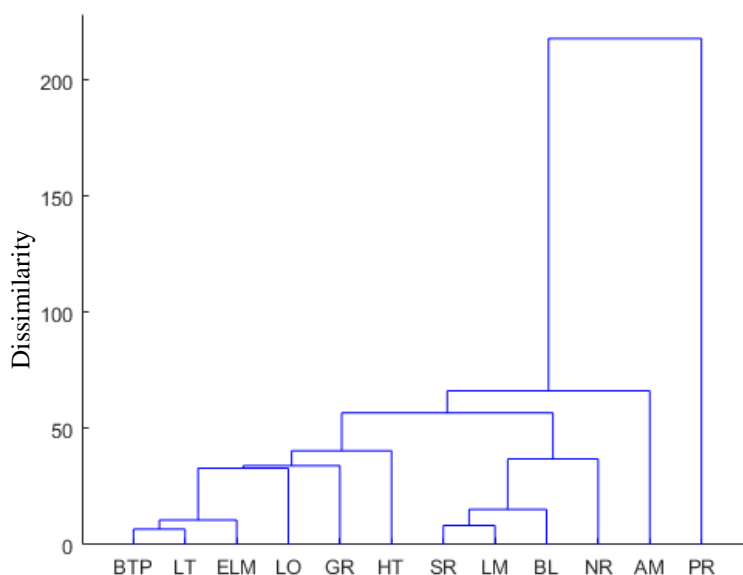




**Figure 2.3.6** DON values for all NOM sources. Sources with sewage inputs are indicated by (\*).

DON concentrations are very low for most of the freshwater sources (below detection), with significant margins of error. PR sewage effluent had significantly higher DON ( $253.8 \pm 8.811 \mu\text{g}/\text{mgC}$ ) and comparatively low error. Intermediately hard ligands such as N-containing groups provide stronger binding sites for hard metals like Cu, Ni, and Zn, thus sewage sources may significantly reduce the bioavailability of these metals.

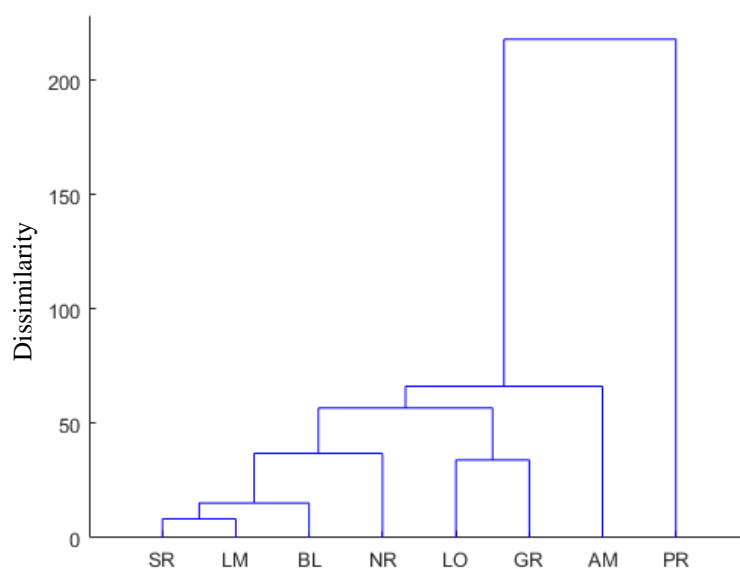
Figure 2.3.7 shows a hierarchical cluster analysis of all NOM sources by considering  $\text{SAC}_{340}$ , FI, %HA, %FA, %Trp, %Tyr, CRS, thiol, and DON for all NOM sources. In cases where thiol values were below detection or a source was not measured, the detection limit of 15 nM (as reported in the assay kit user manual) was used (assuming a DOC of 1 mgC/L) for cluster analysis. For sources where DON values were below detection, the lowest measured DON value of  $1.705 \mu\text{gN}/\text{mgC}$  was used for cluster analysis.



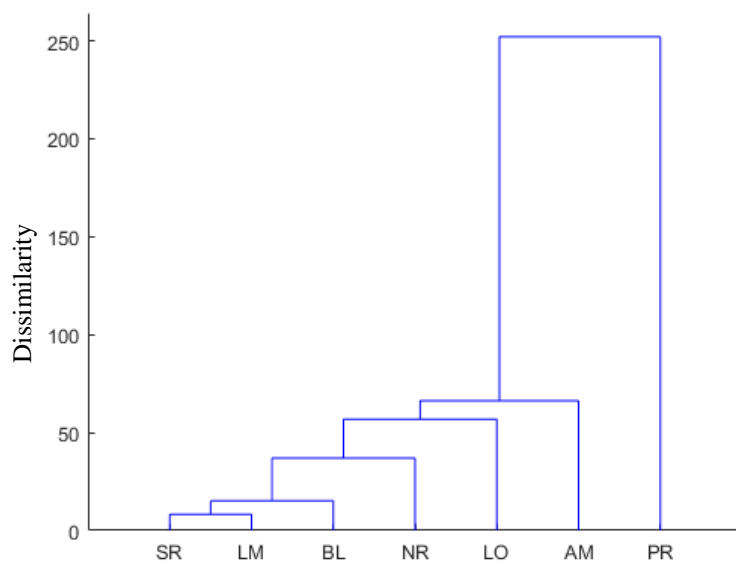
**Figure 2.3.7** Hierarchical cluster analysis of all NOM sources when considering SAC<sub>340</sub>, FI, %HA, %FA, %Trp, %Tyr, CRS, thiol, and DON measurements.

The resulting dendrogram separates the sources into two main groups – freshwater sources with low DON, and saltwater sources. The higher the branch lengths, the more dissimilar the sources. PR and AM are grouped on their own, and are very different from the other sources, especially PR. The most similar pairs are BTP and CCLT, and SR and LM. ELM is different from BTP and CCLT, and although LO and GR are also significantly different from BTP, CCLT, and ELM, they are still similar to one another. CCHT is the most different of the saltwater sources.

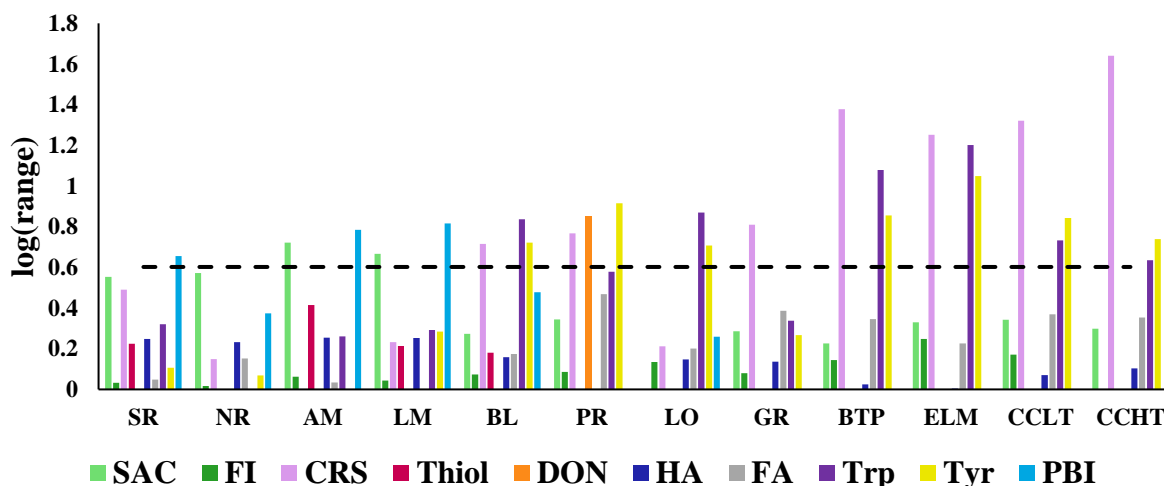
Although different, BL and NR are similar to SR and LM, but are significantly different from the saltwater sources as well as the microbial sources LO and GR. The only tropical freshwater source, AM, is significantly different from the other temperate freshwater and saltwater sources, with PR being the most different from all other sources. Cluster analysis has been further investigated in figures 2.3.8 – 2.3.10 below.



**Figure 2.3.8** Hierarchical cluster analysis of freshwater NOM characterization data in the absence of PBI.



**Figure 2.3.9** Hierarchical cluster analysis of freshwater NOM characterization data with PBI included.



**Figure 2.3.10** Characterization data normalized to the lowest values. A factor of four is indicated by the dashed back line.

The most similar pair of freshwater sources is SR and LM, which can be attributed to their comparable %HA, %FA, %Trp, %Tyr, and FI values. These values for SR and LM are 81.29%, 14.87%, 2.13%, 1.71%, and 1.12%; 82.11%, 13.32%, 2.00%, 2.58%, and 1.15% respectively. However, BL is very similar to these sources as well.

Figure 2.3.10 shows characterization data for all sources and compares the normalized values to a factor of 4 – the typical factor that is employed for BLM metal prediction. Anything above a factor of 4 (as indicated by the black dashed line) could potentially be significantly different. BL has CRS, Trp, and Tyr levels that are potentially significant – 10.6 nmol/mgC, 7.00%, and 7.07% respectively. By contrast, SR and LM have potentially significant PBI (0.4978 and 0.72 respectively), however, they do not appear to have significant levels of Trp or Tyr. Although SAC<sub>340</sub> values for SR and BL do not appear to be significant, it is worth noting that BL has a SAC<sub>340</sub> value of 14.56, meaning it is approximately half as aromatic as SR and LM, which have SAC<sub>340</sub> values of 27.72 and 36.03 respectively.

In the cluster analysis, NR is separated from SR, LM, and BL, indicating it is different from those sources. Although in figure 2.3.10 it appears NR doesn't have any significantly different characteristics, this differentiation may be due to its comparatively low thiol content. NR has a thiol concentration of 71.8 nmol/mgC – an order of magnitude lower than SR, LM, and BL which have thiol concentrations of 120.2, 117.4, and 108.8 nmol/mgC respectively.

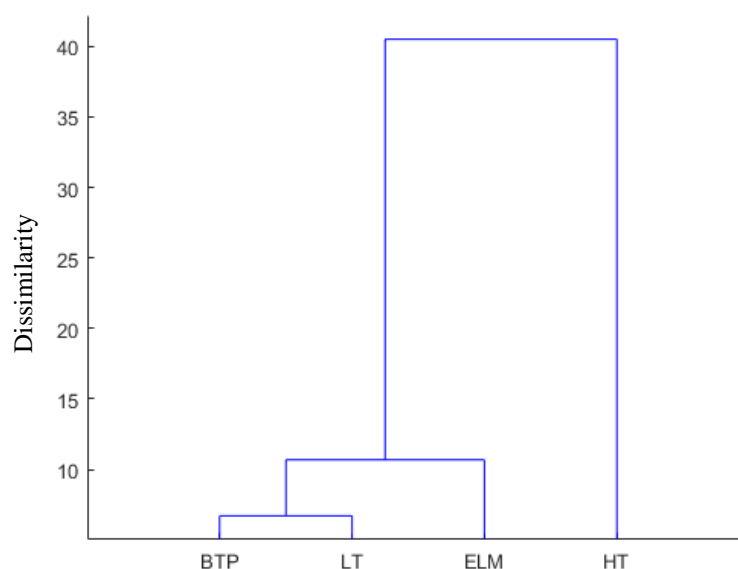
From figure 2.3.10 it appears as though LO is different from SR, LM, and NR due to its comparatively high Trp (7.56%) and Tyr (6.84%), and its comparatively low PBI (0.20). These values are: 2.13%, 1.71%, and 0.4978 for SR; 2.00%, 2.58%, and 0.72 for LM; 1.02%, 1.57%, and 0.26 for NR respectively. It also appears as though LO has significantly lower CRS than BL. LO has a CRS concentration of 3.31 nmol/mgC, while BL has a concentration of 10.6 nmol/mgC – an order of magnitude higher than LO. However, LO is also different from SR, LM, NR, and BL due to its seemingly low thiol content. As previously stated, for cluster analysis thiol was set to 15 nmol/mgC in cases where values were below detection. This is much lower than the values measured for SR (120.2 nmol/mgC), LM (117.4 nmol/mgC), NR (71.8 nmol/mgC), and BL (108.8 nmol/mgC), and could explain why LO has been grouped separately in figures 2.3.7, 2.3.8, and 2.3.9.

In figure 2.3.10 GR appears to have significant levels of CRS (13.1 nmol/mgC), but insignificant levels of Trp (2.22%) when compared to LO (3.31 nmol/mgC and 7.56% respectively). CRS concentrations in GR are an order of magnitude higher than those found in LO. In comparison to the freshwater sources of terrestrial origin, GR and LO seem to have low SAC<sub>340</sub> values. These values (7.76 and 14.96 for LO and GR respectively) are comparable to BL and PR (14.56 and 17.14 respectively), but are approximately have as aromatic as the freshwater

sources of terrestrial origin. By comparison, SR, NR, AM, and LM have SAC<sub>340</sub> values of 27.72, 28.94, 40.84, and 36.04 respectively.

AM may be significantly different from all of the other freshwater sources of terrestrial origin due to its very high thiol concentration of 186.5 nmol/mgC – which is more than double the thiol content of NR (71.8 nmol/mgC), and an order of magnitude greater than the values assumed for sources below detection (15 nmol/mgC). However, from figure 2.3.10, this does not seem to be significant, as it occurs below a factor of four. As previously mentioned, what may be of significance is its high SAC<sub>340</sub> value of 40.84 – an order of magnitude higher than LO (7.76), and more than double the values reported for BL (14.56), PR (17.14), GR (14.96), BTP (13.08), ELM (16.59), CCLT (17.06), and CCHT (15.44). From figure 2.3.10 it also appears that AM has a significant PBI value of 0.67, which separates it from other sources. This is more than double the values reported for NR (0.26), BL (0.33), PR (0.11), and LO (0.20).

