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# The influence of calcium and dissolved organic matter on the acute and chronic toxicity of nickel to Hyalella azteca

Katherine Chan
chan8920@mylaurier.ca

# The influence of calcium and dissolved organic matter on the acute and chronic toxicity of nickel to *Hyalella azteca*

by

**Katherine Chan**

HBSc Biology, University of Toronto at Mississauga, 2010

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# Abstract

This study focuses on the effect of Ca and dissolved organic matter (DOM) on Ni toxicity to *Hyalella azteca* in soft waters (interpreted in the context of the biotic ligand model (BLM)) and is linked to a larger project directed at understanding the recovery of aquatic systems from long term smelter damage. Amphipods (source: Hannah Lake, Sudbury ON) were cultured and tested in soft waters (12 mg $CaCO_3$/L, pH 7.0, 21ºC) following Environment Canada standard method EPS 1/RM/33 (Environment Canada, 1997). Effects of Ca, but not Mg, were observed where a 5-fold increase in protection was a result of an increase of Ca concentrations from 0.1 to 2.0 mM. DOM was collected from sites previously impacted and recovering from long term smelter emissions and also from reference sites in central Ontario. Dissolved organic carbon (DOC) concentrations at 6 mg/L and higher offered protection against Ni toxicity. Acute toxicity tests with different DOM sources (at 6 mg DOC/L) showed variation in protective capacity but no clear links to measured optical characteristics were observed. DOM sources also reduced short term (6h) whole body accumulation of Ni but there was no correlation between the capacity of DOM to reduce accumulation and its ability to mitigate toxicity. Application of the BLM illustrated that acute toxicity could be modelled reasonably well except for some effects of Ca (2.0mM). Chronic (28d) effects on *Hyalella* occurred at much lower Ni concentrations (acute to chronic ratio of approximately 50) and the protective effects of Ca and DOM were proportionally similar. For example, 1mM Ca increased the chronic $LC_{50}$ by 3-fold. Growth (assessed as dry weight) was generally a more sensitive indicator of impacts than survival. In exposures without modifying factors, the $EC_{20}$ and $EC_{50}$ for growth were 1.4 and 12.7 µg Ni/L, respectively, while the $LC_{50}$ was 13.8 (CI: 11.5 - 16.7) µg Ni/L. Contrary to other studies, this research did not show

relationships between DOM quality and toxicity mitigation and this demonstrates the need for an improved understanding of DOM characteristics in relation to the potential impact of Ni.

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# Authors declaration

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Katherine Chan

# Glossary

BLM ..... biotic ligand model

CETIS.....comprehensive environmental toxicity information system

CI.....confidence interval

DOC .....dissolved organic carbon

DOM ..... dissolved organic matter

EC20.....concentration associated with a 20% effect

EC50.....concentration associated with a 50% effect

EEMS ..... excitation emission matrix spectroscopy

FA .....fulvic acid

FI ..... fluorescence index

HA .....humic acid

LA50 .....accumulation associated with 50% lethality

LC50.....concentration associated with 50% lethality

PARAFAC ..... parallel factor analysis

SAC .....specific absorbance coefficient

SD.....standard deviation

SEM..... standard error of mean

TALER ..... Terrestrial Aquatic Linkages for Ecosystem Recovery

UV ..... ultraviolet

WHAM..... Windermere humic aqueous model

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# Chapter 1 Introduction

### *1.1 Hyalella azteca*

*Hyalella azteca* is an omnivorous and benthic amphipod (class Crustacea) (Pennak, 1953). They can be identified by several distinct features including two sets of anterior antennae where the inner set lacks an accessory flagellum and are shorter than the outer set as well as, the presence of teeth (spines) on the abdominal pleon (segments 1 and 2; Environment Canada, 1997). Males are usually larger than females and reach a length of 8mm and 6mm (respectively) as adults (Environment Canada, 1997). *Hyalella* are broadly distributed throughout shallow fresh-water lakes in the Americas (Othman and Pascoe, 2001; Gonzalez and Watling, 2002). They occur in a wide variety of environmental conditions (e.g., hardness of 20 to 250 mg $CaCO_3$/L and pH 6.0 to 8.0; Environment Canada, 1997) and tend to be more abundant in waters that have summer surface temperatures of ≥ 10 ºC (Environment Canada, 1997). They occupy the lower end of the food chain in littoral zones often depending on vegetation for cover (i.e., protection; Environment Canada, 1997). As omnivores, their diet consists of a variety of food sources such as bacteria, algae and detritus (Hargrave, 1970).

The lifecycle of *Hyalella* occurs across nine or more instars (i.e., molting periods) which usually spans over a period of 1 year (Pennak, 1953). The first five instars form the juvenile period and reproduction first occurs during the start of the adult period (i.e., eighth instar; within about 15 days; Pennak, 1953). Generation time will vary according to environmental conditions but under standard culturing conditions in the lab (pH 7, 20ºC), it is approximately 33 days (Environment Canada, 1997). *Hyalella* reproduce sexually (Strong, 1972) where males actively search for and mate with females and attach themselves to females for periods of time that range from hours to days (Othman and Pascoe, 2001). Fertilization and development of eggs occur in the marsupium (brood pouch) and under ideal conditions, about 18 offspring can be produced per

brood and 15 broods over a 152 day period (Pennak, 1953). *Hyalella* adapt well to laboratory cultures, they are resilient and reliably produce viable offspring over the course of their life.

The lifecycle characteristics and ease of culture of *Hyalella* make them good candidates for toxicity testing in the context of environmental risk assessment. As well, they are sensitive to a variety of aquatic contaminants particularly metals (Blockwell et al., 1999; Borgmann, 1998; Environment Canada, 1997; Ingersoll et al., 1998; Nebeker & Miller, 1988). The suitability of *Hyalella* for assessing environmental risks is reflected by the development of standard culture and testing methods. Both Environment Canada and the Environmental Protection Agency have developed standardized methods (EPS 1/RM/33, Environment Canada, 1997; EPA 440/5-85-001, US-EPA, 1986). The standard methods according to Environment Canada (1997) are recommended for performing sediment toxicity testing although alterations can be made for water exposures. Environment Canada (1997) guidelines include: static cultures with weekly water renewals, 96 h acute exposures, 28 day chronic exposures, and the use of 2 – 9 day old *Hyalella* for initiating tests. The guidelines account for the lifecycle, reproduction, and feeding habits of *Hyalella* which are different than other standard invertebrate test species such as *Daphnia*. Understanding the potential impacts for contaminants to aquatic biota using *Hyalella* is therefore possible using toxicity tests following standard methods.

## *1.2 Nickel*

### *1.2.1 Impacts of Ni on the environment*

Nickel is a naturally occurring element in the environment where concentrations can range from 10 to 1000 µg/L in waters (uncontaminated and contaminated respectively; Eisler,

1998). Elevated levels of Ni in aquatic environments can be a result of aerial and effluent emissions associated with mining and smelting operations that have occurred or are occurring in the area, for example, the Sudbury region of central Ontario (Keller, 1992). In the Sudbury area increased concentrations of metals and high acidity have impacted aquatic biota since the turn of the twentieth century (Keller et al., 2007). Sudbury lakes have been severely impacted but as a result of reduced smelter emissions (Keller et al., 1992) and pollution control (Keller et al., 2007), water quality has significantly improved. In spite of these improvements, the recovery of zooplankton diversity is problematic in some lakes and Ni has been suggested to be a limiting factor in this recovery (Yan et al., 1996; Keller et al., 2007)

### *1.2.1 Toxicity of Ni, the influence of geochemistry, and indicators of toxicity*

The impacts of Ni are not as well studied as other metals (e.g. Cu or Zn) however there is a reasonable understanding of acute toxicity, factors that modify its toxicity and the physiological mechanisms of uptake. Ni has been linked to the disruption of Mg balance in invertebrate species such as *Daphnia magna* (Pane et al., 2003a) while it impacts respiratory functions in fish (Pane et al., 2004), resulting in impaired gas exchange at the gill (Pane et al., 2003a). In acute exposures, $LC_{50}$ values in relatively hard waters ranged from 3.1 mg Ni/L for *Hyalella azteca* (Keithly et al., 2004) to 15.3 mg Ni/L for rainbow trout (Pane et al., 2003b). An acute toxic effect caused by Ni, in relatively soft water (30 mg $CaCO_3$/L), was observed at a $LC_{50}$ value of 15.9 µM (0.93 mg Ni/L) with *Daphnia pulex* (Kozlova et al, 2009). In the Kozlova et al. (2009) study a reduction of Ni toxicity occurred in the presence of Ca (tested concentrations ranged from 0.02 to 1.25mM), Mg (0.01 to 1.44 mM) and DOM (0.5 to 41 mg C/L). For Ca, Mg and DOM, $LC_{50}$

values increased by 18-fold, 2.3-fold and 3-fold respectively. Therefore Ca provided greater protection against Ni toxicity than Mg and DOM.

Ni is a metal that can cause acute as well as chronic toxic effects (Kozlova et al., 2009; Schroeder et al., 2010; Keithly et al., 2004). Fourteen day $LC_{50}$ values ranged from 25.1 to >118 µg Ni/L for various invertebrates in hard water (Deleebeeck et al., 2007). In particular, chronic exposures with *Hyalella azteca* resulted in a 14-d $EC_{20}$ growth effect of 61 µg Ni/L (Keithly et al., 2004) and a 7-d $LC_{50}$ value of 0.3 µM Ni/L (17.61 µg Ni/L) (Schroeder et al., 2010). In the study by Schroeder et al. (2010), toxicity impacts of Ni are reduced in the presence of Ca (10 to 130 mM) and Mg (7.2 to 47 mM) where $LC_{50}$ values increased 8.5-fold and 6.7-fold respectively. In the chronic study by Schroeder et al. (2010), both Ca and Mg protected against Ni toxicity although Ca offered more protection than Mg, which was also demonstrated in the acute studies of Kozlova et al. (2009).