In both the cluster analysis, and normalized characterization data (see figures 2.3.7 – 2.3.10) PR appears to be significantly different from all the other sources. What separates PR from all of the other freshwater sources, is its comparatively very high DON concentration of 253.8 mgN/mgC. The majority of the sources were below detection. For these sources, the assumed value of 1.705 mgN/mgC (the lowest measured value) is two orders of magnitude lower. The only other source with measurable DON, GR, had a concentration of 35.76 mgN/mgC, which is an order of magnitude lower than that found in PR. However, from figure 2.3.10 it appears as though CRS and Tyr are also significant parameters for PR.



**Figure 2.3.12** Hierarchical cluster analysis of saltwater NOM characterization data in the absence of PBI.

From figure 2.3.10, it appears as though CRS, Trp, and Tyr are all significant parameters for saltwater sources. Of these saltwater sources, the most similar pair is BTP and CCLT. These sources are different from ELM due to their comparatively low FI and high CRS values. FI values for BTP, CCLT, and ELM are 1.45, 1.54, and 1.84 respectively. Their CRS values are 48.5, 42.7, and 36.3 nmol/mgC respectively. CCHT is the most different saltwater source, which can be attributed to its very low FI of 1.04, and very high CRS concentration of 89.0 nmol/mgC.

Although there is no one parameter, or simple series of properties that can be used to discriminate NOM origin, through the use of these characterization methods sources can be differentiated based on their chemistry. CRS may be the most important characteristic to discriminate freshwater sources from saltwater sources. Figures 2.3.6 and 2.3.10 both demonstrate that CRS levels in saltwater environments are much higher than those found in freshwater – in some cases almost two orders of magnitude greater. Trp and Tyr may also be

significant discriminating factors for saltwater sources, as the variation between sources is not described by a factor of four. In freshwater sources SAC<sub>340</sub>, CRS, thiol, DON, Trp, Tyr, and PBI may all be essential parameters to discriminate NOM source and quality. Sources of terrestrial origin tend to have significant SAC<sub>340</sub> and PBI values, while sources of microbial origin tend to have significant CRS, DON, Trp, and Tyr levels.

## 2.4 Conclusions

The goal of this study was to characterize NOM sources that were as different in origin as possible in order to determine if NOM quality is variable. A wide variation was found in NOM chemistry. SAC<sub>340</sub>, FI, %HA, %FA, %Trp, %Tyr, CRS, thiol, DON, and PBI values ranged from: 7.76 – 40.84, 1.04 – 1.84, 46.41 – 82.41%, 13.32 – 39.21%, 1.02 – 16.21%, 1.34 – 14.99%, 2.03 – 89.0 nmol/mgC, 71.8 – 186.5 nmol/mgC, 35.76 – 253.8 µgN/mgC, and 0.33 – 1.72 respectively. CRS, Trp, and Tyr may be able to discriminate saltwater from freshwater sources, while SAC<sub>340</sub>, CRS, thiol, DON, Trp, Tyr, and PBI may be able to discriminate between freshwater sources. Although this study has shown significant variability in NOM quality, the question remains as to whether or not this variation is of environmental significance. In order to address this question, a computational program such as WHAM could be adjusted to account for variables such as SAC<sub>340</sub>, CRS, thiol, DON, Trp, Tyr, and PBI. Metal speciation predictions could then be compared to experimental data in order to determine if all or any of these parameters allow for better prediction of metal speciation and toxicity.



## 2.5 References

- (1) Sillanpaa, M. E. T. *Natural Organic Matter in Water: Characterization and Treatment Methods*, 1st ed.; 2015.
- (2) Thurman, E. M. *Oceanic Geochemistry of Natural Waters*; 1985.
- (3) Tipping, E.; Hilton, J.; Freshwater, B. J.; Association, B.; Tipping, E.; Hilton, J.; James, B. *Freshw. Biol.* 1988, *19*, 371–378.
- (4) McKnight, D. M.; Boyer, E. W.; Westerhoff, P. K.; Doran, P. T.; Kulbe, T.; Andersen, D. T.; E. W. Boyer; P. K. Westerhoff; P. T. Doran; T. Kulbe; Anderson, D. T. *Limnol. Oceanogr.* 2001, *46* (1), 38–48.
- (5) Hudson, N.; Reynolds, D. *River Res. Appl.* 2007, *23* (6), 631–649.
- (6) Thacker, S. A.; Tipping, E.; Baker, A.; Gondar, D. *Water Res.* 2005, *39*, 4559–4573.
- (7) Gjessing, E. T.; Egeberg, P. K. *Environ. Int.* 1999, *25* (2), 145–159.
- (8) Tait, T. N. Determination of Copper Speciation, Bioavailability and Toxicity in Saltwater Environments, Wilfrid Laurier University, 2013.
- (9) Depalma, S. G. S. Characterization of Dissolved Organic Matter and Reduced Sulfur in Coastal Marine and Estuarine Environments : Implications for Protective Effects on Acute Copper Toxicity by, University of Waterloo, 2009.
- (10) Al-reasi, H. A.; Wood, C. M.; Smith, D. S. *Environ. Int.* 2013, *59*, 201–207.
- (11) Mager, E. M. In *Fish Physiology - Homeostasis and Toxicology of Non-Essential Metals*; Wood, Chris M.; Farrell, Anthony P.; Brauner, C. J., Ed.; 2011; pp 186–225.
- (12) Korak, J. A.; Dotson, A. D.; Summers, R. S.; Rosario-ortiz, F. L. *Water Res.* 2013, *49*, 327–338.
- (13) Schwartz, M. L.; Curtis, J. P.; Playle, R. C. *Environ. Toxicol. Chem.* 2004, *23* (12), 2889–

2899.

- (14) Chen, W.; Westerhoff, P.; Leenheer, J. A.; Booksh, K. *Environ. Sci. Technol.* 2003, *37*, 5701–5710.
- (15) Smith, D. S.; Bell, R. A.; Valliant, J.; Kramer, J. R. 2004, *38* (7), 2120–2125.
- (16) Smith, D. S.; Bell, R. A.; Kramer, J. R. *Comp. Biochem. Physiol. Part C* 2002, *133*, 65–74.
- (17) In *Aquatic Organic Matter Fluorescence*; Coble, P. G., Lead, J., Baker, A., Reynolds, D. M., Spencer, R. G. M., Eds.; Cambridge University Press: New York, 2014; pp 3–124.
- (18) Kramer, J. R.; Bell, R. A.; Smith, D. S. *Appl. Geochemistry* 2007, *22* (8 SPEC. ISS.), 1606–1611.
- (19) Bowles, K. C.; Ernste, M. J.; Kramer, J. R. *Anal. Chem. Acta* 2003, *477* (October 2002), 113–124.
- (20) Mylon, S. E.; Hu, H.; Benoit, G. *Anal. Chem.* 2002, *74* (3), 661–663.
- (21) Al Farawati, R.; Van Den Berg, C. M. G. *Am. Chem. Soc.* 2001, *35* (10).
- (22) Joe-wong, C.; Shoenfelt, E.; Hauser, E. J.; Crompton, N.; Myneni, S. C. B. *Environ. Sci. Technol.* 2012, *46* (18), 9854–9861.
- (23) Rao, B.; Simpson, C.; Lin, H.; Liang, L.; Gu, B. *Talanta* 2014, *119*, 240–247.
- (24) Mangal, V.; Guéguen, C. *Anal Bioanal Chem* 2015, *407*, 4305–4313.
- (25) Al-reasi, H. A.; Wood, C. M.; Smith, D. S. .
- (26) Atalay, Y. B.; Di Toro, D. M.; Carbonaro, R. F. *Geochim. Cosmochim. Acta* 2013, *122*, 464–477.
- (27) Czerwionka, K. *Oceanologia* 2016, *58* (1), 39–45.
- (28) Wang, Q.; Hua, B.; Yang, J.; Liu, F.; Zhu, G.; Deng, B. *J. Chem.* 2015, *2015*.

- (29) Hu, H.; Ren, H. *Chemosphere* 2016, *164*, 299–303.
- (30) Chen, B.; Westerhoff, P.; Zhang, L.; Zhu, A.; Yang, X. I. N.; Wang, C. 2015, 249–276.
- (31) Chen, W. The effects of Dissolved Organic Matter (DOM) sources on Pb 2+ , Zn 2+ and Cd 2+ binding, Trent University, 2017.
- (32) Al-Reasi, H. A. The Direct and Indirect Effects of DOM on Aquatic Organisms, McMaster University, 2012.
- (33) Ohno, T. *Environ. Sci. Technol.* 2002, *36* (4), 742–746.
- (34) Diamond, R. L. Characterizing Dissolved Organic Matter and Cu<sup>2+</sup> , Zn<sup>2+</sup> , Ni<sup>2+</sup> , and Pb<sup>2+</sup> Binding in Salt Water and Implications for Toxicity Characterizing Dissolved Organic Matter and, Wilfrid Laurier University, 2012.
- (35) Ferris, F. G.; Smith, D. S. *Environ. Sci. Technol.* 2001, *35* (23), 4637–4642.
- (36) Cooper, C. A.; Tait, T. N.; Gray, H.; Cimprich, G.; Santore, R. C.; McGeer, J. C.; Wood, C. M.; Smith, D. S. *Environ. Sci. Technol.* 2014, *48* (2), 1213–1221.
- (37) Newton, J. Evidence for Manganese-catalyzed Nitrogen cycling in salt marsh sediments, Georgia Institute of Technology, 2006.
- (38) Carey, E. A. Using plug-flow reactors to determine the role of soluble Fe(III) in the cycling of iron and sulfur in salt marsh sediments, Georgia Institute of Technology, 2003.
- (39) Taillefert, M.; Neuhuber, S.; Bristow, G. *Geochem Trans.* 2007, *8* (6), 1–15.
- (40) Carey, E.; Taillefert, M. *Limnol. Oceanogr.* 2005, No. 50, 1129–1141.
- (41) Resources, M. D. of M. In *Grand Bay National Estuarine Research Reserve (NERR): Final Environmental Impact Statement/Reserve Management Plan*; 1998; p 12.
- (42) Bouchet, S. *Anal. Chem.* 2015, No. 87, 1089–1096.
- (43) Westgate, P. J.; Park, C. *Environ. Sci. Technol.* 2010, *44* (14), 5352–5357.

- (44) Yu, D. Evaluation of effluent organic nitrogen and its impacts on receiving water bodies,  
University of Massachusetts - Amherst, 2012.

## **Chapter 3 – The Impacts of NOM Quality on Lead Speciation**

### 3.0 Abstract

Trace metals play an important role in aquatic systems. While some trace metals are essential for aquatic life, other metals may cause toxicity to biota if present in high enough concentrations. Lead is one such metal, and can be found as a variety of different species in aquatic environments. These different species display different mechanisms and levels of toxicity. This makes metal speciation and the factors that influence it important to consider when addressing toxicity, as water chemistry is very site-specific. Organic matter is one parameter affecting speciation and therefore toxicity, making it important to consider. This study aimed to define Pb speciation in freshwater using both an artificial ligand and natural organic matter (NOM). NOM from different sources were analyzed to determine if binding characteristics are source-dependent. Flow-through titrations using a Pb ion-selective electrode (ISE) were employed in order to determine speciation using an internal calibration method. Ethylenediamine (EN) was used as a model ligand in both artificial freshwater (AFW) and artificial seawater (ASW). Free Pb was calculated and modelled using certified logK values from NIST database. In both AFW and ASW, the ISE accurately measured  $\text{Pb}^{2+}$  within a factor of two. However, when an internal calibration method was used in NOM, the fundamental assumption that Pb-NOM binding will not occur at low pH disagreed with WHAM, which predicted Pb-NOM binding would indeed occur. Thus the internal calibration method is not effective at determining Pb speciation in NOM, although it is effective when only EN is present. Among NOM samples,  $\text{Pb}^{2+}$  speciation was reproducibly different, indicating source-dependent Pb-NOM binding. In all cases, the Windermere Humic Aqueous Model (WHAM) did not adequately predict the variation in Pb speciation within a factor of two. DON and  $\text{SAC}_{340}$  for the titrated sources are significantly different, and may potentially explain differences in source-dependent Pb speciation in

freshwater environments. This is of immense industrial and environmental importance, as current water quality guidelines (WQG) do not account for NOM, DON, or SAC<sub>340</sub>.

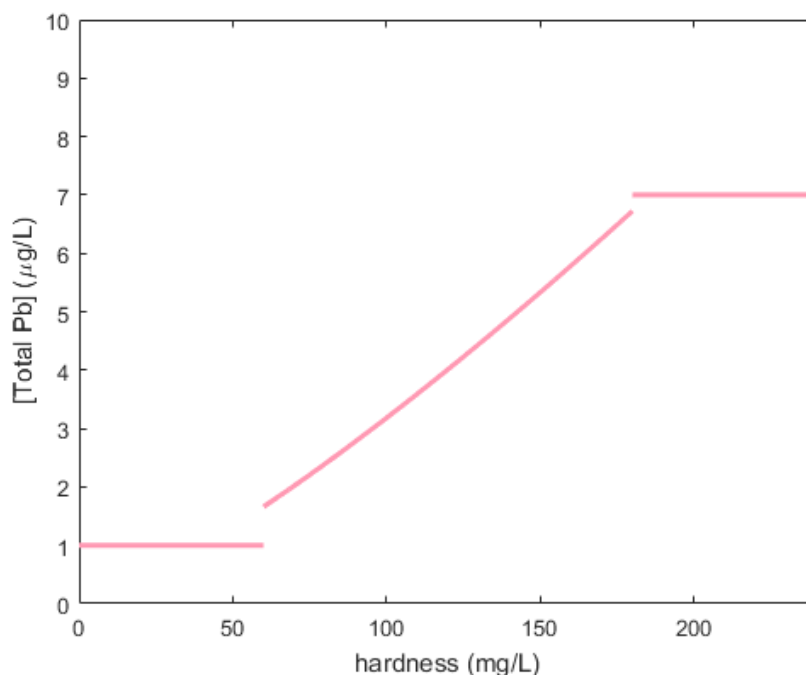
### 3.1 Introduction

Lead is a non-essential metal with an extensive toxic history<sup>1</sup>. In previous years, it was used for plumbing material, cookware, as a food additive<sup>1</sup>. More recently (1923-2014), it has been used as an antiknock additive in gasoline<sup>2,3</sup>. Currently, the greatest contributors to anthropogenic lead input are lead mining, smelting, and refining; domestic wastewater; and sewage sludge<sup>4</sup>. Anthropogenic sources contribute significantly more Pb to aquatic ecosystems than natural inputs, accounting for an estimated 138,000 metric tons annually<sup>3,4</sup>. Natural sources contribute a mere 12,000 metric tons per year<sup>3,4</sup>. Background concentrations of Pb in surface waters have been reported to be in the 1-23 ng/L range, however, as a result of human activity, current concentrations can be up to several hundred µg/L of dissolved lead, or in the mg/L range for total lead<sup>3</sup>.

In aquatic environments, Pb can be bound to colloidal particles, organic and inorganic ligands, or it may occur as the free metal ion – which has the greatest mobility and bioavailability<sup>3,5</sup>. In freshwater with pH < 7.5, Pb partially exists as the free metal ion<sup>6</sup>. However, under basic conditions, Pb forms insoluble carbonate complexes<sup>6</sup>. Under acidic conditions, lead is more soluble and is typically found as the free metal ion, PbSO<sub>4</sub>, PbCl<sub>4</sub><sup>2-</sup>, Pb(OH)<sub>2</sub>, and PbOH<sup>+</sup><sup>3,7</sup>. In seawater, Pb speciation is dominated by PbCl<sup>+</sup>, PbCO<sub>3</sub>, and Pb(CO<sub>3</sub>)<sub>2</sub><sup>2-</sup><sup>6</sup>. Lead is also able to form complexes with natural organic matter (NOM) binding sites such as thiol, amino, carboxyl and phenolic functional groups<sup>8</sup>. These different species display different levels and modes of toxicity, making speciation (and the factors that influence it) important to consider when assessing toxicity<sup>3,9</sup>.

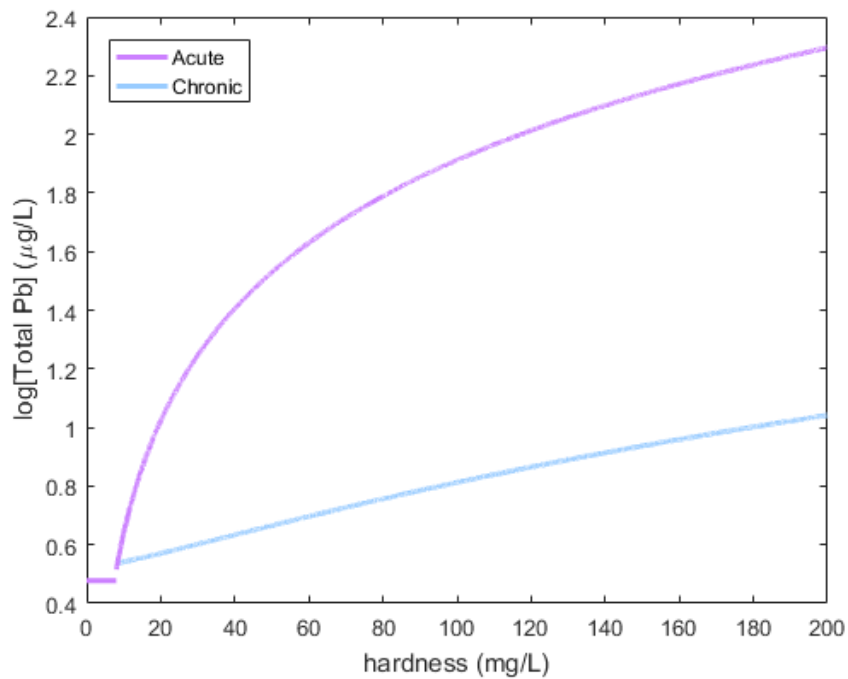


In Canada, current national water quality guidelines (WQG) only address freshwater environments, and only take water hardness into consideration<sup>10</sup>. A graphical representation of the current WQG is shown in figure 3.1.1 below.



**Figure 3.1.1** CCME 1987 Lead freshwater chronic water quality guidelines for the protection of aquatic life<sup>10</sup>.

Aquatic biota can have different toxicity thresholds for different metals<sup>11</sup>. These thresholds are known to be influenced by water hardness in freshwater systems, and once reached, toxicity will occur<sup>11</sup>. However, parameters other than water hardness – such as pH and dissolved organic carbon (DOC) – have also been shown to significantly impact metal toxicity in freshwater<sup>12</sup>. Saltwater guidelines are even less common, and do not account for differences in water chemistry. The CCME currently does not have national WQG's for Pb in marine waters, however, provincial criteria do exist (ex. both acute and chronic criteria in British Columbia)<sup>3,13</sup>. Freshwater and saltwater provincial criteria for BC are shown in figure 3.2.1 below.



**Figure 3.1.2** BC provincial lead water quality criteria for the protection of freshwater aquatic life<sup>13</sup>.

**Table 3.1.1** BC provincial lead water quality criteria for the protection of marine and estuarine aquatic life<sup>13</sup>.

| Acute [Pb <sub>T</sub> ] (μg/L) | Chronic [Pb <sub>T</sub> ] (μg/L) |
|---------------------------------|-----------------------------------|
| 140                             | ≤2                                |

WQGs in BC are given as acute (maximum values never to be exceeded) and chronic (30-day average values not to be exceeded) values<sup>13</sup>.

The biotic ligand model (BLM) is based on the concept that metal toxicity can be predicted through complex chemical equilibrium modelling, making it capable of accounting for variation in water chemistry<sup>14</sup>. BLM inputs include simple parameters such as pH, [DOC], alkalinity, major cations and anions<sup>14</sup>. Metal binding at the biotic ligand results in toxicity, and

the effects are directly proportional to its accumulation<sup>9,14</sup>. Since  $\text{Pb}^{2+}$  is thought to be the most bioavailable, and therefore the most toxic species, it is the species used for modelling<sup>9</sup>.

The BLM is able to address hardness, salinity, and metal complexation by accounting for cation competition and inorganic ligands/DOC<sup>3,9,15,22</sup>. The resulting metal toxicity is then defined in terms of lethal accumulation concentrations ( $\text{LA}_{50}$ ), or a lethal concentration ( $\text{LC}_{50}$ ), where 50% mortality occurs<sup>9,15</sup>. Although BLM's for Pb in freshwater have been proposed in the literature, none have been implemented<sup>12,16,17</sup>.

The Windermere Humic Aqueous Model (WHAM) version VII is a computational tool capable of predicting metal speciation at equilibrium, and can be used to predict free metal concentrations<sup>18</sup>. WHAM computations can be combined with toxicity (ex.  $\text{LC}_{50}$  or  $\text{LA}_{50}$  values) in order to determine toxic levels of metals to biota.

### 3.2 Methods

All freshwater samples for Pb titrations were prepared 24-h in advance with a background electrolyte concentration of 0.01 M  $\text{KNO}_3$  (99.999%, Sigma Aldrich, St. Louis, MO), and allowed to equilibrate at room temperature in a volumetric flask covered with parafilm. Model ligand titrations were performed using lead standard solution (1000 mg/L lead atomic spectroscopy standard concentrate, Sigma Aldrich, St. Louis, MO), 15 M ethylenediamine (EN) (ReagentPlus  $\geq 99\%$ , Sigma Aldrich, St. Louis, MO), trace metal grade  $\text{KNO}_3$ , and organization for Economic Cooperation and Development (OECD) artificial freshwater (AFW)<sup>19</sup>. The recipe used for OECD AFW can be found in table 3.2.1 below. All salts were dissolved to a total volume of 1L in ultrapure water (18.2 M $\Omega$ , MilliQ).

**Table 3.2.1** OECD recipe for 1L of artificial freshwater<sup>19</sup>.

| Salt                                  | Mass (g) |
|---------------------------------------|----------|
| CaCl <sub>2</sub> · 2H <sub>2</sub> O | 0.2490   |
| MgSO <sub>4</sub> · 7H <sub>2</sub> O | 0.1232   |
| NaHCO <sub>3</sub>                    | 0.0648   |
| KCl                                   | 0.0058   |

Samples for model ligand titrations in saltwater were prepared in the same way as the freshwater samples, with two exceptions: no additional background electrolyte was added, and 32 ppt artificial seawater (ASTM 2004) was used instead of AFW. The recipe for 32 ppt ASW can be found in table 3.2.2 below.

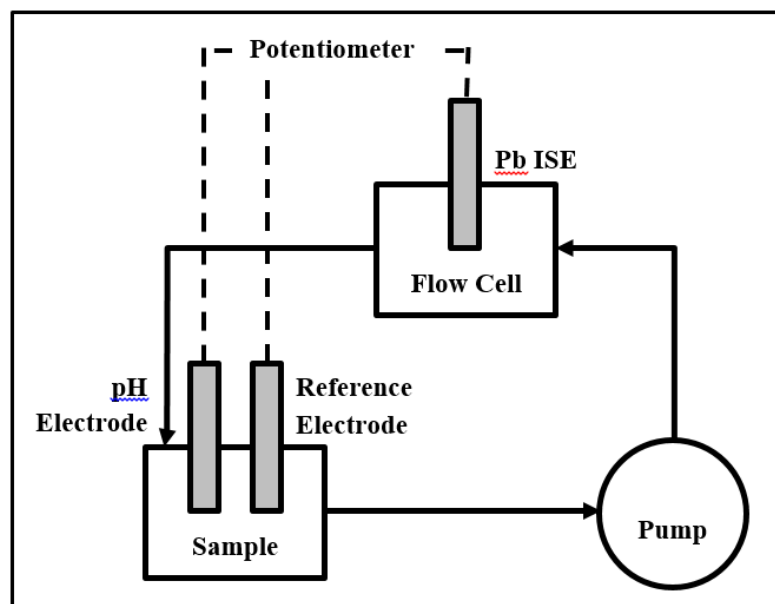
**Table 3.2.2** ASTM recipe for 1L of artificial saltwater.

| Salt                                  | Mass (g) |
|---------------------------------------|----------|
| NaCl                                  | 26.013   |
| CaCl <sub>2</sub>                     | 1.242    |
| MgCl <sub>2</sub> · 6H <sub>2</sub> O | 4.531    |
| MgSO <sub>4</sub> · 7H <sub>2</sub> O | 5.497    |
| NaHCO <sub>3</sub>                    | 0.391    |
| KCl                                   | 0.828    |

NOM samples were prepared by diluting NOM concentrates (refer to 2.2.1 for sample collection details) to a [TOC] of 20 mgC/L in ultrapure water (18.2 MΩ, MilliQ), with 0.01M KNO<sub>3</sub> as a background electrolyte.

A Mantech Pb ISE (Model PCE-80-PB1001, Mandel, Guelph, ON) was polished daily before use with aluminum oxide powder (<10micron, 99.7%, Sigma Aldrich, St. Louis, MO) and ultrapure water (18.2 MΩ, MilliQ). After polishing, the electrode was soaked for 15 minutes in a 1 μM Pb conditioning solution made from 1000 mg/L standard (Sigma Aldrich, St. Louis, MO) and MilliQ water.

A flow-through system similar to that described by Tait *et al.* was used to titrate all NOM sources and the model ligand <sup>20</sup>. However, the sample was recycled continuously, and a fast flow rate (~160 mL/h) was used. A schematic can be seen below.



**Figure 3.2.1** Schematic of the flow-through system used for Pb titrations, adapted from Tait *et al.*<sup>20</sup>

The Pb ISE was held in a micro-Flowcell (FIALab Bellevue, WA) wrapped in wire and connected to an electrical ground. An Orion Ag/AgCl double junction reference electrode (Model 900200, Boston, MA, USA) and Mettler Toledo half-cell pH electrode (Model 51343195, Mississauga, ON) were housed in a beaker containing the sample solution. Both the Pb ISE and the reference electrode were filled with 0.01 M KNO<sub>3</sub> outer filling solution (99.999%, Sigma Aldrich, St. Louis, MO). The electrodes were connected to a potentiometer (Tanager, Model 9501, Ancaster, ON), and a Cerampump FMI “Q” Pump (GQ6, Fluid Metering Inc., Syosset, NY) was used to pump the sample through the system.