Metal toxicity is influenced by water chemistry (Richards et al., 2001; Kozlova et al., 2009; Clifford and McGeer, 2008) where the competition of $Ca^{2+}$ and $Mg^{2+}$ with $Ni^{2+}$ as well as complexation with DOM provide protective effects. Ca and Mg content are often expressed in terms of water hardness, defined as the concentration of multivalent cations and expressed in units of mg/L (weight/volume) or parts per million (ppm) of calcium carbonate ($CaCO_3$). Generally, concentrations of the two most prevalent cations (i.e., calcium and magnesium) determine hardness although other cations (i.e., sodium and potassium) are present and may interact with Ni in a different manner that influences bioavailability. Waterborne $Ca^{2+}$ and $Mg^{2+}$ compete with $Ni^{2+}$ for uptake on the biotic ligand therefore reducing toxic effects (Kozlova et al., 2009; Schroeder et al., 2010). DOM reduces toxicity because it binds free ions ($Ni^{2+}$) thus reducing overall Ni bioavailability (Schroeder et al., 2010). This complexation results in an

inverse relationship between DOM concentration and Ni toxicity (i.e., concentration dependent mitigation of toxicity). Studies suggest that different sources of DOM have different metal-binding capabilities and therefore vary in their capacity to protect against metals (Richards et al., 2001; Kozlova et al., 2009).

The effects of water chemistry on metal toxicity can be quantified by endpoints such as survival, growth, and reproduction, but there has been interest in using bioaccumulation as an indicator of toxicity as well. In theory, bioaccumulation accounts for differences in exposure bioavailability and uptake and thus potentially could be a good indicator of toxicity (Borgmann et al., 2001). However, other studies have demonstrated clearly that whole body metal bioaccumulation cannot be linked to chronic impacts (McGeer et al., 2003; Adams et al., 2010). Linking metal accumulation to observed toxic effects in chronic exposures is difficult since some organisms have the ability to eliminate, store or detoxify metals through metallothionein or metallothionein-like proteins (Adams et al., 2010). Not all accumulated metal will be associated with toxicity and much of it may be in detoxified forms. When measuring bioaccumulation, especially on a whole body basis, all of these different forms are measured together. To date it has not been possible to definitely measure the fraction of bioaccumulated metal associated with toxicity.

### *1.2.2 Effects of water chemistry on Ni speciation*

Ni in solution can occur in a variety of different forms (known as species). For example, in the culture medium that was used for *Hyalella* (0.1mM $CaCl_2$-$2H_20$, 0.1mM $NaHCO_3$, 0.025mM $MgSO_4$-$7H_2O$, 0.005mM KCl, and 0.001mM NaBr), species such as $Ni^{2+}$, $NiHCO_3^+$,

$NiOH^+$, $NiOH_2$, $NiCl^+$, $NiSO_4$ and $NiCO_3$ will be present. According to the Windermere Humic Aqueous Model (WHAM, v. 7.0), the main species present in solution are $Ni^{2+}$ (88.8%), $NiHCO_3^+$ (7.8%), $NiSO_4$ (0.4%) and $NiCO_3$ (3.6%). The presence of elevated anion concentrations may also influence Ni species formed in solution. In general, the free ion form ($Ni^{2+}$) is considered to be the most available and most toxic (Pagenkopf, 1983).

Different water chemistries will result in different speciation profiles and these have been linked to bioavilability and toxicity. The addition of anions such as chloride ($Cl^-$), bicarbonate ($HCO_3^-$) and sulfate ($SO_4^{2-}$) may alter $Ni^{2+}$ concentrations and therefore possibly affect the degree of toxic impacts (Kozlova et al., 2009). For example, daphnids exposed to enriched bicarbonate (150-250 mg/L) were extremely sensitive to Ni where $LC_{50}$ values decreased from 6.7 to 3.4 µg Ni/L (Puttaswamy and Liber, 2012) suggesting that Ni-carbonate species may induce toxic effects. On the contrary, no significant protective effects on Ni toxicity to daphnids occurred with increased levels of chloride and sulfate since $LC_{50}$s were similar between groups (Puttaswamy and Liber, 2012). This observation was also demonstrated other studies where chloride showed no effect on acute Ni toxicity (Kozlova et al., 2009) and the addition of Ni did not disturb $Cl^-$ balance (Pane et al., 2003a).

### *1.2.3 Development of the Biotic Ligand Model*

The biotic ligand model (BLM) is used as a tool to calculate metal speciation and predict site-specific toxicity in aquatic systems (Keithly et al., 2004; Kozlova et al., 2009; Clifford and McGeer, 2010). It accounts for the metal concentration as well as cations, anions and species of metal in solution giving estimates of metal bioavailability based on the physiological

mechanisms of toxicity (Keithly et al., 2004; Kozlova et al., 2009; Clifford and McGeer, 2010). The model can be used to characterize accumulation from a specific exposure but it is important to note that the model oversimplifies the complex interactions associated with metal uptake and accumulation (McGeer et al., 2010). The BLM modelling approach has been used in the deriviation of water quality criteria.

The BLM model predicts interactions at the site of uptake and the degree of toxic impact (McGeer et al., 2010). The site of uptake for *Hyalella* is unknown. Through the consideration of biological aspects of the organism, it can be speculated that the site of uptake (and even toxic action) may be the gill. Although the actual sites of uptake and toxic impact cannot be defined in *Hyalella azteca*, the BLM is still able to assess and predict metal to binding site (i.e., biotic ligand) interactions and these can be correlated to the measured toxic effects. As such the biotic ligand is a virtual entity and modelled endpoint although its origins are based on measurements of accumulation effect relationships in fish (Di Toro et al., 2001).

The free metal ion can cause detrimental effects (Pagenkopf, 1983) as a result of metal interactions (e.g., binding) at the site of toxicity (e.g., biotic ligand). The BLM is conceptually based on toxicity and it's relation to the metal concentration in solution, competition between cations and free metal ions at the biotic ligand, and metal-ligand complexation (Di Toro et al., 2001). It has been demonstrated that toxicity is reduced in the presence of cations ($Ca^{2+}$ and $Mg^{2+}$) since toxic metal cations (e.g., $Ni^{2+}$) compete with other dissolved cations for uptake (Kozlova et al., 2009). For example, Kozlova et al. (2009) found a significant decrease in Ni toxicity with increased concentrations of calcium and magnesium. Complexation of free metal ions, such as $Ni^{2+}$, to DOM significantly reduces toxic effects (Kozlova et al., 2009). Furthermore, the concentration of metal affects the ratio between bioavailable and complexed

forms of the metal, and therefore influences the potential for toxicity.

The BLM was initially developed around Cu, Cd, Zn (Di Toro et al., 2001), Ag (McGeer et al., 2000) on its effect of metal to aquatic biota, and has recently been extended to the effects of Ni (Meyer et al., 1999; Keithly et al., 2004; Deleebeeck et al., 2007; Kozlova et al., 2009). The principles of the BLM were initially demonstrated to be applicable to Ni through studies performed by Meyer et al. (1999) with fathead minnow. A BLM was developed by Deleebeeck et al. (2007) to predict the toxic effects of Ni with different degrees of water hardness for various invertebrates (e.g., *Ceriodaphnia quadrangula*, *Daphnia longispina*, and *Ceriodaphnia pulchella*). The research by Deleebeeck et al. (2007) demonstrated that the protective effects caused by water hardness need to be incorporated into the bioavilability model for Ni. Another BLM (Kozlova et al., 2009) was developed to predict the toxicity of free $Ni^{2+}$ on *Daphnia pulex* with experiments involving different water chemistries where concentrations of $Ca^{2+}$, $Mg^{2+}$, $K^{+}$, $Na^{+}$, pH and DOM were varied. This model can be adjusted to match the observed effect of Ni on *Hyalella* by changing modeling parameters (critical value (i.e., $LA_{50}$ value) or logK values) to find a model of 'best fit' for this study. Although the BLM is mainly used as a tool to predict acute metal toxicity, this approach has been successfully extended to the chronic toxicity of Ni on *Hyalella* by Schroeder et al. (2010). Adjustments to the Kozlova et al. (2009) model may lead to a better understanding of the interactions between toxicity modifying factors and Ni for *Hyalella* in soft waters.

## *1.3 Natural dissolved organic matter*

### *1.3.1 Toxicity mitigating abilities of different DOM sources*

DOM protects against metal toxicity in a concentration dependent manner (typically assessed based on dissolved organic carbon (DOC) content). There is also evidence that the composition of each DOM source can be related to the quality of DOM. For example, the relative humic acid (HA) and fulvic acid (FA) composition may vary among DOM sources (Morel and Hering, 1993). Composition differences may result in different metal binding characteristics and therefore potentially affecting the protective capacity for metal toxicity (Richards et al., 2001). Different sources of DOM (Suwannee River DOM and Nordic Reservoir DOM) demonstrated different protective effects at 15 mg DOC/L ($EC_{50}$ values of 50 µM (2.9 mg Ni/L) and 90 µM (5.2 mg Ni/L) respectively) (Kozlova et al., 2009). The differences in toxicity mitigation have been correlated to optical characteristics (absorbance and fluorescence measurements) of DOMs as shown with Cu (Al-Reasi et al., 2012; Schwartz et al., 2004; Ryan et al., 2004). Relationships between DOM optical characteristics and protection against $Ni^{2+}$ toxicity have not been studied.

### *1.3.2 Characterization of DOM sources*

DOM consists of decomposed organic molecules from different origins (plant and animal matter) found within aquatic systems as well as terrestrial surroundings (McKnight et al., 1983; Morel and Hering, 1993). These organic compounds are complex and heterogeneous due to their formation from various precursors (McKnight and Aiken, 1998) and typically consist of carbon (45-55%), oxygen (35-45%), hydrogen (3-5%), and nitrogen (1-4%). DOM concentration is

typically measured as DOC. Concentrations in natural waters range from 0.1 to 200 mg DOC/L (Kinniburgh et al., 1996). DOM is important to the aquatic ecosystem since it provides a source of carbon and nutrients at the base of the food web (Wetzel, 2001).