Model ligands were titrated at intervals between pH 5-9. In freshwater, EN was titrated at pH 9, 8, 7, 6.5, 6, and 5. Subsequent model ligand titrations for saltwater omitted pH 6.5 for efficiency. All of the NOM sources were then titrated at pH 8, 7, 6, and 5. The pH was kept stable ( $\pm 0.1$  pH units) by manual addition of 0.1 M NaOH and HCl made from diluted 1 M standards (1M Volumetric, Sigma Aldrich, St. Louis, MO) and (1M BioReagent, Sigma Aldrich, St. Louis, MO) respectively. An internal calibration method was used as described by Tait *et al.*, where the free Pb was set to be the total Pb concentration at low pH (in this case pH 5)<sup>20</sup>. The reference potential can then be calculated using the Nernst equation (see equation 3.2.1), and used to determine free Pb concentrations at any pH.

$$E = E^0 + \frac{0.0592}{2} \log_{10}[Pb^{2+}] \quad \text{equation 3.2.1}$$

Speciation models for model ligand titrations were calculated using an in-house MATLAB code developed by Smith *et al.* and certified NIST logK values. In-house modelling with computer programs like MATLAB is an appealing option as it is able to predict metal speciation under any conditions. To account for activity, logK values from NIST were corrected for an ionic strength of 0.0189 M (combined ionic strength of AFW and 0.01 M KNO<sub>3</sub>) and 0.6505 M (ionic strength of ASW) for freshwater and saltwater titrations respectively. If available, a range of logK values (above and below the desired ionic strength) were used to interpolate for the appropriate ionic strength. In cases where certified logK values were not available for an ionic strength above or below the desired value, logK values were extrapolated from available values using equation 3.2.2 below.

$$\log_{10} \gamma_i = -Az_i^2 \sqrt{I} \quad \text{equation 3.2.2}$$

Where  $\gamma_i$  is the activity coefficient,  $z_i$  is the charge,  $I$  is the ionic strength, and  $A$  is a constant (0.509 Kg<sup>1/2</sup>mol<sup>-1/2</sup>). Free Pb speciation was then calculated in MATLAB based on the

chemical equilibria expressed as tableaus in appendix B. For the purpose of this study, inorganic species included:  $H^+$ ,  $Pb^{2+}$ ,  $Cl^-$ ,  $SO_4^{2-}$ ,  $CO_3^{2-}$ ,  $OH^-$ ,  $PbOH^+$ ,  $Pb(OH)_2$ ,  $Pb(OH)_3^-$ ,  $PbCl^+$ ,  $PbCl_2$ ,  $PbCl_3^-$ ,  $PbSO_4$ ,  $PbHCO_3^+$ ,  $PbCO_3$  and  $Pb(CO_3)_2^{2-}$ . Organic species of interest included:  $PbEN$  and  $PbEN_2$ .  $EN$  was used as an organic component as it has been well defined in literature (i.e. its logK values for Pb binding have been well established), does not bind Pb too strongly, and is readily commercially available.

The system was solved by using mass action (logK values), mass balance ( $Pb_T$ ,  $Cl_T$ ,  $SO_{4T}$  and  $C_T$ ) and electroneutrality. Precipitation was also considered for supersaturated systems by creating a tableau (found in appendix B) for solid species using their respective  $K_{sp}$  values.

Once the method was validated via model ligand titrations, NOM samples were titrated and their speciation compared to WHAM models. WHAM models were calculated using WHAM VII and the following inputs found in table 3.2.3 below.

**Table 3.2.3** Input parameters for WHAM VII modelling of Pb speciation in freshwater.

| <b>SPM</b> | <b>Temperature</b> | <b>pCO<sub>2</sub></b> | <b>pH</b> | <b>[Pb<sub>T</sub>]</b> | <b>[K<sup>+</sup>]</b> | <b>[NO<sub>3</sub><sup>-</sup>]</b> | <b>Colloidal FA</b> |
|------------|--------------------|------------------------|-----------|-------------------------|------------------------|-------------------------------------|---------------------|
| mg/L       | deg C              | atm                    |           | M                       | M                      | M                                   | mg/L                |
|            | 25                 |                        | 8         |                         | 0.01                   | 0.01                                | 24                  |
|            | 25                 |                        | 7         |                         | 0.01                   | 0.01                                | 24                  |
|            | 25                 |                        | 6         |                         | 0.01                   | 0.01                                | 24                  |
|            | 25                 |                        | 5         |                         | 0.01                   | 0.01                                | 24                  |
|            | 25                 |                        | 4.5       |                         | 0.01                   | 0.01                                | 24                  |
|            | 25                 |                        | 4         |                         | 0.01                   | 0.01                                | 24                  |

SPM and pCO<sub>2</sub> were left at default settings (zero),  $Pb_T$  varied from  $10^{-8}$  M to  $10^{-6}$  M Pb depending on the total Pb concentration, and colloidal FA was calculated as shown in equation 3.2.3<sup>21</sup>.

$$[Colloidal\ FA] = 2[TOC] * 0.65 \quad \text{equation 3.2.3}$$

[TOC] is assumed to be 100% fulvic acid, multiplied by two to convert [TOC] to [NOM], and multiplied by 0.6 (the default factor) to determine the fraction available for metal-binding.

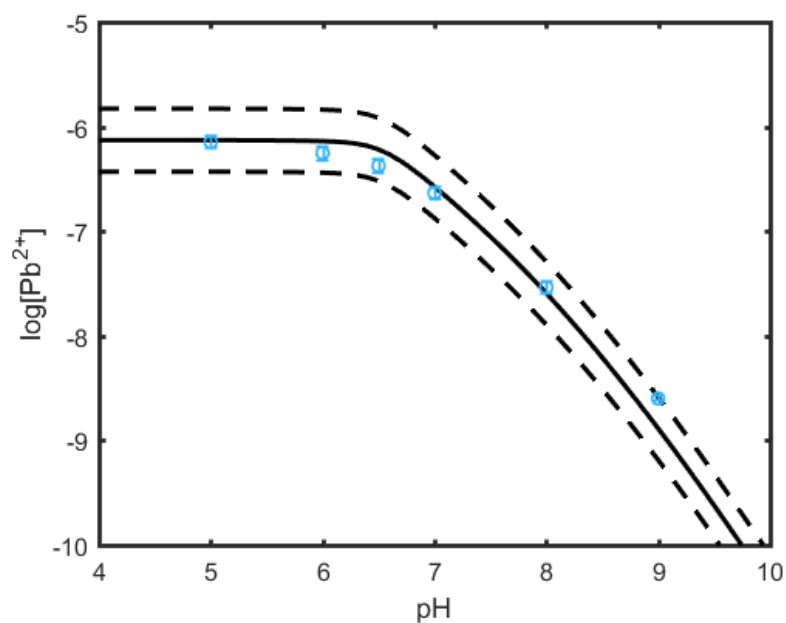
The data output was then plotted in MATLAB against experimental speciation data for comparison.

Sources of each type (IHSS standard, terrestrial, and microbial sources) that showed the greatest differences in hierarchical cluster analysis (see section 2.3) were chosen for titration in order to test for source-dependent Pb-binding. AM was chosen to represent highly aromatic terrestrial NOM, SR was chosen both as a reference source and as a less aromatic terrestrial freshwater source, while BL and PR were chosen to represent sewage effluent sources with lower aromaticity and greater FI, CRS, and (in the case of PR) high organic Nitrogen.

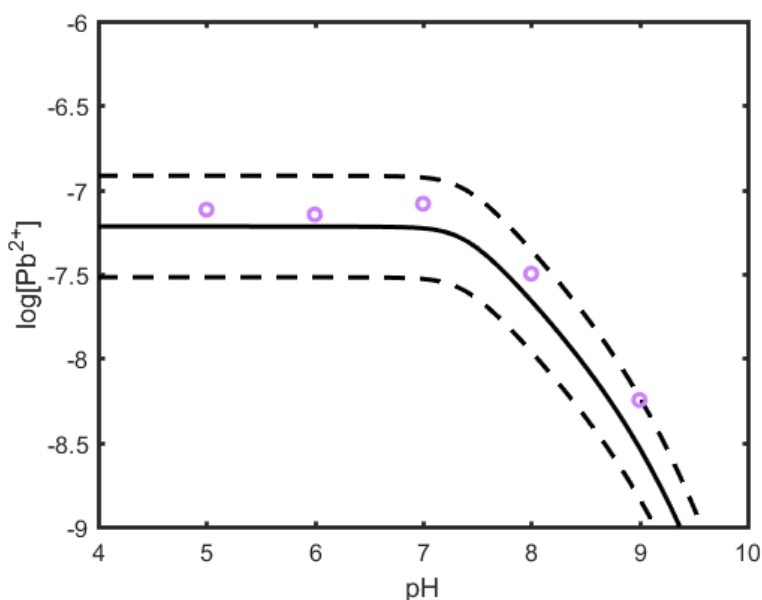
### **3.3 Results and Discussion**

Figures of measured free Pb speciation compared to a model curve for Pb-EN binding can be found in figures 3.3.1 and 3.3.2 below.





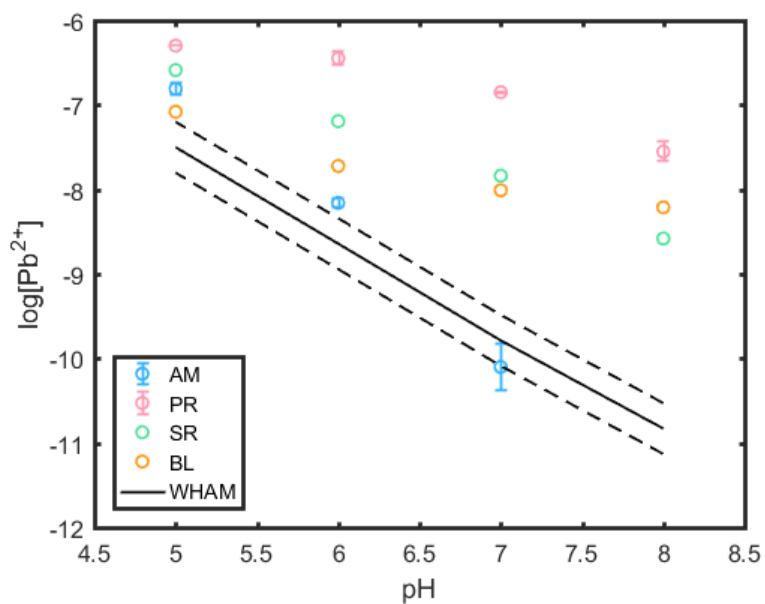
**Figure 3.3.1** pH-dependent flow-through titration of EN in OECD AFW. The black solid curve represents un-optimized modelled  $\text{Pb}^{2+}$  speciation using certified  $\log K$  values, the black dashed curves represent a factor of 2 above and below, and the blue circles with error bars are experimental data.



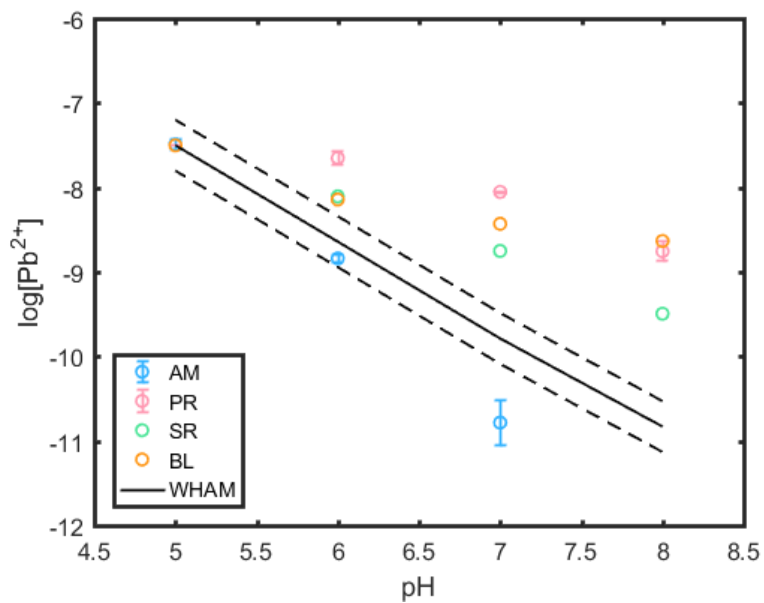
**Figure 3.3.2** pH-dependent flow-through titration of EN in 32 ppt ASW. The black solid curve represents un-optimized modelled  $\text{Pb}^{2+}$  speciation using certified logK values, the black dashed curves represent a factor of 2 above and below, and the purple circles with error bars are experimental data.

Free Pb speciation for the model was calculated using certified logK values from the NIST database. Free Pb values measured via ISE very closely match the model, and are well within a factor of two, as indicated by the dashed black lines. Replicate data were not obtained for Pb in seawater, however, in freshwater the observed values are within one standard deviation of the model predictions (with the exception of pH 8). Thus it can be concluded that the commercially available Manntech Pb ISE is an accurate and reliable means of determining  $\text{Pb}^{2+}$  in freshwater (though potentially not at  $\text{pH} > 8$ ), and may also be practical for application in saltwater.

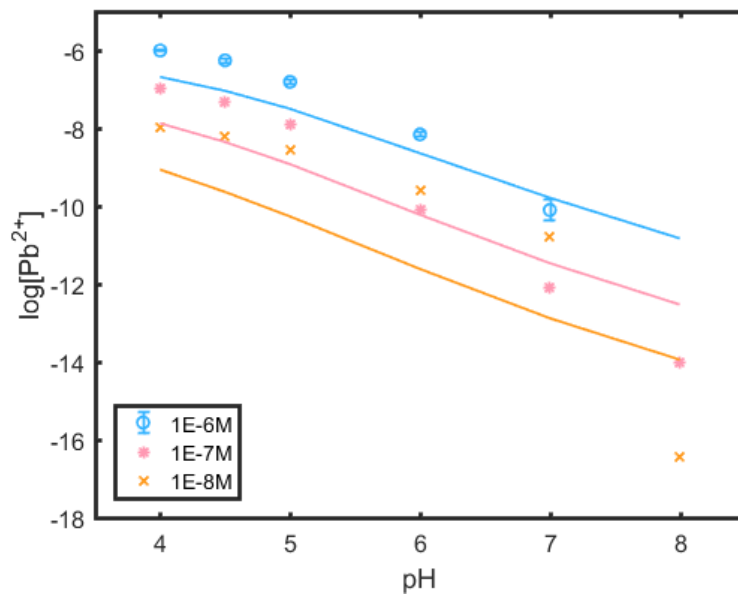
Figures 3.3.3 – 3.3.8 show the extent of Pb-NOM binding as a function of pH. Lower levels of  $\text{Pb}^{2+}$  indicate strong binding, while higher concentrations indicate weaker binding.



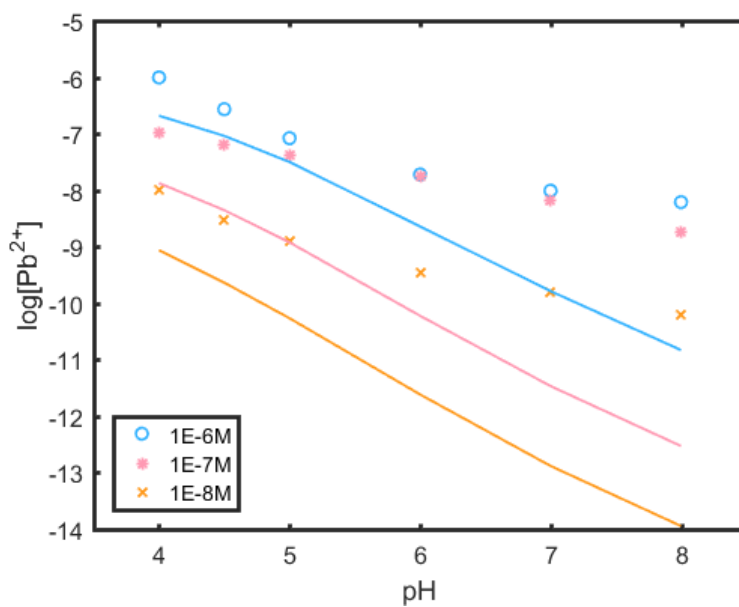
**Figure 3.3.3** pH-dependent flow-through titration titration data (calculated using internal calibration) for NOM sources at  $10^{-6}$  M total Pb. Lead speciation as predicted by WHAM VII is represented by the solid black line. Dashed black lines represent a factor of two above and below WHAM predicted speciation.



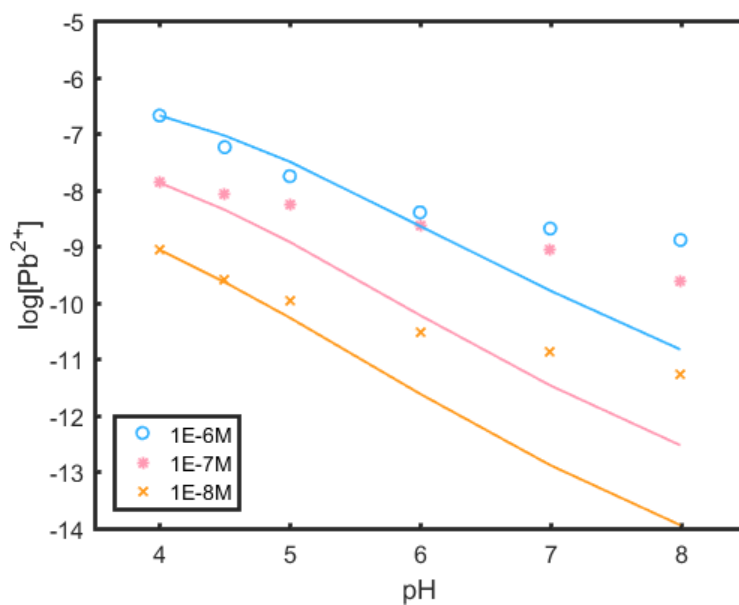
**Figure 3.3.4** pH-dependent flow-through titration data (low pH forced to agree with WHAM) for NOM sources at  $10^{-6}$  M total Pb. Lead speciation as predicted by WHAM VII is represented by the solid black line. Dashed black lines represent a factor of two above and below WHAM predicted speciation.



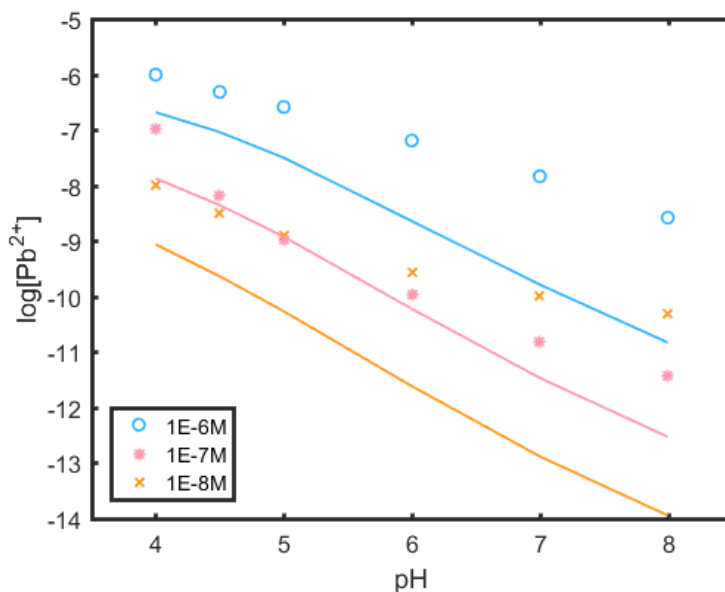
**Figure 3.3.5** pH-dependent flow-through titration data (calculated using internal calibration) for AM NOM with different concentrations of total Pb. Solid coloured lines represent Pb speciation as predicted by WHAM VII.



**Figure 3.3.6** pH-dependent flow-through titration data (calculated using internal calibration) for BL NOM with different concentrations of total Pb. Solid coloured lines represent Pb speciation as predicted by WHAM VII.



**Figure 3.3.7** pH-dependent flow-through titration data (low pH forced to agree with WHAM) for BL NOM with different concentrations of total Pb. Solid coloured lines represent Pb speciation as predicted by WHAM VII.



**Figure 3.3.8** pH-dependent flow-through titration data (calculated using internal calibration) for SR NOM with different concentrations of total Pb. Solid coloured lines represent Pb speciation as predicted by WHAM VII.

In all of the NOM sources,  $\text{Pb}^{2+}$  increased with decreasing pH and increasing total Pb. Figures 3.3.4 – 3.3.8 demonstrate free Pb speciation as a function of pH for the various sources with varying total Pb. WHAM predictions are indicated by solid curves, and decrease proportionally with decreasing total Pb. AM had the lowest  $\text{Pb}^{2+}$  concentrations, especially at high pH, and therefore demonstrated the strongest Pb-NOM binding. BL, SR, and PR all showed similar binding curves, however, PR showed the weakest binding of all the sources as it had the highest measured free Pb values. Figure 3.3.4 shows the same data, but recalibrated such that it is forced to agree with WHAM at low pH (pH 5).

When an internal calibration was used to calibrate the data, the measured speciation was consistently higher than WHAM predictions at low pH. This internal calibration method is based on the assumption that all Pb is unbound at low pH. However, WHAM predictions demonstrate

that Pb-NOM binding is still anticipated at low pH. This suggests that although the internal calibration method proved effective with a model ligand, it is not effective for quantification in the presence of much larger and more complex molecules, such as those found in NOM.

The data was recalibrated by forcing the  $\text{Pb}^{2+}$  at low pH to agree with WHAM predictions. Speciation curves for all sources at all concentrations using this method can be found in appendix B. Figures 3.3.6 and 3.3.7 show a comparison of the two different calibration methods for the same source – BL. When using this alternate calibration method, the data is better described by WHAM. AM in particular is much better described by WHAM when using this calibration method.

However, it is clear from figure 3.3.4, that even when the data is forced to agree with WHAM at low pH, WHAM does not adequately describe the majority of the speciation data as the majority falls outside the factor of 2 acceptable range (as indicated by dashed lines). It is clear that in order to accurately predict free lead speciation in freshwater, WHAM predictions for Pb need to account for more than just inorganic salts, pH, and TOC as fulvic acid.

As discussed in section 2.3,  $\text{SAC}_{340}$ , CRS, thiol, DON, Trp, Tyr, and PBI may all be important parameters for discriminating NOM quality, and may allow for better prediction of source-dependent Pb-NOM binding. In figure 3.3.3, Pb speciation appears to be very comparable for SR and BL. Pb-PR binding is also similar to the binding observed in SR and BL, however Pb-NOM binding is slightly weaker in PR. Pb-NOM binding for AM appears to be significantly different from all the other sources, however, it cannot be confirmed to be statistically significant from SR and BL without replication. Replicates were obtained for both AM and PR (see figure 3.3.3), and these two sources clearly demonstrate statistically significant source-dependent Pb-NOM binding, as the data points do not fall within one standard deviation of one another.



From the hierarchical cluster analysis and range plots found in section 2.3, it was found that SAC<sub>340</sub>, PBI, CRS, DON, Trp, and Tyr may all be significant factors to address for accurate prediction of metal speciation. Sources of terrestrial origin had significant SAC<sub>340</sub> and PBI values, while sources of microbial origin had significant CRS, DON, Trp, and Tyr levels. From figure 2.3.10, it is clear that SR and BL are different due to SR's significant PBI, but insignificant CRS, Trp, and Tyr. Conversely, BL has insignificant PBI, but significant CRS, Trp, and Tyr. PR has significant levels of CRS, Tyr, and DON. Since SR and BL have such comparable Pb-NOM binding curves, PBI, CRS, and Tyr may not be of importance when predicting source-dependent binding. What makes PR unique from SR and BL is its significant levels of DON, suggesting that DON may be an important parameter when predicting source-dependent Pb speciation.

It is also clear from figure 2.3.10 that the AM has significant PBI and SAC<sub>340</sub> values. However, despite having significant PBI, similar to SR, the speciation curves are very different for these two sources, further suggesting that PBI may not be a significant factor for assessing source-dependent Pb speciation. None of the other titrated sources (SR, BL, and PR) have significant SAC<sub>340</sub> values. It is therefore conceivable that SAC<sub>340</sub> and DON are the two factors that best describe the source-dependent Pb-NOM binding observed in figure 3.3.3, and may be important factors to include in WHAM modelling in order to allow for accurate prediction of Pb speciation in natural environments.

### **3.4 Conclusions**

As a non-essential metal, lead may cause toxicity, and poses potential risks to aquatic organisms. Although the speciation of inorganic Pb complexes is well defined and certified logK values exist, the chemistry of Pb interactions with NOM is not as well understood. In order to

better understand how Pb interacts with NOM, both characterization and speciation data is needed. This study has been able to confirm the accuracy and precision of the Pb ISE as a means of efficiently determining  $\text{Pb}^{2+}$  speciation in both freshwater and saltwater using a model ligand. However, using an internal calibration method, the speciation measurements in NOM did not agree with WHAM at low pH. WHAM predicted Pb-NOM binding at low pH, while this calibration method assumed no Pb-NOM binding would occur. This suggests that while an internal calibration method is effective for predicting speciation in the presence of a model ligand, it is not effective at predicting speciation in the presence of a much more complex matrix. An alternate calibration method – forcing speciation to agree with WHAM at low pH) – allowed for more reasonable measurements. However, regardless of the calibration method used, Pb-NOM binding displayed source-dependence. Titration data was compared to a WHAM VII model, which was not representative of the Pb-NOM binding within a factor of two. This has immense industrial and environmental implications, as source-dependent binding is not currently accounted for in WQGs.

### 3.5 References

- (1) Lewis, A.G; Cave, W. . In *Oceanography and Marine Biology*; Barnes, H., Ed.; 1982; pp 471–695.
- (2) Kitman, J. . *The Nation* **2000**, 270 (11), 11.
- (3) Mager, E. M. In *Fish Physiology - Homeostasis and Toxicology of Non-Essential Metals*; Wood, Chris M.;Farrell, Anthony P.;Brauner, C. J., Ed.; 2011; pp 186–225.
- (4) Nriagu, Jerome O.;Pacyna, J. M. *Nature* **1988**, 333, 134–139.
- (5) Abbasse, G. A.; Uddane, B. O.; Ischer, J. C. F. *Anal. Sci.* **2003**, 19 (4), 529–535.
- (6) UNEP. *Final review of scientific information on lead*; 2010.
- (7) DePalma, S. G. S.; Arnold, W. R.; McGeer, J. C.; Dixon, D. G.; Smith, D. S. *Appl. Geochemistry* **2011**, 26 (3), 394–404.
- (8) Sillanpaa, M. E. T. *Natural Organic Matter in Water: Characterization and Treatment Methods*, 1st ed.; 2015.
- (9) Di Toro, Dominic M; Allen, H. E.; Bergman, H. L.; Meyer, J. S.; Paquin, P. R.; Santore, R. C. *Environ. Toxicol. Chem* **2001**, 20 (10), 2383–2396.
- (10) CCME. In *Canadian Water Quality Guidelines*; Sheffer, M. G., Chong Chu, B., Andrews, J., Windle, W., Eds.; CCME, 2008.
- (11) Besser, B. J. M.; Leib, K. J. In *Integrated investigations of environmental effects of historical mining in the Animas River watershed, San Juan County, Colorado*; Church, S. E., von Guerard, P., Finger, E. S., Eds.; USGS, 2007.
- (12) Arche, Ghent University, K. and I. *Lead BLM SSD Normalization Tool*; Vol. Version 1.
- (13) Ministry of Environment and Parks. *Water Quality Criteria for Lead*; Victoria, BC, 1987; Vol. 2.