Due to the complexity of the compounds, DOMs are categorized by source type: allochthonous and autochthonous. Allochthonous sources are organic materials of terrestrial origin that are exported from land into the aquatic system. Composition varies with the characteristics of the catchment (e.g., area, cover, and types of vegetation, etc., Schiff et al., 1990). Autochthonous DOM is produced within aquatic systems and are a result primarily from the decomposition of algae and plant matter. These DOMs usually contain more carbohydrates and nitrogen-containing groups and are lightly colored (Richards et al., 2001) compared to allochthonous sources which are enriched with humic substances (HA and FA) and are highly colored (Buffle, 1988). Lakes typically contain a mixture of allochthonous and autochthonous materials, therefore giving each DOM source unique characteristics.

Humic substances are usually the main component of DOM, typically between 50% and 90% (Thurman, 1985). The term humic substances is used to describe the HA and FA content within organic matter. The proportion of HA affects metal speciation since free metal ions complex to this compound (Buffle, 1988). Protective effects against metals may be different between sources due to varying amounts of HA within each source of DOM. Characterizing DOM usually consists of determining the proportions of HA and FA and therefore differences between sources can be distinguished (Buffle, 1988). Understanding the differences between DOM sources through characterization may lead to a better comprehension of the variation in protective effects on toxic metals (Al-Reasi et al., 2012).

Characteristics of DOM sources can be identified by measurements of absorbance and

fluorescence. Absorbance at wavelengths of 254 and 340 nm are commonly used to distinguish differences of DOM since the absorption of light in this range is typically caused by the presence of HA and FA compounds (Abbt-Braun et al., 2004). The specific absorption coefficient measured at 340 nm ($SAC_{340}$) can serve as an indicator of HA within DOM (Curtis and Schindler, 1997; Richards et al., 2001). The amount of HA present in the DOM is related to the color and therefore $SAC_{340}$ also serves as a measure of color (light vs. dark DOMs).

Fluorescence excitation-emission matrix spectroscopy (EEMS) can also be used to characterize DOM (Coble, 1996). The fluorescence index (FI) is calculated as a ratio of emission intensities at 450 and 500 nm at an excitation wavelength of 370 nm. These indices are used to distinguish between DOM sources. A higher FI value indicates a more autochthonous DOM while a lower FI represents a source that is more terrestrially derived (McKnight et al., 2001).

Along with FI values, EEMS also creates matrices of data that can be transformed into contour plots (Winter et al., 2007). These contour plots act like a finger print for DOM; each source being unique from the other (Winter et al., 2007). These plots can be interpreted with parallel factor analysis (PARAFAC; Stedmon and Markager, 2005). PARAFAC can identify common components in the DOM samples (Stedmon and Markager, 2005). These components are HA-like, FA-like, tryptophan-like, and tyrosine-like (measured at excitation:emission of 360-390 nm:460-520 nm, 320-340 nm:400-450 nm, 280 and 230 nm:340-350 nm, and 280 and 230 nm:300 nm, respectively) (Winter et al., 2007). For the purposes of this research, HA-like and FA-like components were used to compare differences between DOMs.

### *1.4 Objectives*

The objectives of this research are to understand the role of cations and DOM in Ni toxicity to the freshwater invertebrate *Hyalella azteca*. Understanding the role of these potential protective mechanisms on Ni toxicity will contribute to understanding the processes involved in the recovery of smelter damaged aquatic ecosystems in the Sudbury area. The approach developed in this research occurs in three phases: 1) determining the role of cationic competition ($Ca^{2+}$ and $Mg^{2+}$) in mitigation of Ni toxicity, 2) deriving relationships between DOM quantity and reduction in toxic impact and 3) understanding the role of DOM source quality in relation to its toxicity mitigating capabilities. Sources for DOM from reference locations (Muskoka, ON), previously smelter impacted lakes (Sudbury, ON), and other impacted sites (logged and burned; White River, ON) are compared.

The hypotheses to be tested in these experiments include:

1. $Ca^{2+}$ and $Mg^{2+}$ cations compete with $Ni^{2+}$ for uptake into *Hyalella* such that:

    1a) increased levels of waterborne Ca results in an increase in the $LC_{50}$ of $Ni^{2+}$.

    1b) increased levels of waterborne Mg results in an increase in the $LC_{50}$ of $Ni^{2+}$.

2. DOM complexes with $Ni^{2+}$ in test solutions in a concentration-dependent-manner but with differences between sources.

    2a) as the concentration of DOM increases, Ni bioaccumulation decreases.

    2b) when compared at the same DOC concentration different sources of DOM offer different levels of protection against Ni toxicity.

3. Develop a hypothesis around Ni bioaccumulation and toxicity

One of the goals of this research is to integrate the results into existing toxicity-prediction models in order to test/validate cationic competition (between $Ca^{2+}$ or $Mg^{2+}$ and $Ni^{2+}$) and DOM complexation principles.

# Chapter 2
# Factors modifying acute Ni toxicity

### *2.1 Introduction*

Acute toxicity is variable in aquatic environments due to its dependence on organism sensitivity and exposure conditions. Understanding the sensitivity of an organism as well as the speciation of metals that are influenced by water chemistry can lead to a better understanding of acute toxic effects. The development of toxicity prediction models, such as the BLM, are influenced by our knowledge of the toxic abilities of metal species, their bioavailability, and its physiological mechanism for toxic impact (Di Toro et al. 2001; Keithly et al., 2004). The BLM uses these fundamental principles to predict acute toxic effects of metals to specific organisms.

In these studies, *Hyalella azteca* were exposed to various test solutions with increased levels of Ca, Mg and DOM to assess the acute toxic impacts of Ni. These organisms are small freshwater amphipods found in lakes across the Boreal Shield (Othman and Pascoe, 2001) and are used due to their sensitivity to contaminants (Blockwell et al., 1999; Borgmann, 1998; Environment Canada, 1997; Ingersoll et al., 1998; Nebeker & Miller, 1988). Contaminants such as nickel naturally occur in aquatic environments but can be found at elevated concentrations that range from 10 to 1000 µg Ni/L (Eisler, 1998). These elevations are generally associated with both aerial (stack) emissions and effluents (e.g., the mining and smelting operations in the Sudbury area; Keller, 1992). Metals (i.e., Ni) have affected the aquatic biota in the Sudbury region since the turn of the twentieth century (Keller et al., 2007) although water quality has improved in the recent years as a result of reduced smelter emissions (Keller et al., 1992) due to pollution control measures (Keller et al., 2007).

Acute Ni toxicity, factors that modify toxicity and physiological mechanisms are reasonably understood although Ni is not as well studied as other metals (eg. Cu or Zn). Elevated

levels of Ni cause a disturbance to the balance of Mg in *Daphnia* (Pane et al., 2003a) as well as disrupt the respiratory functions in fish (Pane et al., 2004). A concentration of 15.9 µM (0.93 mg Ni/L) caused a toxic effect to *Daphnia pulex* in relatively soft water (30 mg $CaCO_3$/L) (Kozlova et al., 2009). Waterborne $Ca^{2+}$ and $Mg^{2+}$ compete with $Ni^{2+}$ for the site of uptake therefore reducing toxicity with an 18-fold and 2.3-fold increase in protection, respectively (Kozlova et al., 2009). A reduction in Ni toxicity did occur (3-fold protective effect) with the addition of DOM (Buffle, 1988; Kozlova et al., 2009). Different sources of DOM have demonstrated various metal-binding abilities and therefore protection can alter depending on DOM source (Richards et al., 2001). Therefore, alterations to water chemistry (i.e., addition of Ca, Mg and DOM) can affect the degree of protection against Ni toxicity.

Protective effects are linked to different indicators of toxicity such as survival, growth, and reproduction. Recently, Borgmann et al. (2001) has suggested the use of bioaccumulation as an indicator of toxicity since it is a more reliable measure of toxic impacts than exposure concentrations in water medium or sediment. Bioaccumulation is reliable and accurate due to the dynamic nature of metal uptake where changes in exposure conditions and duration influence metal accumulation (Adam et al., 2010). Accumulation of metal is associated with a toxic effect (Borgmann et al., 2001), therefore bioaccumulation in these studies should correlate with the degree of toxic impact.

The free metal ion is generally considered to be the most toxic and most bioavailable form of metal in aquatic media (Pagenkopf, 1983). Detrimental effects occur as a result of metal interaction at the site of toxicity (e.g., the biotic ligand). Bioavailability of the metal, and therefore toxicity, is reduced when cations (e.g., $Ca^{2+}$ and $Mg^{2+}$) compete with $Ni^{2+}$ for uptake into the organism assuming that the cations are taken up at the same binding site (Di Toro et al.,

2001). When DOM is present, complexation with $Ni^{2+}$ occurs and therefore the metal ion is no longer available for uptake (Di Toro et al., 2001; Richards et al., 2001; Kozlova et al., 2009). The degree of competition and complexation is directly related to the concentrations of these components (i.e., Ni, competing cations, and DOM) (Di Toro et al., 2001). The relationship between the interactions of these factors and toxic effects have been integrated into the BLM for the acute effects of toxicity.

DOMs are composed of organic molecules of different origins found within and around aquatic systems (McKnight et al., 1983; Morel and Herring, 1993) resulting in complex and heterogeneous compounds (McKnight and Aiken, 1998). These carbon compounds are a result of decomposition processes that break down plant and animal matter. The DOM of lakes are therefore a mixture of these materials resulting in a unique set of characteristics for each DOM source. The degree of metal binding capacities are associated with the composition of DOM (Richards et al., 2001). The differences between DOMs can be optically characterized through measurements of absorbance and fluorescence through EEMS and PARAFAC (i.e., $SAC_{340}$, FI, HA-like and FA-like components). DOM binding abilities to Ni may vary if optical characteristics differ between sources. These differences affect the toxicity mitigating abilities of DOM to Ni toxicity (Al-Reasi et al., 2012).

To understand the role of Ca, Mg and DOM on the acute toxicity of Ni to the freshwater invertebrate *Hyalella azteca*, our objectives were to: 1) determine the effect of Ca and Mg on acute Ni toxicity, 2) derive relationships between DOM quantity and toxicity mitigation, and 3) test DOM sources for differences in their capacity to influence the impact of Ni.