- (14) Paquin, P. R.; Santore, R. C.; Wu, K. B.; Kavvadas, C. D.; Di Toro, D. M. *Environ. Sci. Policy* **2000**, *3* (Supplement 1), 175–182.
- (15) Hatano, A.; Shoji, R. *Comp. Biochem. Physiol. Part C* **2010**, *151* (1), 25–32.
- (16) Nys, C.; Janssen, C. R.; Mager, E. M.; Esbaugh, A. J.; Brix, K. V.; Grosell, M.; Stubblefield, W. A.; Holtze, K.; De Schamphelaere, K. A. C. *Environ. Toxicol. Chem.* **2014**, *33* (2), 394–403.
- (17) De Schamphelaere, K. A. C.; Nys, C.; Janssen, C. R. *Aquat. Toxicol.* **2014**, *155*, 348–359.
- (18) A, S. L.; A, E. T. **2011**, 501–516.
- (19) OECD. In *OECD Guidelines for the Testing of Chemicals, Section 2*; 1992; p 9.
- (20) Tait, T. N. Determination of Copper Speciation, Bioavailability and Toxicity in Saltwater Environments, Wilfrid Laurier University, 2013.
- (21) Chen, W. The effects of Dissolved Organic Matter (DOM) sources on Pb <sup>2+</sup> , Zn <sup>2+</sup> and Cd <sup>2+</sup> binding, Trent University, 2017.

## **Chapter 4 – Conclusions and Future Work**

This chapter will include a brief summary of the objectives of this project and their corresponding results. Objectives I, II, and III have been restated below.

- I. To characterize NOM sources of varying composition and origin in terms of their functional group content using fluorescence and absorbance spectroscopy, acid-base titrations, HTCO/Hach kits, CRS, and thiol assay measurements. NOM sources were defined in terms of FI, PARAFAC, SAC<sub>340</sub>, PBI measurements; DON, CRS, and thiol content.*

NOM chemistry was found to vary greatly with source. CRS, Trp, and Tyr may be able to discriminate saltwater from freshwater sources, while SAC<sub>340</sub>, CRS, thiol, DON, Trp, Tyr, and PBI may be able to discriminate between freshwater sources. However, no one single parameter, or series of parameters was able to explain all of the variation between NOM sources.

- II. To validate the commercially available Pb ISE as a practical and effective method for determining trace level Pb speciation in freshwater and saltwater environments.*

The commercially available Pb ISE was found to be both an accurate and precise tool for measuring Pb<sup>2+</sup> speciation (using an internal calibration method) in both AFW and ASW in the presence of a model ligand. Experimental data was consistently within a factor of two when compared to the modelled Pb<sup>2+</sup> speciation as calculated using certified NIST logK values. However, when NOM sources were titrated using an internal calibration, experimental data disagreed considerably with WHAM predictions. WHAM predicted Pb-NOM binding would occur at low pH, which disagrees with the assumption made in the internal calibration (that Pb-NOM binding will not occur at low pH). Consequently, the ISE is useful for application in Pb<sup>2+</sup>

measurement in freshwater to seawater in the presence of a model ligand, but using an internal calibration method, it is not effective at determining Pb speciation in NOM.

*III. To determine Pb speciation in various NOM sources using a commercially available Pb ISE, compare it to Pb speciation modelled by the Windermere Humic Aqueous Model (WHAM), determine if WHAM accurately predicts Pb speciation, and deduce which NOM characteristics best explain Pb-NOM binding.*

Sources of varying origin were tested to determine if Pb-NOM binding was source dependent. Free lead speciation was different for the varied sources, indicating that Pb-NOM binding is indeed source-dependent. Experimental speciation data was then compared to a WHAM VII model, and the variation in speciation was not adequately represented by WHAM within a factor of two. The two characteristics that best described differences in the measured Pb speciation in freshwater NOM were DON and SAC<sub>340</sub>. This indicates that current regulations should be modified in order to account for site-specific speciation, and WHAM VII should be adjusted to incorporate DON and SAC<sub>340</sub>.

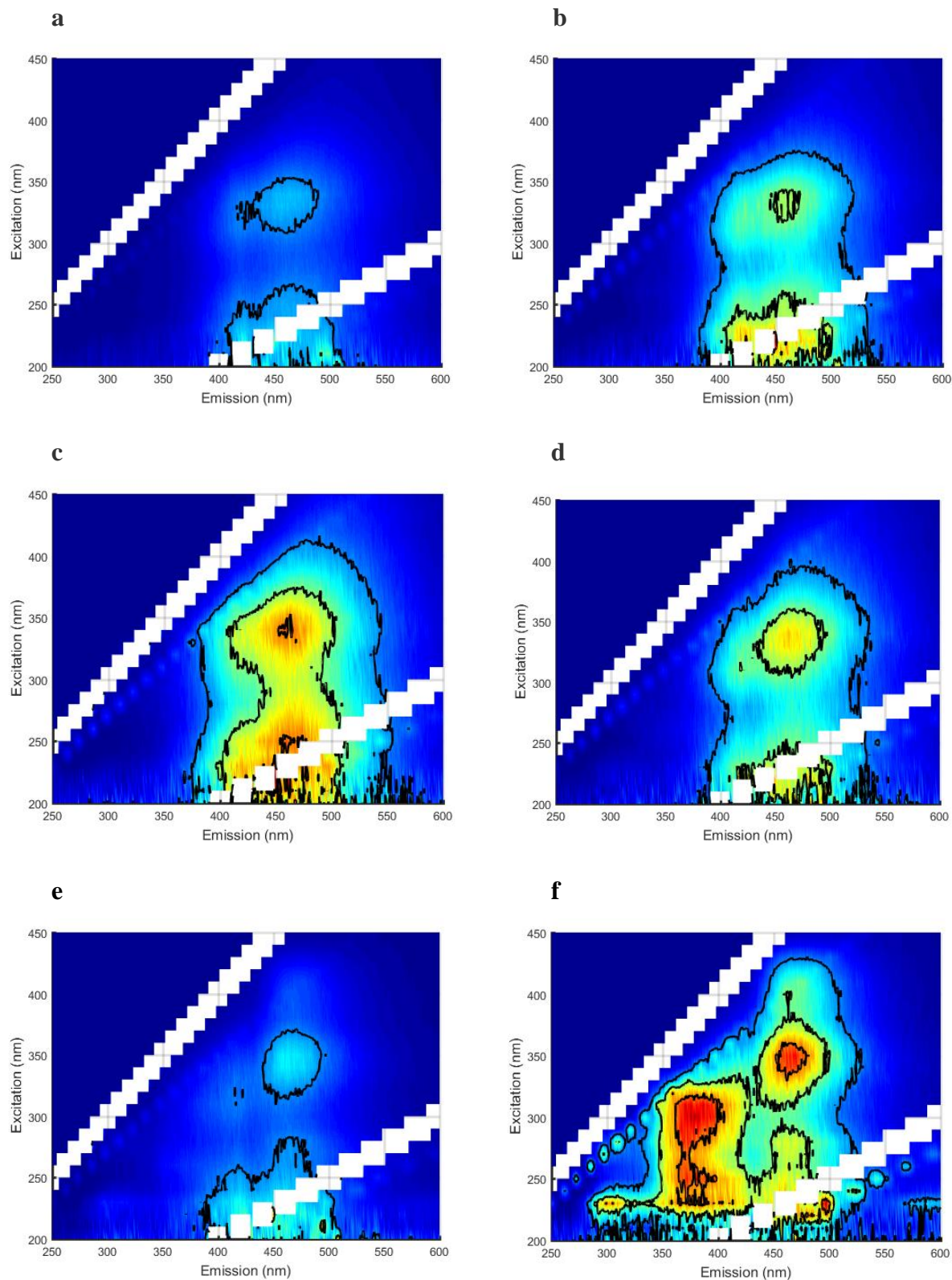
This study has proposed variation in NOM quality, potential NOM quality indicators, and NOM source-dependent Pb binding. However, the possibility of source-dependent Pb-NOM binding has not been explored in saltwater sources. Future work could thus entail:

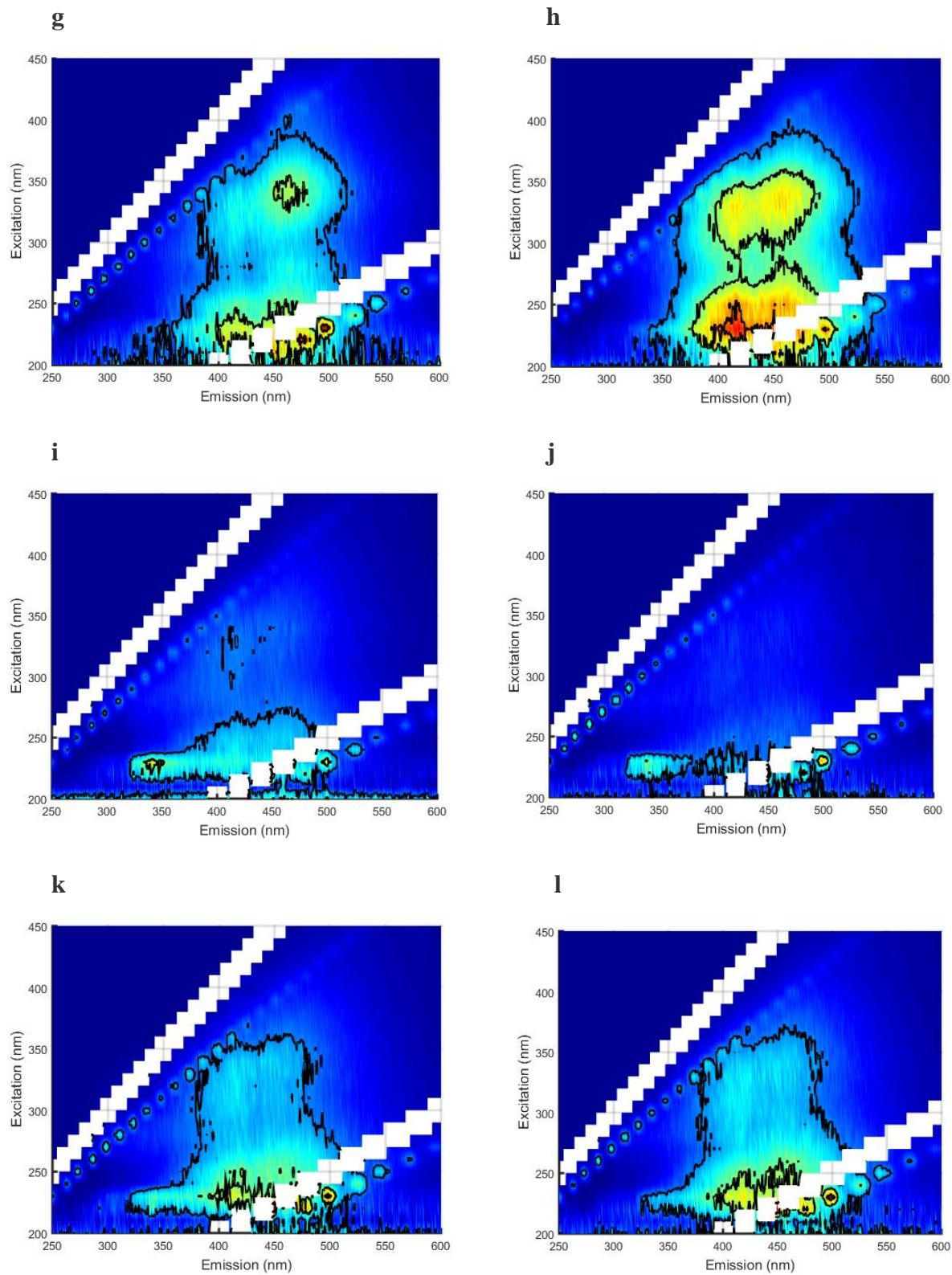
*I. Pb titrations in saltwater sources in order to determine if Pb-NOM binding is significantly different from that observed in freshwater, and if WHAM adequately predicts this speciation.*

- II. Toxicity tests using these NOM sources in order to determine  $LC_{50}$  values. If  $Pb^{2+}$  speciation is the same for all of these NOM sources at their respective  $LC_{50}$  values, then source dependent  $Pb^{2+}$  protectivity could be confirmed.



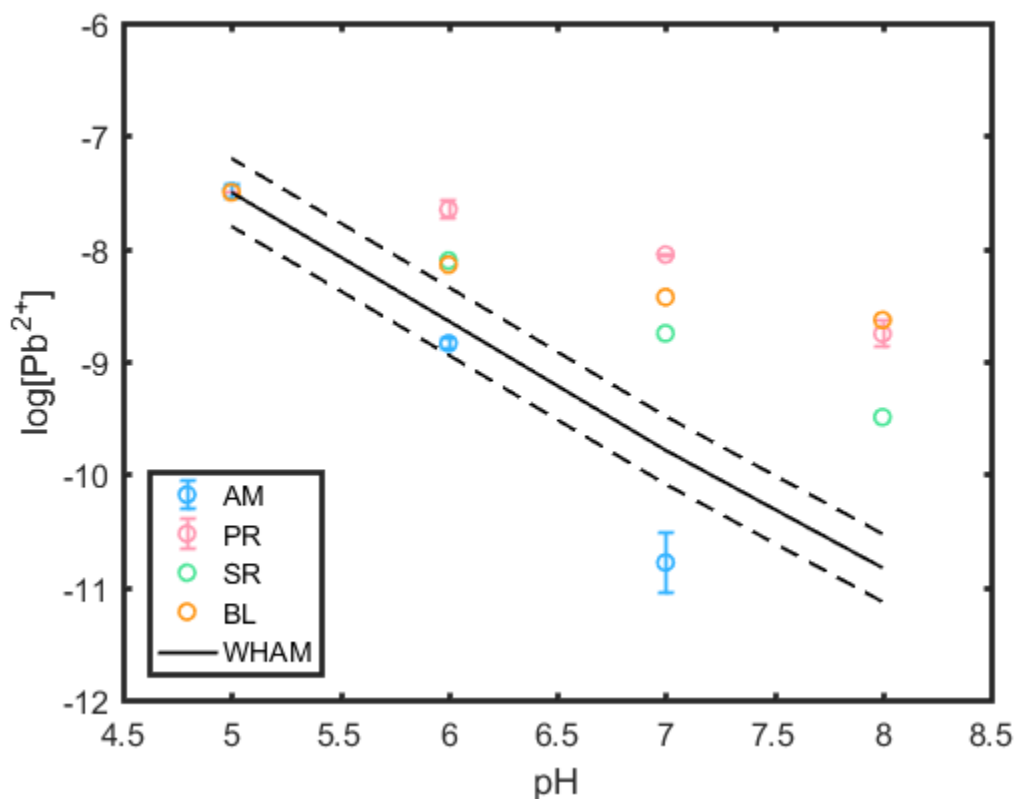
## Appendix A: Fluorescence Excitation-Emission Matrices for all NOM Sources



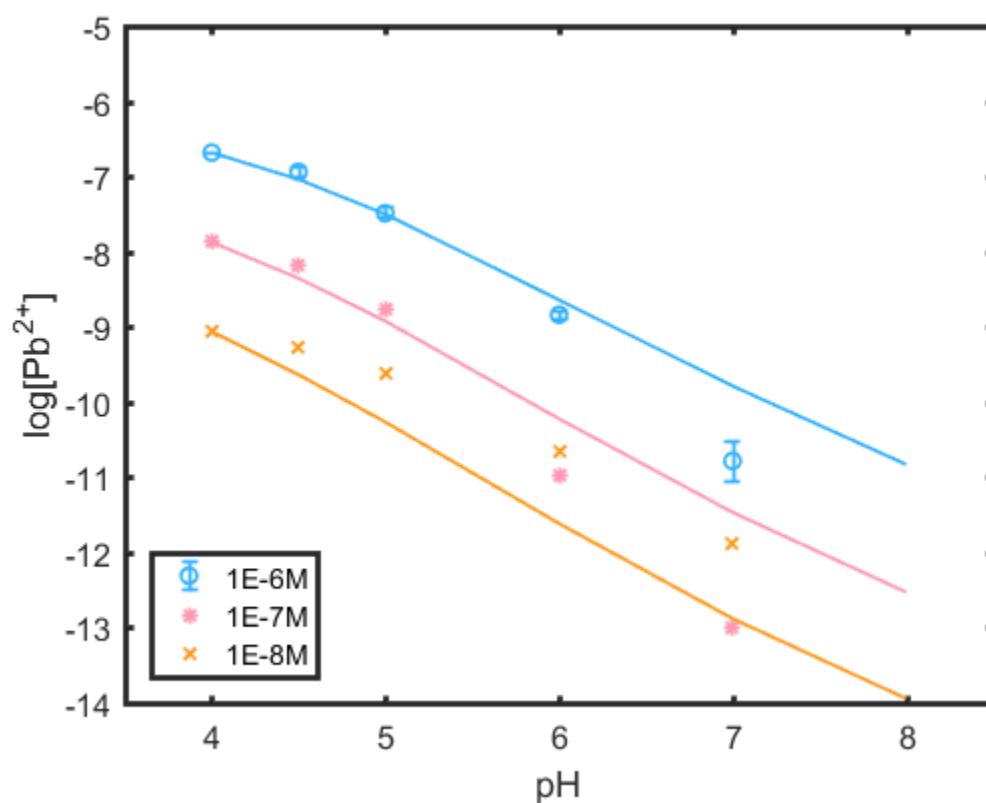


**Figure A.1** FEEMs of NOM sources of different origins: SR (a), NR (b), AM (c), LM (d), BL\* (e), PR\* (f), LO (g), GR\* (h), BTP\* (i), ELM (j), CCLT (k), CCHT (l).

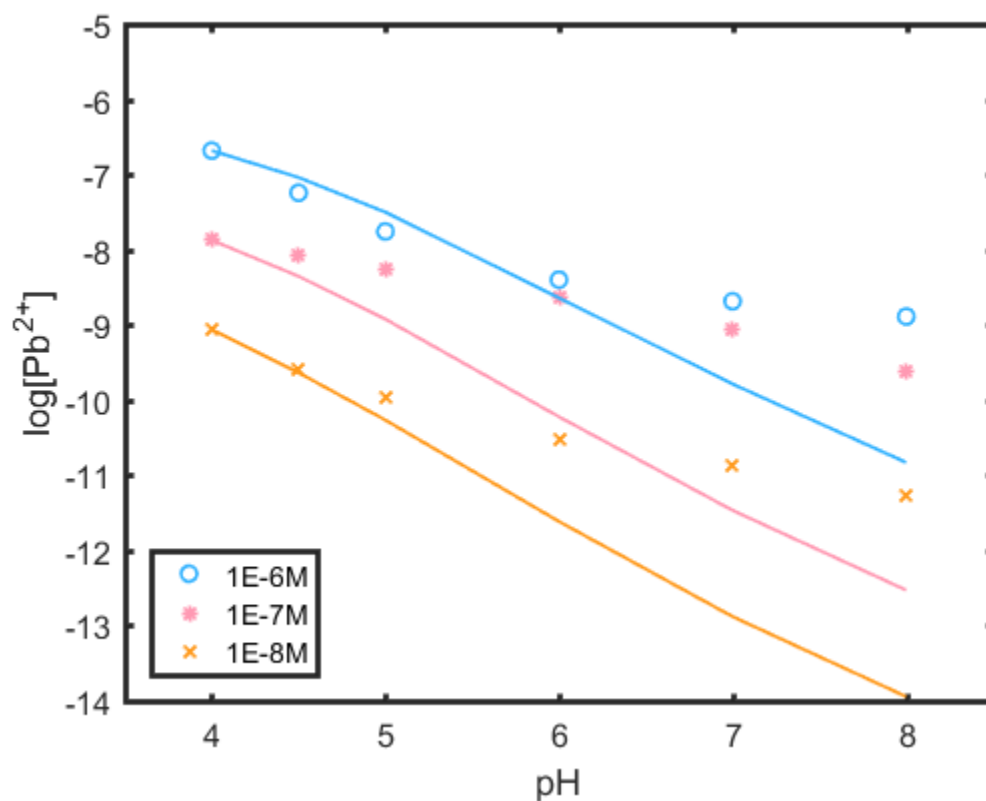
## Appendix B: Flow-Through Titration Data for Pb in NOM Adjusted to Agree with WHAM at low pH



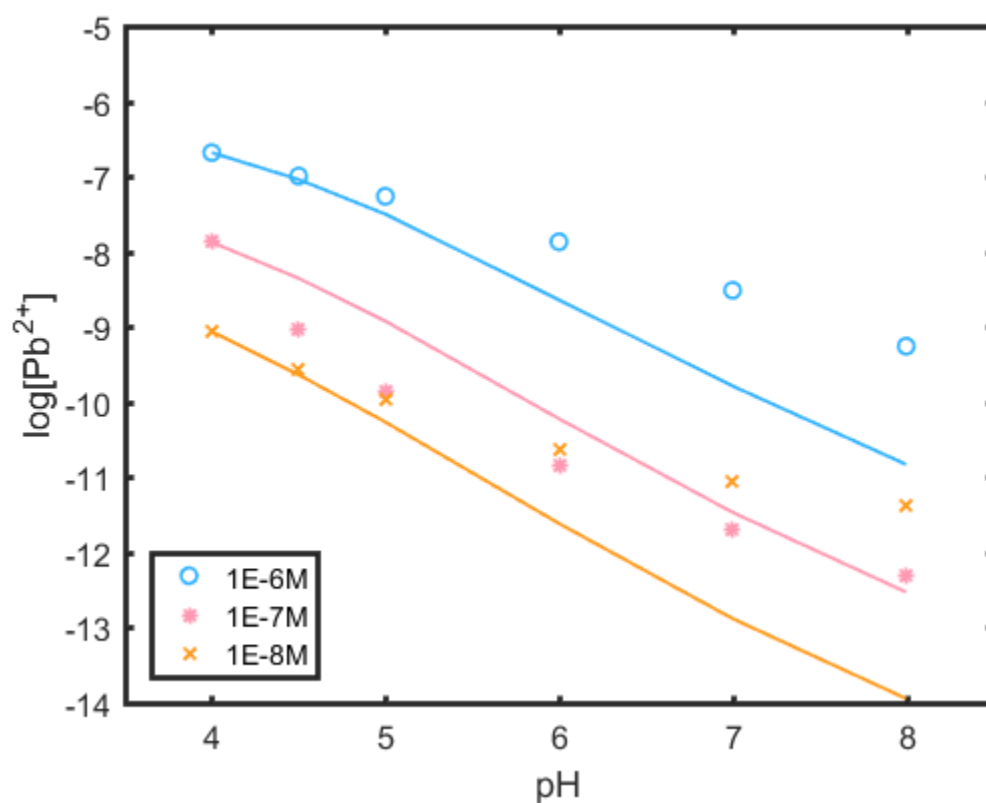
**Figure B.1** pH-dependent flow-through titration data (low pH forced to agree with WHAM) for all NOM sources at  $10^{-6}$  M total Pb. The solid line represents Pb speciation as predicted by WHAM VII, while the black dashed lines represent a factor of two above and below.



**Figure B.2** pH-dependent flow-through titration data (low pH forced to agree with WHAM) for AM NOM at varying concentrations of total Pb. Solid coloured lines represent Pb speciation as predicted by WHAM VII.



**Figure B.3** pH-dependent flow-through titration data (low pH forced to agree with WHAM) for BL NOM at varying concentrations of total Pb. Solid coloured lines represent Pb speciation as predicted by WHAM VII.



**Figure B.4** pH-dependent flow-through titration data (low pH forced to agree with WHAM) for SR NOM at varying concentrations of total Pb. Solid coloured lines represent Pb speciation as predicted by WHAM VII.

## **Appendix C: Tableaus Describing Chemical Equilibria used To Calculate Lead Spectiaion for Freshwater and Saltwater Model Ligand Titrations**

**Table C.1** Tableau for the aqueous interactions between Pb, inorganic species, and EN in AFW.

| <b>H<sup>+</sup></b> | <b>Pb<sup>2+</sup></b> | <b>Cl<sup>-</sup></b> | <b>SO<sub>4</sub><sup>2-</sup></b> | <b>CO<sub>3</sub><sup>2-</sup></b> | <b>EN<sup>4-</sup></b> | <b>logK</b> | <b>species</b>                                     |
|----------------------|------------------------|-----------------------|------------------------------------|------------------------------------|------------------------|-------------|--|
| 1                    | 0                      | 0                     | 0                                  | 0                                  | 0                      | 0           | <b>H<sup>+</sup></b>                               |
| 0                    | 1                      | 0                     | 0                                  | 0                                  | 0                      | 0           | <b>Pb<sup>2+</sup></b>                             |
| 0                    | 0                      | 1                     | 0                                  | 0                                  | 0                      | 0           | <b>Cl<sup>-</sup></b>                              |
| 0                    | 0                      | 0                     | 1                                  | 0                                  | 0                      | 0           | <b>SO<sub>4</sub><sup>2-</sup></b>                 |
| 0                    | 0                      | 0                     | 0                                  | 1                                  | 0                      | 0           | <b>CO<sub>3</sub><sup>2-</sup></b>                 |
| 0                    | 0                      | 0                     | 0                                  | 0                                  | 1                      | 0           | <b>EN<sup>2-</sup></b>                             |
| -1                   | 0                      | 0                     | 0                                  | 0                                  | 0                      | -13.9454    | <b>OH<sup>-</sup></b>                              |
| -1                   | 1                      | 0                     | 0                                  | 0                                  | 0                      | -7.5681     | <b>PbOH<sup>+</sup></b>                            |
| -1                   | 2                      | 0                     | 0                                  | 0                                  | 0                      | -17.0104    | <b>Pb(OH)<sub>2</sub></b>                          |
| -1                   | 3                      | 0                     | 0                                  | 0                                  | 0                      | -27.9376    | <b>Pb(OH)<sub>3</sub><sup>-</sup></b>              |
| 1                    | 0                      | 0                     | 0                                  | 1                                  | 0                      | 16.5316     | <b>HCO<sub>3</sub><sup>-</sup></b>                 |
| 2                    | 0                      | 0                     | 0                                  | 1                                  | 0                      | 10.2308     | <b>H<sub>2</sub>CO<sub>3</sub></b>                 |
| 1                    | 1                      | 0                     | 0                                  | 1                                  | 0                      | 13.3262     | <b>PbHCO<sub>3</sub><sup>+</sup></b>               |
| 0                    | 1                      | 0                     | 0                                  | 1                                  | 0                      | 7.1320      | <b>PbCO<sub>3</sub></b>                            |
| 0                    | 1                      | 0                     | 0                                  | 2                                  | 0                      | 10.2664     | <b>Pb(CO<sub>3</sub>)<sub>2</sub><sup>2-</sup></b> |
| 0                    | 1                      | 0                     | 1                                  | 0                                  | 0                      | 2.6620      | <b>PbSO<sub>4</sub></b>                            |
| 0                    | 1                      | 1                     | 0                                  | 0                                  | 0                      | 1.4532      | <b>PbCl<sup>+</sup></b>                            |
| 0                    | 1                      | 2                     | 0                                  | 0                                  | 0                      | 1.8081      | <b>PbCl<sub>2</sub></b>                            |
| 0                    | 1                      | 3                     | 0                                  | 0                                  | 0                      | 1.6966      | <b>PbCl<sub>3</sub><sup>-</sup></b>                |
| 1                    | 0                      | 0                     | 0                                  | 0                                  | 1                      | 9.9253      | <b>HEN<sup>3-</sup></b>                            |
| 2                    | 0                      | 0                     | 0                                  | 0                                  | 1                      | 6.9076      | <b>H<sub>2</sub>EN<sup>2-</sup></b>                |
| 0                    | 1                      | 0                     | 0                                  | 0                                  | 1                      | 5.0500      | <b>PbEN<sup>2-</sup></b>                           |
| 0                    | 1                      | 0                     | 0                                  | 0                                  | 2                      | 18.8215     | <b>PbEN<sub>2</sub><sup>4-</sup></b>               |