## *2.2 Materials and methods*

### *2.2.1 Invertebrate cultures*

*Hyalella azteca* were collected in August 2010 from Hannah Lake, a previously acid and metal impacted lake near Sudbury, Ontario. Collected organisms were identified as *Hyalella azteca* according to Pennak (1953). Culture and testing at Wilfrid Laurier University followed Environment Canada standard method EPS1/RM/33 (1997). Groups of 30 adults were cultured in 1L polyethylene beakers with 800mL of an artificial soft water medium (hardness of 12 mg $CaCO_3$/L) consisting of: 0.1mM $CaCl_2$-$2H_20$, 0.1mM $NaHCO_3$, 0.025mM $MgSO_4$-$7H_2O$, 0.005mM KCl, and 0.001mM NaBr (all salts from Sigma-Aldrich Inc. Mississauga, ON) at pH 7.0, temperature of 20 $^oC \pm 1\ ^oC$ with a 16h light: 8h dark photoperiod. Br was added to the soft water medium since it is an essential element for the reproduction of *Hyalella azteca* (Borgmann, 1996). Organisms in each beaker was fed 5 mg of ground up Tetramin ™ flakes (Tetra Werke, Blacksburg, VA, USA) three times a week on non-consecutive days with weekly water renewals (100%). A piece of cheese cloth (5 cm x 5 cm) was placed in each beaker to act as substrate for the amphipods. Neonates were harvested weekly by screening through mesh sizes 650 and 275 µm (for catching adults and neonates, respectively; Environment Canada 1997) and viable organisms were used for testing or maintaining cultures.

### *2.2.2 DOM collection site descriptions*

Twelve sites were chosen for DOM collection and testing in this study (Fig 2.1). These sites include Lake Laurentian, Laurentian wetland, Daisy Lake, White River reference, logged and burned, Clearwater Lake, Harp Lake, HP3 (a stream of Harp Lake), Plastic Lake, PC01 and

P108 (an outflow and inflow of a wetland above Plastic Lake, respectively). The Sudbury lakes (Laurentian, Daisy and Clearwater Lakes) as well as Laurentian Wetland have all been impacted by smelter emissions; the lakes closest to the smelters (Laurentian and Daisy) received a high degree of acidification as well as metal contamination (Keller et al., 2004). Laurentian Lake is a reservoir located southwest of the Copper Cliff smelter. Laurentian Wetland is located above the reservoir and drains into it. Daisy Lake is also southwest of Copper Cliff but in close proximity to the Coniston smelter. Lakes that are close in proximity to these smelter operations have recovered to varying degrees. For example, Daisy Lake has recovered to the point where fish and loon populations have been re-established although not much is known about the recoveries (Keller et al., 2004). Also, Clearwater Lake is further from the smelter operations but was also impacted by smelter emissions and is now much like the pristine lakes in the area (Stokes, 1984).

White River DOM sources were collected from low-order boreal forest watersheds across a gradient of recovery from disturbances. These disturbances include an anthropogenic disturbance (logging, 2002), a natural disturbance (burned, 1999), as well as a site with no recent disturbance (reference). All sites are located within a 20-35 minute drive from the town of White River (along the TransCanada Hwy). The reference site is 35 minutes northwest of town, the burned site is 20 minutes northeast of town and the logged site is 20 minutes southeast of town. Vegetation around the riparian areas of the reference site consist of raspberry spp., alder spp., and red osier dogwood while honeysuckle spp. and blueberry spp. are present in the upland areas of this site. Alder spp., honeysuckle spp., and raspberry spp. are found in the riparian areas of the burned site while upland areas consist of blueberry spp., and white birch (all vegetation data on White River sites provided by David Kreutzweiser (CFS NRCAN), personal communication). The vegetation in both areas (riparian and upland) of these sites contribute to the unique

composition of each DOM source. Unfortunately, not much information about the logged site is available since it was a new sampling site but logged sites in the surrounding area mainly consisted of alder spp., raspberry spp., and balsam fir in the riparian areas and honeysuckle spp. and blueberry spp. in the upland areas (David Kreutzwiser (CFS NRCAN), personal communication).

The lakes in the Muskoka region where DOM was collected (Harp Lake and Plastic Lake) are natural lakes that have not been subject to dramatic disturbance such as logging, fire or smelter emission. The selected Muskoka lakes also have relative streams where samples were collected as well. HP3 is a stream that drains into Harp Lake and is known to have a large amount of DOM. Plastic Lake is an oligotrophic lake in south-central Ontario (Watmough et al., 2007) with two associated streams; PC01 and P108. PC01 is the major stream that drains through a wetland before connecting to Plastic Lake (Watmough et al., 2007) and flows all year around in years with more precipitation but has a tendency to dry up during the summer (Eimers et al., 2007). P108 is a short stream that drains from the wetland above Plastic Lake (Watmough et al., 2007). The vegetation in the Plastic lake wetland is made up primarily of white cedar although white pine, eastern hemlock, red maple, striped maple, and black spruce are present as well (Landre et al., 2009).

### *2.2.3 Natural DOM collection and preparation*

DOM was collected from July 2010 to Oct 2011 by reverse osmosis concentration, followed by resination and refrigeration (Schwartz et al., 2004). Water was collected a few meters from shore of the sites and pumped through a 5µM filter to remove any particulate debris before transferring the water into the stainless steel portable reverse osmosis unit. The reverse

osmosis unit was equipped with XYZABC membranes. Collecting approximately 350 L (200 to 500 L, source dependent) of lake water is reduced to about 8 L of DOM concentrate (ranged from 6 to 10 L). The DOM concentrate was collected in pre-washed 4 L polyethylene containers or 10 L polypropylene containers that were previously rinsed with 0.1% $HNO_3$.

During the process of resination, the DOM collected is poured into a stainless steel pot and activated resin (USF C-211 H cation resin, U.S. Filter Corporation, Rockford IL) is added to lower the pH and strip the DOM of any cations or residual metals. To activate resin, 600 mL of 4N HCl (Fisher Scientific, Nepean, ON) was added to 3.6 L of resin for 5 minutes then rinsed 5 times with deionized water and then another 5 times with MilliQ filtered water (18mΩ.cm; MilliQ A10, Millipore, Mississauga, ON). Properly resinated DOM has a stable pH that does not change when more activated resin is added to the solution (usually a pH of 2). These samples were measured for DOC content and then refrigerated and stored at 4ºC. Before using DOM in exposures, aliquots of concentrate were adjusted to pH 7.0 by adding 0.1 M NaOH (Sigma-Aldrich Inc. Mississauga, ON). Appropriate dilutions of DOM concentrate were then done to achieve the desired test concentrations of DOC.

### *2.2.4 Exposure conditions*

Acute toxicity exposures were 96 h, performed in duplicate, with 10 neonates (2 – 9 d of age per replicate), in standardized volumes (200 ml of solution in a 400 mL polyethylene beaker) and organisms were not fed (Environment Canada, 1997). Immobility at 96h was used as the test endpoint. Test solutions were made by adding aliquots of $NiCl_2 \cdot 6H_2O$ (1 g/L stock; Sigma-Aldrich Inc. Mississauga, ON) to prepared aquatic media resulting in concentrations that range

from 0.25 to 10 mg Ni/L. In each toxicity test, the concentration range was adjusted to achieve 100% survival at the lowest Ni concentration and 100% mortality at the highest Ni concentration. Test solutions were prepared with culture media and adjustments to toxicity modifying factors (Ca concentration, Mg concentration or the addition of DOM) were made and equilibrated for 24 h before initiating tests. The pH, temperature and photoperiod during exposures were similar to the cultures (pH 7.0, 20 ± 1 $^{o}$C, 16:8 photoperiod). Cheese cloth substrate pieces (5 cm x 5 cm), presoaked with test solution for 24 h in a separate beaker, were placed in each test beaker just prior to adding neonates. At least 5 Ni concentrations were used in each test along with controls (no additional Ni). These controls consist of culture medium only and modified culture medium (addition of Ca, Mg, or DOM). In different toxicity test series, the potential protective effects of Ca, Mg, DOM quantity and DOM quality on acute Ni toxicity were determined. An independent Ni only test (with no modifying factors) was performed for each series to assess the ongoing health and viability of *Hyalella* neonates over time.

An assessment of protective effects caused by waterborne Ca is conducted through the addition of $CaCl_2$-$2H_20$ (Sigma-Aldrich Inc. Mississauga, ON) to achieve desired Ca concentrations of 0.1, 0.3, 0.75, and 2 mM. The protective effect of Mg was tested by increasing Mg concentration from 0.025 to 0.5 mM through the addition of $MgSO_4$-$7H_20$ (Sigma-Aldrich Inc. Mississauga, ON). Effects of DOM (Harp Lake DOM) quantity on Ni toxicity were tested at three concentrations of DOM (3, 6, or 12 mg DOC/L) based on naturally occurring amounts of DOM observed in soft water lakes around central Ontario (David et al., 1997). The effect of DOM quality (source) on Ni toxicity was tested by conducting acute toxicity tests with each source at a nominal concentration of 6 mg DOC/L.

### *2.2.5 Ni bioaccumulation*

To better understand potential linkages to the toxicity modifying influences of DOM, additional volumes of each test concentration were prepared and equilibrated for short term whole body bioaccumulation assessments. Two to nine day old *Hyalella azteca* (n = 6 at each concentration and controls) were exposed to test conditions following protocols for acute exposures in 200 mls of solution. Individual organisms were sampled at 6h. To assess Ni bioaccumulation, a Ni only exposure (no added modifying factors) and six sources of DOM were used: Laurentian Lake, Laurentian wetland, Daisy Lake, and the three sites from White River (reference, burned, and logged).