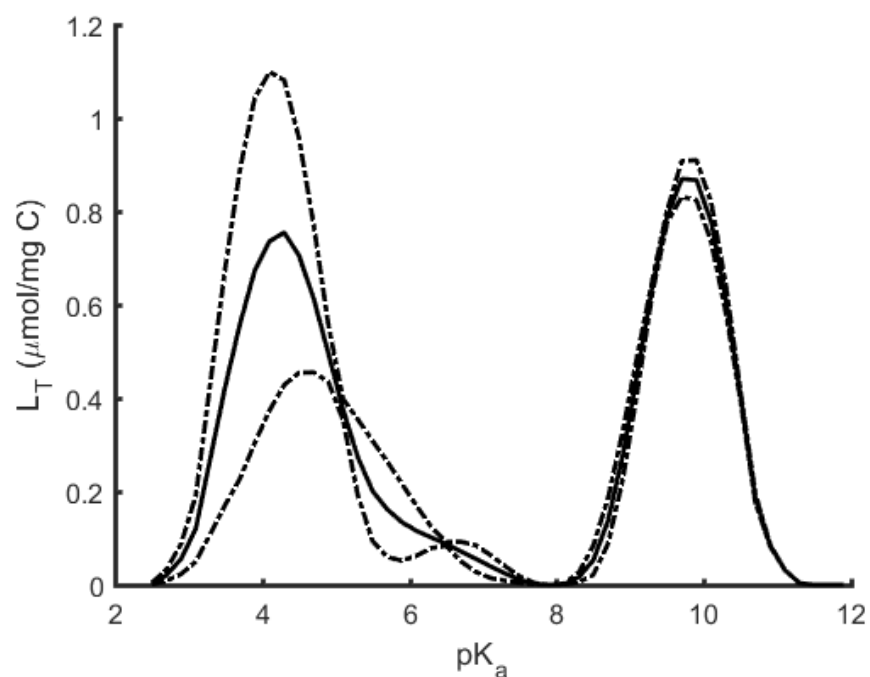
**Table C.2** Tableau for the aqueous interactions between Pb, inorganic species, and EN in ASW.

| <b>H<sup>+</sup></b> | <b>Pb<sup>2+</sup></b> | <b>Cl<sup>-</sup></b> | <b>SO<sub>4</sub><sup>2-</sup></b> | <b>CO<sub>3</sub><sup>2-</sup></b> | <b>EN<sup>4-</sup></b> | <b>logK</b> | <b>species</b>                                     |
|----------------------|------------------------|-----------------------|------------------------------------|------------------------------------|------------------------|-------------|--|
| 1                    | 0                      | 0                     | 0                                  | 0                                  | 0                      | 0           | <b>H<sup>+</sup></b>                               |
| 0                    | 1                      | 0                     | 0                                  | 0                                  | 0                      | 0           | <b>Pb<sup>2+</sup></b>                             |
| 0                    | 0                      | 1                     | 0                                  | 0                                  | 0                      | 0           | <b>Cl<sup>-</sup></b>                              |
| 0                    | 0                      | 0                     | 1                                  | 0                                  | 0                      | 0           | <b>SO<sub>4</sub><sup>2-</sup></b>                 |
| 0                    | 0                      | 0                     | 0                                  | 1                                  | 0                      | 0           | <b>CO<sub>3</sub><sup>2-</sup></b>                 |
| 0                    | 0                      | 0                     | 0                                  | 0                                  | 1                      | 0           | <b>EN<sup>2-</sup></b>                             |
| -1                   | 0                      | 0                     | 0                                  | 0                                  | 0                      | -13.7447    | <b>OH<sup>-</sup></b>                              |
| -1                   | 1                      | 0                     | 0                                  | 0                                  | 0                      | -7.7447     | <b>PbOH<sup>+</sup></b>                            |
| -1                   | 2                      | 0                     | 0                                  | 0                                  | 0                      | -17.2615    | <b>Pb(OH)<sub>2</sub></b>                          |
| -1                   | 3                      | 0                     | 0                                  | 0                                  | 0                      | -27.3773    | <b>Pb(OH)<sub>3</sub><sup>-</sup></b>              |
| 1                    | 0                      | 0                     | 0                                  | 1                                  | 0                      | 15.5123     | <b>HCO<sub>3</sub><sup>-</sup></b>                 |
| 2                    | 0                      | 0                     | 0                                  | 1                                  | 0                      | 9.5395      | <b>H<sub>2</sub>CO<sub>3</sub></b>                 |
| 1                    | 1                      | 0                     | 0                                  | 1                                  | 0                      | 16.5394     | <b>PbHCO<sub>3</sub><sup>+</sup></b>               |
| 0                    | 1                      | 0                     | 0                                  | 1                                  | 0                      | 4.8582      | <b>PbCO<sub>3</sub></b>                            |
| 0                    | 1                      | 0                     | 0                                  | 2                                  | 0                      | 5.4008      | <b>Pb(CO<sub>3</sub>)<sub>2</sub><sup>2-</sup></b> |
| 0                    | 1                      | 0                     | 1                                  | 0                                  | 0                      | 1.7273      | <b>PbSO<sub>4</sub></b>                            |
| 0                    | 1                      | 1                     | 0                                  | 0                                  | 0                      | 0.8847      | <b>PbCl<sup>+</sup></b>                            |
| 0                    | 1                      | 2                     | 0                                  | 0                                  | 0                      | 1.2045      | <b>PbCl<sub>2</sub></b>                            |
| 0                    | 1                      | 3                     | 0                                  | 0                                  | 0                      | 1.1309      | <b>PbCl<sub>3</sub><sup>-</sup></b>                |
| 1                    | 0                      | 0                     | 0                                  | 0                                  | 1                      | 10.0870     | <b>HEN<sup>3-</sup></b>                            |
| 2                    | 0                      | 0                     | 0                                  | 0                                  | 1                      | 7.3667      | <b>H<sub>2</sub>EN<sup>2-</sup></b>                |
| 0                    | 1                      | 0                     | 0                                  | 0                                  | 1                      | 5.0500      | <b>PbEN<sup>2-</sup></b>                           |
| 0                    | 1                      | 0                     | 0                                  | 0                                  | 2                      | 17.9838     | <b>PbEN<sub>2</sub><sup>4-</sup></b>               |

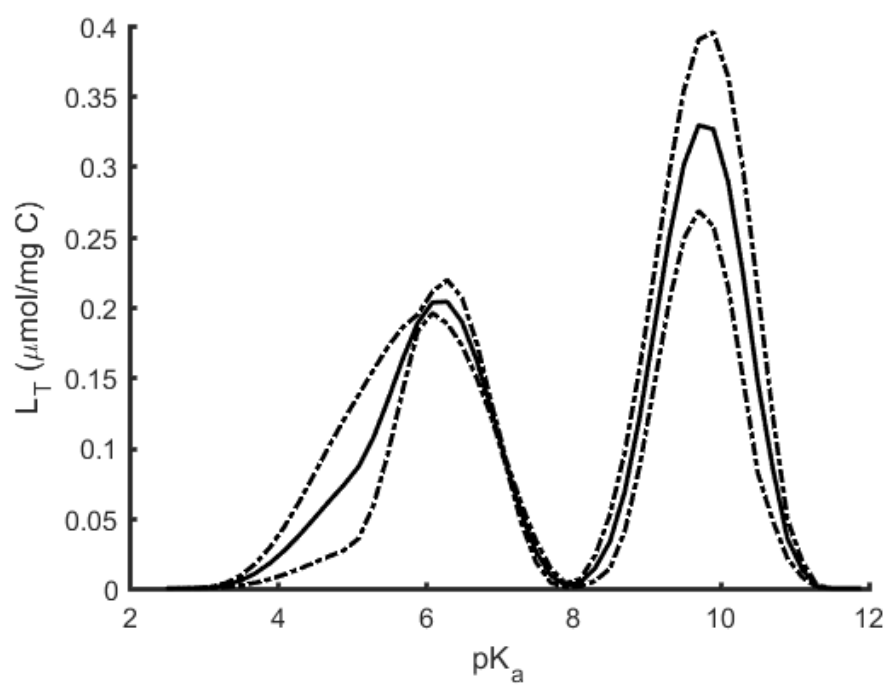
**Table C.3** Tableau for the solid phase interactions between Pb, inorganic species and EN.

| <b>H<sup>+</sup></b> | <b>Pb<sup>2+</sup></b> | <b>Cl<sup>-</sup></b> | <b>SO<sub>4</sub><sup>2-</sup></b> | <b>CO<sub>3</sub><sup>2-</sup></b> | <b>EN<sup>4-</sup></b> | <b>logK</b> | <b>species</b>               |
|----------------------|------------------------|-----------------------|------------------------------------|------------------------------------|------------------------|-------------|------------------------------|
| -2                   | 1                      | 0                     | 0                                  | 0                                  | 0                      | -12.6       | <b>Pb(OH)<sub>2(s)</sub></b> |
| 0                    | 1                      | 0                     | 0                                  | 1                                  | 0                      | 13.1        | <b>PbCO<sub>3(s)</sub></b>   |
| 0                    | 1                      | 2                     | 0                                  | 0                                  | 0                      | 4.8         | <b>PbCl<sub>2(s)</sub></b>   |
| 0                    | 1                      | 0                     | 1                                  | 0                                  | 0                      | 7.8         | <b>PbSO<sub>4(s)</sub></b>   |

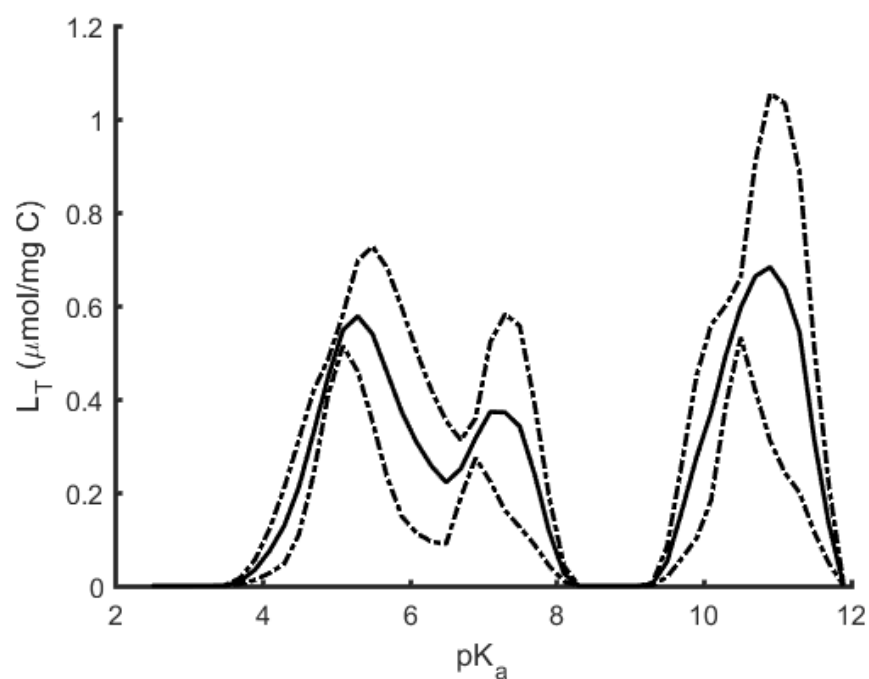
## Appendix D: Proton Affinity Spectra for Freshwater NOM Sources



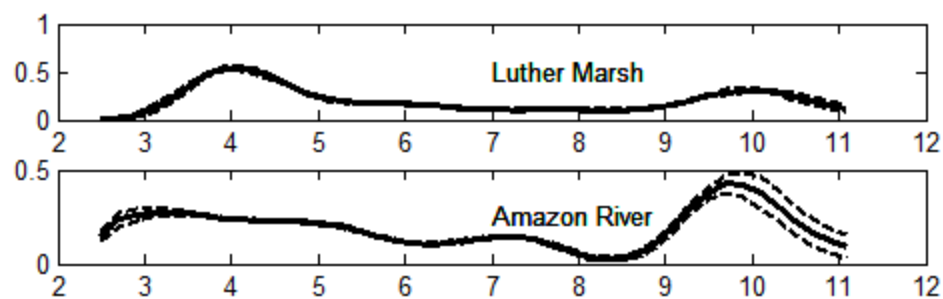
**Figure D.1** Proton affinity spectrum (proton binding capacity ( $L_T$ ) vs.  $\text{pK}_a$ ) for BL (determined by fully optimized model (FOCUS) of proton titration data). The solid line represents the mean spectra while the dashed lines represent standard error.



**Figure D.2** Proton affinity spectrum (proton binding capacity ( $L_T$ ) vs.  $\text{pK}_a$ ) for SR (determined by fully optimized model (FOCUS) of proton titration data). The solid line represents the mean spectra while the dashed lines represent standard error.



**Figure D.3** Proton affinity spectrum (proton binding capacity ( $L_T$ ) vs.  $\text{pK}_a$ ) for NR (determined by fully optimized model (FOCUS) of proton titration data). The solid line represents the mean spectra while the dashed lines represent standard error.



**Figure D.4** Proton binding spectrum for LM and AM. The solid lines represent average spectra, while the dashed lines represent standard errors.