### *2.2.6 Sampling and digestion – water and organisms*

Water samples (10 ml) were collected before and after exposures to ensure consistency of the solution throughout exposure. Both filtered (0.45 um HT tuffryn, Pall, Ann Arbor, MI) and unfiltered water samples were sampled for dissolved and total (respectively) Ni concentrations. Each sample was taken with a 10 mL disposable syringe (NORM-JECT®, Henke Sass Wolf, Germany) and stored in 20 mL scintillation vials (VWR International, LLC, Mississauga, ON). All samples were acidified to 1% by adding 16N $HNO_3$ (Trace Metal Grade, Fisher Scientific, Mississauga, ON). For toxicity tests with DOM, additional 50 mL samples were taken for DOC analysis. These water samples were filtered (0.45 um HT tuffryn, Pall, Ann Arbor, MI) and the sample tube was filled to minimize head space. Subsequently, they were stored in the dark at $4^{\circ}C$.

*H. azteca* sampled for bioaccumulation were removed from exposures with a disposable pipette, transferred briefly into deionized water, and then blotted on a kimwipe to remove excess moisture. The organisms were then placed in individual 0.6 mL tubes and dried at $80^{o}C$

(Isotemp500 series, Fisher Scientific, Nepean, ON) for 48 hrs. Each organism was then weighed to the nearest 0.1 µg (SE2 Ultra Micro Balance, Sartorius Mechantronics Corp., Bohemia, NY) and placed back into its individual tube for digestion. The protocol for digestion followed Neumann et al. (1999), beginning with the addition of 25 µL of 16N $HNO_3$ (Trace Metal Grade, Fisher Scientific, Mississauga, ON). After 6 days at room temperature, 20 µL of 30% $H_2O_2$ (Sigma-Aldrich Inc. Mississauga, ON) was added and after 24 hrs, solutions were brought up the final volume of 250 µL with the addition of MilliQ water (18mΩ cm; MilliQ A10 Millipore, Mississauga, ON). This digested sample was then analyzed for Ni content.

### *2.2.7 Sample Analysis – bioaccumulation and water samples*

Ni content in *Hyalella* was measured by graphite furnace atomic absorption spectroscopy (AAS SpectraAA 880 GTA 100 atomizer, Varian, Mississauga, ON). Ni, Ca and Mg in water samples was measured by AAS in flame mode. Both filtered and unfiltered samples were measured for Ni. Standards were made from $NiCl_2$•$6H_2O$, $CaCl_2$•$2H_2O$ and $MgSO_4$•$7H_2O$ (all Sigma-Aldrich Inc., Mississauga, ON) and verified with an aqueous certified reference standard (TM-26.3, National Water Research Institute, Burlington, ON).

DOM samples were filtered and measured for DOC content using a TOC analyzer (Shimadzu TOC-V with ASI-V autosampler, Mandel Scientific, Guelph, ON) at Wilfrid Laurier University (Waterloo, ON). A subset of the samples, those from the DOM quantity experiment, were measured by the same method but with a different instrument (5050A TOC Analyzer, Shimadzu, Mandel Scientific Columbia, MD) at McMaster University (Hamilton, ON).

### *2.2.8 Optical characterization*

All DOM sources were analyzed for optical characteristics, specifically $SAC_{340}$, FI values, HA-like content, and FA-like content via EEMS and PARAFAC. The $SAC_{340}$ values of DOM sources were measured and analyzed (Christine Geiger, York University, ON) following the method of Al-Reasi et al. (2012) using the equation provided by Curtis and Schindler (1997):

$$SAC_{340} = [2303 \times (Abs340)] / DOC \quad \text{Equation 1}$$

where Abs340 is the absorbance value at 340nm and DOC represents the concentration of DOM that was used for measurements. All DOM samples were increased to a pH of 7 by the addition of 0.1 NaOH then diluted to a concentration of 10 mg DOC/L with MilliQ filtered water (18mΩ.cm; MilliQ A10, Millipore, Mississauga, ON) before analysis. These samples were then placed in a 1cm quartz cell (Helma Canada Ltd. Concord, On, Canada) and measured three times. Measuring the absorbance of MilliQ filtered water (18mΩ.cm; MilliQ A10, Millipore, Mississauga, ON) was used as a reference (i.e., blank). A correlation of $SAC_{340}$ values and measured $LC_{50}$ values will be made to determine if absorbance coefficients can explain differences in protective effects from Ni toxicity.

FI was conducted (Kelly Livingstone, Wilfrid Laurier University, ON) following the methods of Gheorghiu et al. (2010) and was calculated via (McKnight et al., 2001):

$$FI = (370:450)/(370:500) \quad \text{Equation 2}$$

where FI is the ratio of intensities at 370:450 and 370:500 (excitation : emission wavelengths in

nm). The concentration of DOM used for these measurements was 5 mg DOC/L. DOM samples were also analyzed for HA-like and FA-like components using EEMS and PARAFAC. PARAFAC analyzes the data produced by EEMS and yields three-dimensional contour plots that distinguish different components found within DOM including HA-like and FA-like content (Smith and Kramer, 1999)

### *2.2.9 Calculations and statistical analysis*

Survival data from toxicity exposures were used to calculate $LC_{50}$ values using the Comprehensive Environmental Toxicity Information System (CETIS, v.6.0, Tidepool Scientific Software) and this was done on a measured total and dissolved Ni basis. The Spearman-Karber statistical test was used to approximate the $LC_{50}$ value based on survival data. Significant differences in $LC_{50}$s were assessed by using overlaps of the 95% confidence intervals (CI). In cases where intervals overlapped, the statistical method developed by Litchfield and Wilcoxon (1938) as described by Gillis et al. (2010) was used to assess significant differences.

Ni bioaccumulation was measured on a whole body dry weight basis as µg Ni/ g dry weight. Differences among treatment groups were tested by one-way ANOVA followed with, when appropriate, the Fischer LSD test using Sigmaplot™ (v. 11), a scientific data analysis and graphing software.

### *2.2.10 Modelling*

The BLM was altered from the model for *Daphnia pulex* in soft water developed by

Kozlova et al. (2009) for the prediction of toxic effects caused by Ni to *Hyalella azteca*. The conditional equilibrium constants describing the toxic interaction of $Ca^{2+}$, $Mg^{2+}$, and $Ni^{2+}$ on the biotic ligand remained unmodified ($logK_{CaBL}$ = 4.2, $logK_{MgBL}$ = 3.6, and $logK_{NiBL}$ = 4.87). The output created from this model was compared to the measured $LC_{50}$ values. Through adjustments of values to the model, only the critical value (also known as the $LA_{50}$) was changed to achieve a model of 'best fit' between the measured and predicted values. These adjustments achieved a model that can be used to predict Ni toxicity in soft waters with *Hyalella azteca*. Three models were produced (the effect of Ca, the effect of DOM quantity, and the effect of DOM quality/source) using the comparison of predicted Ni toxicity output created by the model and the measured Ni toxicity. For all models, measured water chemistry was used and a 10% humic acid component was assumed for DOC based on previous studies (Deleebeeck et al., 2008; Wu et al., 2003).

### *2.3 Results*

#### *2.3.1 Water chemistry*

Water chemistry was measured for Ni (mg/L), Ca (mM), Mg (mM), and DOC (mg/L) when necessary (Table 2.2). For measurements of Ni, unfiltered and filtered water samples were analyzed for total Ni and dissolved Ni, respectively. Dissolved Ni was calculated to be 99.9 ± 7 % (mean ± SEM, n = 250) of total Ni; consequently, all Ni concentrations are reported on a total Ni basis. Ni concentrations at 96h were calculated to be an average of 96.6 ± 4% (mean ± SEM, n = 250) of Ni concentrations at day 0 (range from 92 to 107%). To monitor changes (if any) between the start (0 h) and end of exposure (96 h) in total Ni, dissolved Ni, and DOC (when necessary), a paired t-test was performed and no significant difference occurred (t = 0.858, P =

0.392; t = 1.639, P = 0.108; t = -0.489, P = 0.630, respectively). In acute exposures with culture medium and added Ni (no additional modifying factors), 88.1% of total Ni is calculated to be free $Ni^{2+}$ ion by the Windermere Humic Aqueous Model (WHAM, ver.7.0.2) at the measured $LC_{50}$ value. Other Ni species were predicted to be 7.8%, 0.4%, and 3.6% for $NiHCO_3$, $NiSO_4$, and $NiCO_3$ respectively. The BLM, on the other hand, predicted the same solution to be 82.7% $Ni^{2+}$ ion while Ni species $NiHCO_3$, $NiSO_4$, and $NiCO_3$ were predicted to be 8%, 0.3%, and 1.8%, respectively. When the two models are compared, WHAM predicts more of these specific Ni species ($NiHCO_3$, $NiSO_4$ and $NiCO_3$) by 1% than the BLM as well as more $Ni^{2+}$ ions.

### *2.3.2 Ni toxicity*

Six toxicity tests with only Ni were performed in the artificial soft water medium and $LC_{50}$ values were consistent over time ranging from 0.55 ± 0.31 to 0.89 ± 0.10 mg Ni/L (± 95% CI). The mean $LC_{50}$ value was 0.70 ± 0.10 mg Ni/L (± SEM, n = 6).

### *2.3.3 Cationic competition on acute Ni toxicity*

The addition of Ca increased $LC_{50}$ values (Fig 2.2a). This reduction in toxicity has a positive linear trend increasing to a Ca concentration of 0.75 mM then remained relatively constant at higher Ca concentrations (Fig 2.2a). Contrary to results in the Ca series, the increase of Mg concentrations from 0.025 to 0.5 mM did not cause $LC_{50}$ values to change significantly. However there were problems in the Mg series as unexposed controls for the elevated Mg testing (0.5 mM) mostly did not meet the acceptable minimal survival of 80%, given in the test method (Environment Canada, 1997). This test was repeated three times with mortality rates of 45 %, 35% and 30% before an acceptable survival rate for testing was achieved.

### *2.3.4 DOM complexation on acute Ni toxicity*

The 12 DOM sources (differing in levels of impact) were tested for their relative ability to protect *Hyalella azteca* from the effects of waterborne Ni. DOM quantity as well as quality was tested. $LC_{50}$ values increased with increasing DOM concentration (Fig 2.2b). Protective effects were observed to be statistically significant when nominal concentrations of DOM were at 6 mg DOC/L and higher (Fig 2.2b) and therefore, this threshold concentration (i.e., 6 mg DOC/L) was chosen for exposures where source quality was assessed.

When the different sources of DOM were compared at a common measured concentration of 7 mg DOC/L, the $LC_{50}$ values differed by approximately 2-fold (Fig 2.3). The DOM source with the highest level of protection was Plastic Lake (2.04 ± 0.6 mg Ni/L (± 95% CI)) while the lowest was Clearwater Lake (1.00 ± 0.4 mg Ni/L (± 95% CI)). Ten of the twelve DOM sources tested offered significant protective effects (at 7.0 ± 1.2 mg DOC/L (mean ± SEM)) compared to a corresponding Ni only spiked exposure (Fig 2.3). DOM from Clearwater Lake and P108 offered no significant protective effect against Ni toxicity (Fig 2.3).

### *2.3.5 Bioaccumulation*

Ni accumulation in *Hyalella azteca* during $LC_{50}$ tests was measured after 6 h of exposure to assess possible links between short term accumulation (whole body basis) and subsequent 96 h survival. Bioaccumulation of Ni was much lower in the presence of DOM compared to the Ni-only exposure (Fig 2.4). Without any added DOM, Ni accumulation was as high as 2200 µg Ni/g dry weight (n = 1) at 4 mg Ni/L (Fig 2.4). When DOM was added to exposure, Ni accumulation reached a high of 166 ± 14.5 µg Ni/g dry weight (mean ± SEM, n = 5) at 4 mg Ni/L (Fig 2.3). Different DOM sources provided different accumulation patterns where accumulation ranged

from 32 ± 4.0 µg Ni/g dry weight (mean ± SEM, n = 6) to 166 ±14.5 µg Ni/g dry weight (mean ± SEM, n = 5) at 4 mg Ni/L (Fig 2.4). Ni accumulation at the respective $LC_{50}$ values were observed to vary where bioaccumulation ranged from 20 µg Ni/ g dry weight (Lake Laurentian) to 49 µg Ni/ g dry weight (Daisy Lake). Ni accumulation did not correspond to the protective abilities observed in $LC_{50}$ values.

### *2.3.6 Optical characteristics*

$SAC_{340}$ values were calculated (Christine Geiger, York University, ON) and these results were then compared to the observed $LC_{50}$ values and were determined to have no correlation with both summer and fall collected samples ($r^2$ = 0.11, $p > 0.05$) (Fig 2.5a) . $LC_{50}$ values remained consistent when compared to FIs (Kelly Livingstone, Wilfrid Laurier University, ON) where no correlation was observed in summer or fall samples ($r^2$ = 0.34, $p > 0.05$) (Fig 2.5d). PARAFAC analysis was performed on all twelve DOM sources (Kelly Livingstone, Wilfrid Laurier University) where the analysis of HA-like and FA-like components were used to make correlations to the measured acute $LC_{50}$ values. No significant correlations with $LC_{50}$ values were demonstrated with either component: HA-like components ($r^2$ = 0.31, $p > 0.05$) (Fig 2.5b) and FA-like components ($r^2$ = -0.41, $p > 0.05$) (Fig 2.5c).

### *2.3.7 Modelling*

The BLM developed by Kozlova et al. (2009) for Ni and *Daphnia pulex* was tested for the ability to predict acute Ni toxicity in *Hyalella azteca*. The original model (Kozlova et al.,

2009) predicted a $LC_{50}$ value of 0.5 mg Ni/L, which was less than the average measured $LC_{50}$ for *Hyalella* (0.70 ± 0.10 mg Ni/L (mean ± SEM, n = 6)). The Kozlova model critical accumulation threshold value (in the modeling input matrix) was adjusted from 5.1 to 6.75 (nmol Ni/g) to account for this reduced sensitivity in *Hyalella*, and to bring the predicted $LC_{50}$ to 0.7 mg Ni/L.

This adjusted BLM was then compared to the Ca series of exposures to determine if the prediction model reflected the measured $LC_{50}$ values. There was a good match between the predicted and measured $LC_{50}$ values from 0.1 mM to 0.75 mM of Ca (Fig 2.2a). At 2.0 mM, there was a significant difference between the measured and predicted value where the protective measure was over-predicted by a factor of 2.5 (Fig 2.2a).

This BLM model was also used to compare predicted to measured $LC_{50}$ values for DOM exposures, both quantity and quality (difference between sources). In the DOM quantity series, the model under-predicted the protective effects of Harp Lake DOM at 3 and 6 mg DOC/L but did show a perfect prediction at 12 mg DOC/L (Fig 2.2b). For the DOM quality series, the predicted $LC_{50}$ value at a measured concentration of 7 mg DOC/L was 1.4 mg Ni/L and this is an intermediate value within the range of $LC_{50}$ tests with various DOM sources (Fig 2.3).

### *2.4 Discussion*

This study shows that *Hyalella azteca* are sensitive to Ni and that Ca and DOM provide protection against Ni toxicity (Fig 2.2 and 2.3). It was demonstrated by this study that Ni does cause a toxic effect to *Hyalella azteca*. Unexpectedly (in comparison to Kozlova et al., 2009 and Pane et al., 2003a), Mg did not provide significant protection. The protective effects of DOM against Ni toxicity were associated with reduced bioaccumulation patterns where metal accumulation in the organism decreased drastically (Fig 2.4). Different sources of DOM

provided varying protective effects (Fig 2.3), but these differences could not be explained by optical characteristics (i.e., $SAC_{340}$, FI, HA-like component and FA-like component) (Fig 2.5 a – d). The mitigating effects of Ca and DOM were predicted reasonably (but not at high Ca) within the BLM, especially after adjustments had been made to account for the relative sensitivity of *Hyalella azteca* (Fig 2.2 and Fig 2.3).

Cations, such as Ca and Mg, provide protection against Ni toxicity in *D. pulex* (Kozlova et al., 2009), *D. magna* (Pane et al., 2003a), and in this experiment, Ca provided protection to *Hyalella azteca* (Fig 2.2a). Protection was not provided by Mg and this deviates from the conclusion that main water hardness cations (e.g., $Mg^{2+}$) protect against Ni toxicity and are primary toxicity modifying factors (Pane et al., 2003a; Keithly et al., 2004; Hoang et al., 2004). In the Ca series of the Kozlova et al. (2009) study, the 48 h $EC_{50}$ at 0.1 mM Ca (1.3 mg Ni/L) was approximately double that of our observed 96 h $LC_{50}$ at the same Ca concentration. The difference between these values may represent a higher sensitivity of *Hyalella* to Ni. A longer duration of exposure (48h for *D. pulex* and 96h for *H. azteca*), or perhaps differences due to water chemistry (other than Ca, e.g., Mg or $HCO_3$) may also explain differences in $LC_{50}$ values between studies. Although the responses to Ni (between Kozlova et al., 2009 and our study) were different (quantitatively), the protective effect of elevated Ca was comparable between the Ca concentrations of 0.1 and 0.75 mM where a 6-fold increase in protection against Ni toxicity was observed for both studies. In addition to the similar trend of protection, the BLM model for *Daphnia* (adjusted for sensitivity) provided reasonable estimates of protection against Ni toxicity for *Hyalella* (also see BLM discussion).

The protective effect of Ca on Ni toxicity indicates an interaction between $Ca^{2+}$ and $Ni^{2+}$ and likely reflects the competition between the two ions for uptake sites (Meyer et al., 1999).

This competitive interaction is consistent with the mechanistic assumptions underpinning the BLM, although the mechanism of Ni uptake to *Hyalella* is not well known. While Ca protection may signify a direct $Ca^{2+}$ and $Ni^{2+}$ interaction for uptake, it is also possible that physiological effects of elevated Ca indirectly protect against Ni toxicity (Schubauer-Berigan et al., 1993; Meyer et al.,1999). This is supported by the study of Pane et al. (2003a) where Ca protection against Ni toxicity to *D. magna* was indirect. In that same study (Pane et al., 2003a), Ni uptake was directly related to the inhibition of Mg uptake (i.e., $Mg^{2+}/Ni^{2+}$ competition for uptake).

The uptake of Ca within an organism is important since it leads to the regulation of major functions. It is hypothesized in fish that Ca is required for the synthesis of vitellogenin, which is required for oocyte production (Hogstrand and Wood, 1996). The decrease in Ca uptake can lead to an imbalance within the system resulting in a decrease of Ca in the plasma which eventually leads to hypocalcemia, a deficiency of calcium in the bloodstream (Santore et al., 2002). Ca is important in crustaceans since it is a major component of the carapace (Cameron and Wood, 1985). $Ca^{2+}$ ion regulation has been observed to be disturbed in many organisms by metals such as Zn (Santore et al., 2002; Clifford and McGeer, 2008), and Cd (Clifford and McGeer, 2010). However, it has been suggested by Pane et al. (2003a) that Ni differs from other metals since antagonism of $Ca^{2+}$ homeostasis was not observed following an acute waterborne Ni exposure (although it was indicated that the results were difficult to interpret due to the process of molting and calcification of the carapace in daphnids). Contrary to the study of Pane et al. (2003a), our study shows that Ca does protect against Ni toxicity but whether this represents direct competition for uptake between Ca and Ni and a physiological difference between *Daphnia* and *Hyalella* is unknown but is worthy of further study.

The effect of Mg on Ni toxicity observed in these studies was unexpected because other

studies (Pane et al., 2003a, 2004; Kozlova et al., 2009) have shown Mg to have protective effects. Surprisingly, the increased Mg concentration in our study seemed to create a stressful response causing a decrease in survival of organisms even though no Ni was present (i.e., controls). These results differed from that observed in Kozlova et al. (2009) with *Daphnia pulex* where a protective effect of Mg was observed and the reported $LC_{50}$ value was 2.5-fold higher than our observed values (30 µM of Ni (1.76 mg Ni/L) compared to 0.66 mg Ni/L, respectively) at an elevated Mg level of 0.5 mM. Similar to that of the Ca exposures, differences in physiology between the organisms might explain the variation in $LC_{50}$ values. Differences between the protective abilities of Ca and Mg may also be explained by Ca having a stronger influence on Ni toxicity than Mg (Schroeder et al., 2010).

An interaction between Ni and Mg is well characterized in mammalian literature. It has been observed that Ni is chemically similar to Mg (Weast, 1973) and therefore able to be taken up in the same manner. In fact, Mg is able to mimic carcinogenic processes such as genotoxicity, cell transformation as well as tumor induction caused by Ni substances (Costa, 1991; Sunderman, 1989). Similarly, Ni is able to inhibit the function of DNA polymerase, a Mg requiring enzyme (Costa, 1991). The mechanism of inhibition in invertebrates is not well characterized but the uptake of Mg is directly inhibited by Ni through a blockade of Mg channels or Ni may indirectly hinder an active transporter of Mg (Pane et al., 2003a). Ni antagonizes Mg in an acute waterborne exposure with *D. magna* where whole body Mg concentrations were significantly reduced in the presence of Ni (Pane et al., 2003a). Clearly, the Mg concentration in our exposure was sufficiently elevated which induced detrimental effects (where controls did not survive well) and therefore few conclusions can be drawn about potential interactions between Ni and Mg to *Hyalella azteca*.

Aside from the addition of cations, elevated concentrations of DOM in this study caused a reduction of Ni toxicity (Fig 2.2b); similar reductions are reported for other species such as fathead minnow (Wu et al., 2003), *Daphnia magna* (Deleebeeck et al., 2007), and *Daphnia pulex* (Kozlova et al., 2009). The protective effect observed is most likely caused by the complexation of DOM and $Ni^{2+}$ ions (Livens, 1991). This study shows that different sources of DOM offer different degrees of protection (Fig 2.3) similar to the study by Richards et al. (2001). However a 2-fold variation in protective effects were provided and this is generally less than that provided for Cu (for example); where it can range over four-fold or more. Differences in the capacities of DOMs to reduce the toxicity of metals have been linked to variations in their optical characteristics, as measured by parameters such as $SAC_{340}$, FI, HA-like and FA-like content (Schwartz et al., 2004). In this study, there were no meaningful correlations between optical characteristics and measured $LC_{50}$ values (Fig 2.5a – d).

$SAC_{340}$ can serve as an indicator of aromatic compounds within DOM (Curtis and Schindler, 1997; Richards et al., 2001). Aromatic compounds in DOM mainly consist of HA-like content which has the ability to bind to metals and therefore can be correlated to toxicity mitigation abilities. A higher $SAC_{340}$ value correlates with a darker color of DOM which is related to a higher amount of aromatic groups (Abbt-Braun et al., 2004). DOM sources that reduce metal toxicity to a great degree are usually characterized to have a high protein-to-carbohydrate ratio, low nitrogen and phosphorus concentrations and a high degree of aromaticity represented as a high $SAC_{340}$ value (Richards et al., 2001). For example, the mitigation of Cu toxicity has been significantly and positive correlated to $SAC_{340}$ values (Al-Reasi et al., 2011) but these correlations were not observed in our studies with Ni toxicity (Fig 2.5a)

In addition to absorbance measurements (i.e., $SAC_{340}$), fluorescence measures such as the

FI and those derived via EEMS and PARAFAC (yielding estimates of HA-like, and FA-like components) have been related to toxicity mitigation of metals such as Cu to fat head minnow (Ryan et al., 2004), rainbow trout (Schwartz et al., 2004), and *Daphnia magna* (Al Reasi et al., 2012) or Ag to fathead minnow (VanGenderen et al., 2003). Unfortunately, these optical characteristics did not provide protective correlations to Ni toxicity mitigation in this study (Fig 2.5). Measurements of FI ranged from 0.23 to 1.58 (Clearwater Lake and HP3 respectively) suggesting that most of our DOM sources were terrestrially derived in varying degrees. FI is an optical characteristic that provides information about the origin of DOM where a value of ~1.9 describes a source that is autochthonous and a value of ~1.4 represents a source that is terrestrially derived (McKnight et al., 2001). It has been widely accepted that autochthonous sources are lightly colored due to the enriched nitrogen and carbohydrate content in comparison to allochthonous DOM which are darker due to the richness in aromatic HA and FA (Richards et al., 2001). Therefore, a greater reduction of metal toxicity should be associated with a lower FI value, which in this study did not occur. Similarly, HA-like and FA-like components did not correlate with $LC_{50}$ values (Fig 2.5b and Fig 2.5c). The percentage of HA content is positively related to the protective effects of DOM source on metal-toxicity more so than FA (Guthrie et al., 2003) and this effect was not observed for Ni.

The lack of relationships between optical characteristics and the mitigation of Ni toxicity by DOM may be explained by relative concentration differences between binding interactions and acute toxicity. Another way of considering this is that the strength of the binding interaction (as expressed by Log K values for example) to DOM and the biological surface are comparable within similar waterborne concentration ranges. For example, it appears that for Cu, the complexation by DOM and the acute toxicity to aquatic organisms occurs within approximately

the same concentration range (e.g., Schwartz et al., 2004). There may not be the same chemical and biological synchrony as the strong binding of Ni to DOM occurs at a low concentration relative to the toxicity of Ni, which is in the range of 1-2 mg Ni/L (Town and Filella, 2002). Therefore, optical characteristics that are important predictors of strong binding to Cu do not provide the same predictive abilities for Ni. At higher concentrations, there is still some binding of Ni as toxicity mitigation and reduction in bioaccumulation were observed. To understand the mechanisms involved, it would be necessary to have an improved understanding of the characteristics of DOM.

A reduction of metal accumulation (a 10-fold decrease) was observed when DOM at a measured concentration of 7 mg DOC/L was added (Fig 2.4). It is expected that accumulation of metal (e.g., Ni) will decrease with added DOM since metal ions (e.g., $Ni^{2+}$) complex with organic matter decreasing the amount of free $Ni^{2+}$ ions available for uptake (Di Toro et al., 2001). Our results are supported through other studies where the addition of DOM decreases Ni accumulation into the organism and therefore reduces toxic effects (e.g., Livens, 1991; Kozlova et al., 2009). Although the addition of DOM reduced Ni accumulation, patterns of bioaccumulation were different than those observed by Borgmann et al. (2001). Ni accumulation in *Hyalella* at 0.5 mg Ni/L was 10-fold higher in our study when compared to the study of Borgmann et al. (2001). The difference in results may be caused by length of time for accumulation (6 hr vs. 7 days), age of *Hyalella* used (2 – 9 days vs. 4 - 6 weeks), or even the effect of a 24 h gut clearance in the Borgmann et al. (2001) study. Although the addition of DOM produced a difference in accumulation patterns, it was not possible to tease out accumulation-toxicity relationships among the different sources of DOM. It is hypothesized that DOM sources which provide less protection will produce high levels of Ni bioaccumulation (and vice versa)

but this was not observed. Therefore, the results do not support the theory that Ni accumulation is directly related to a toxic effect (Borgmann et al., 1991, 1998, and 2001), except from the general perspective of DOM presence in exposure.

The development of a BLM for *Hyalella azteca* in soft waters showed that toxicity can be predicted reasonably well (Fig 2.2a, Fig 2.2b and Fig 2.3). Although the model provided reasonable predictions of competitive interactions when compared to the measured values for the Ca series, there was an over prediction by a factor of 2.5 at 2.0 mM of Ca (Fig 2.2a). This suggests that the model lacks the ability to account for the potential for Ca to induce physiological effects in *Hyalella* that may have altered Ni toxicity responses. The prediction of the Ca series was observed in the DOM quantity series as well (i.e., increasing DOM concentrations reduces Ni toxicity) but the predicted effects were underestimated (Fig 2.2b). A prediction of toxicity with DOM at a measured concentration of 7 mg DOC/L was intermediate (Fig 2.3) and it is clear that the measurement of DOC as one value (10% humic acid; Deleebeeck et al., 2008; Wu et al., 2003) does not account for source differences since various protective effects related to different DOM sources are observed in our studies similar to that of Kozlova et al. (2009). It is demonstrated that an adjustment in HA content (from 10% to the measured value) (Wood et al., 2011) or FA content (Deleebeeck et al., 2008) will result in a better prediction due to a ‘better fit’. This holds true for metals such as Cu since there is a good correlation between optical characteristics of DOM and their ability to reduce toxicity, but this is not the case for Ni (Fig 2.5). Therefore further study in the toxicity of Ni and its interaction with DOM is needed to understand the relationship between optical characteristics and DOM source.

**Table 2.1.** All sources of DOM used in toxicity testing are listed below. The location as well as time of collection and GPS co-ordinates of each site are noted. The respective $LC_{50}$ values associated with each DOM source and well as its optical characteristics are present.

| Site name | Location | Collection date | GPS co-ordinates | $LC_{50}$ (mg Ni/L) | $SAC_{340}$ | HA % | FA % | FI |
|---|---|---|---|---|---|---|---|---|
| Laurentian Lake | Sudbury | July 2010 | 46447'N, 80.961'W | 1.8 (1.47 – 2.32) | 26.8 | 53 | 26 | 1.27 |
| Laurentian Wetland | Sudbury | July 2010 | 46450'N, 80942'W | 1.1 (0.73 – 1.59) | 38.0 | 65 | 65 | 1.20 |
| Daisy Lake | Sudbury | July 2010 | 46450'N, 80888'W | 1.4 (1.14 – 1.69) | 16.4 | 57 | 27 | 1.44 |
| Clearwater Lake | Sudbury | October 2011 | 46370'N, 81050'W | 1.0 (0.87 – 1.15) | 9.7 | 0 | 78 | 0.23 |
| White R. Reference | White River | September 2011 | 48.751442, -85.17329 | 1.7 (1.02 – 2.62) | 19.4 | 53 | 35 | 1.20 |
| White R. Burned | White River | September 2011 | 48.653532, -85.36495 | 1.3 (0.96 – 1.65) | 9.9 | 35 | 43 | 1.35 |
| White R. Logged | White River | September 2011 | 48.431849, -85.350316 | 1.3 (1.12 – 1.47) | 22.3 | 61 | 39 | 1.40 |
| Harp Lake | Muskoka | October 2011 | 45228'N, 79085'W | 1.9 (1.48 – 1.73) | 27.9 | 34 | 66 | 1.36 |
| HP3 | Muskoka | October 2011 | 45224'N, 79084'W | 1.8 (1.48 – 2.24) | 27.9 | 100 | 0 | 1.58 |
| Plastic Lake | Muskoka | October 2011 | 45107'N, 78496'W | 2.0 (1.53 – 3.04) | 10.4 | 67 | 23 | 1.13 |
| PC01 | Muskoka | October 2011 | 45107'N, 78497'W | 1.3 (0.75 – 1.98) | 30.4 | 69 | 11 | 1.21 |
| P108 | Muskoka | October 2011 | 45109'N, 78497'W | 1.0 (0.8 – 1.23) | 13.8 | 64 | 37 | 1.38 |

**Table 2.2.** Water chemistry for each series along with $LC_{50}$ values from 96 h toxicity tests with *Hyalella azteca*. Calculated LC50 values are based on measure Ni concentration (with lower confidence limit (LCL) and upper confidence limit (UCL)) were calculated from measured concentrations (mean ± standard deviation (n)).

| **Series** | **Ca (mM)** | **Mg (mM)** | **pH** | **DOC (mg/L)** | **$LC_{50}$ (mg Ni/L)** |
|---|---|---|---|---|---|
| Ca | 0.09 ± 0.01 (48) | 0.03 ± 0.004 (48) | 7.11 ± 0.03 (22) | 0.83 ± 0.11 (10) | 0.56 (0.33 – 0.94) |
| | 0.28 ± 0.03 (48) | 0.03 ± 0.007 (48) | 7.12 ± 0.02 (22) | 0.82 ± 0.11 (10) | 1.08 (0.84-1.39) |
| | 0.75 ± 0.09 (48) | 0.03 ± 0.006 (48) | 7.15 ± 0.02 (22) | 0.81 ± 0.11 (10) | 2.69 (2.09 – 3.47) |
| | 2.19 ± 0.24 (48) | 0.03 ± 0.004 (48) | 7.21 ± 0.02 (22) | 0.83 ± 0.11 (10) | 3.16 (2.48 – 4.03) |
| Mg | 0.09 ± 0.01 (48) | 0.03 ± 0.004 (48) | 7.17 ± 0.02 (22) | 0.83 ± 0.11 (10) | 1.08 (0.77 – 1.32) |
| | 0.09 ± 0.01 (48) | 0.51 ± 0.005 (48) | 7.14 ± 0.02 (22) | 0.82 ± 0.11 (10) | 0.66 (0.27 – 1.61) |
| Harp Lake DOM | 0.09 ± 0.01 (48) | 0.03 ± 0.005 (48) | 7.15 ± 0.02 (22) | 2.76 ± 0.39 (10) | 1.41 (1.15 – 1.73) |
| | 0.09 ± 0.01 (48) | 0.03 ± 0.004 (48) | 7.12 ± 0.02 (22) | 6.48 ± 0.17 (10) | 1.88 (1.48 – 2.32) |
| | 0.09 ± 0.01 (48) | 0.03 ± 0.004 (48) | 7.11 ± 0.02 (22) | 12.46 ± 0.52 (10) | 2.01 (1.66 – 2.44) |
| Lake Laurentian DOM | 0.09 ± 0.01 (48) | 0.03 ± 0.003 (48) | 7.13 ± 0.02 (22) | 6.32 ± 0.36 (10) | 1.85 (1.47 – 2.32) |
| Laurentian wetland DOM | 0.09 ± 0.01 (48) | 0.03 ± 0.005 (48) | 7.18 ± 0.02 (22) | 6.58 ± 0.38 (10) | 1.11 (0.73 – 1.59) |
| Daisy Lake DOM | 0.09 ± 0.01 (48) | 0.03 ± 0.004 (48) | 7.15 ± 0.02 (22) | 7.12 ± 0.36 (10) | 1.38 (1.14 – 1.69) |
| White R. Reference DOM | 0.09 ± 0.01 (48) | 0.03 ± 0.004 (48) | 7.19 ± 0.02 (22) | 7.11 ± 0.30 (10) | 1.65 (1.02 – 2.62) |
| White R. Burned DOM | 0.09 ± 0.01 (48) | 0.03 ± 0.003 (48) | 7.11 ± 0.02 (22) | 7.15 ± 0.98 (10) | 1.27 (0.96 – 1.65) |
| White R. Logged DOM | 0.09 ± 0.01 (48) | 0.03 ± 0.004 (48) | 7.11 ± 0.02 (22) | 6.99 ± 0.30 (10) | 1.28 (1.12 – 1.47) |
| HP3 DOM | 0.09 ± 0.01 (48) | 0.03 ± 0.004 (48) | 7.11 ± 0.02 (22) | 7.19 ± 0.21 (10) | 1.81 (1.48 – 2.24) |
| Clearwater Lake DOM | 0.09 ± 0.01 (48) | 0.03 ± 0.005 (48) | 7.11 ± 0.02 (22) | 6.73 ±0.33 (10) | 1.00 (0.87 – 1.15) |
| Plastic Lake DOM | 0.09 ± 0.01 (48) | 0.03 ± 0.004 (48) | 7.11 ± 0.02 (22) | 7.19 ± 0.18 (10) | 2.03 (1.53 – 3.04) |
| P108 DOM | 0.09 ± 0.01 (48) | 0.03 ± 0.006 (48) | 7.11 ± 0.02 (22) | 6.46 ± 0.23 (10) | 1.00 (0.8 – 1.23) |
| PC01 DOM | 0.09 ± 0.01 (48) | 0.03 ± 0.004 (48) | 7.11 ± 0.02 (22) | 7.01 ± 0.35 (10) | 1.27 (0.75 – 1.98) |

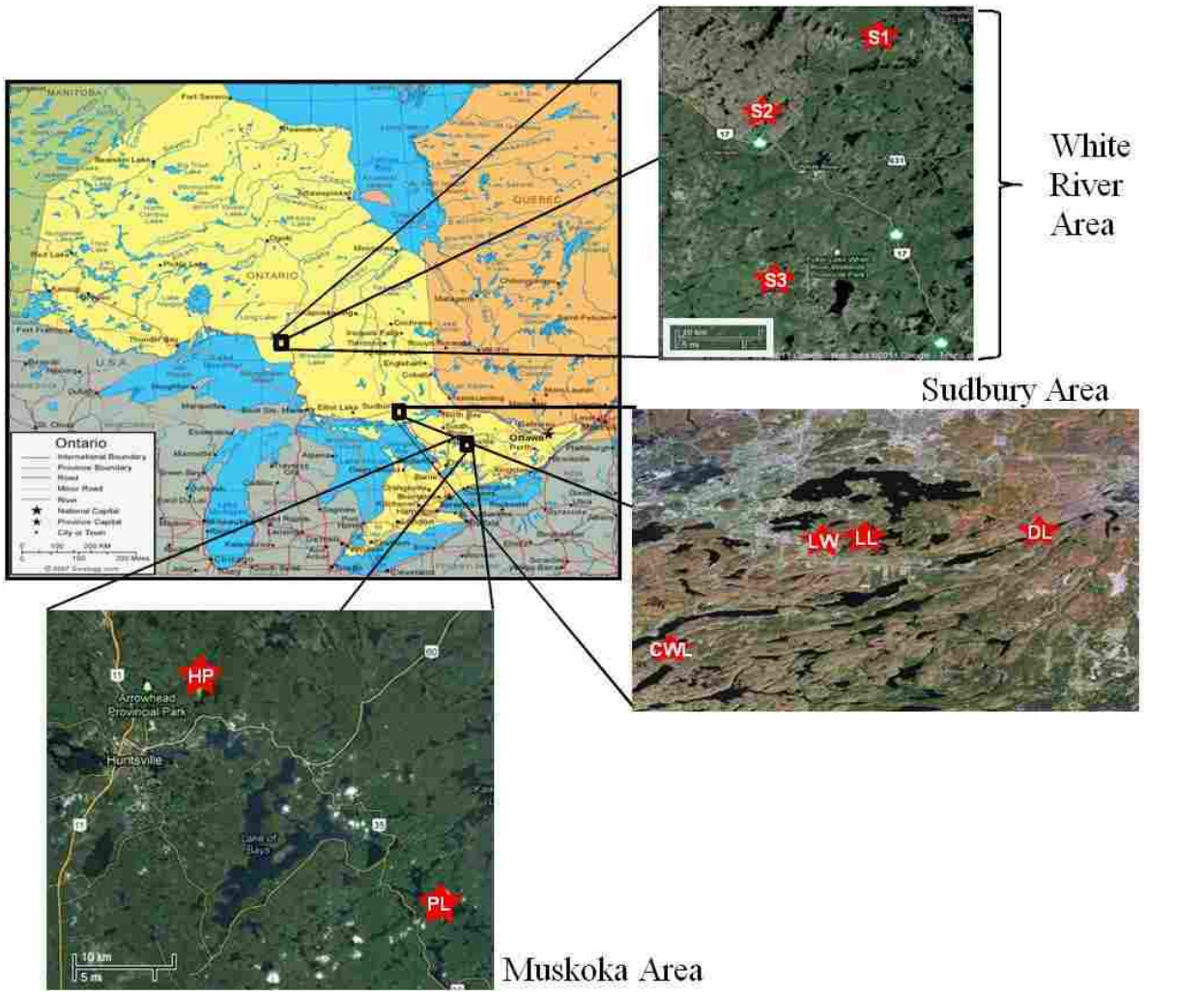


**Figure 2.1.** Map of DOM collection sites in central Ontario. In the White River area, 3 sites were collected (s1 = reference, s2 = burned, s3 = logged). Four sites were collected from the Sudbury area (CWL = Clearwater Lake, LW = Laurentian Wetlands, LL = Laurentian Lake, DL = Daisy Lake) and 5 sites were collected from the Muskoka area (HP = Harp Lake and HP3, PL = Plastic Lake, PC01, P108).

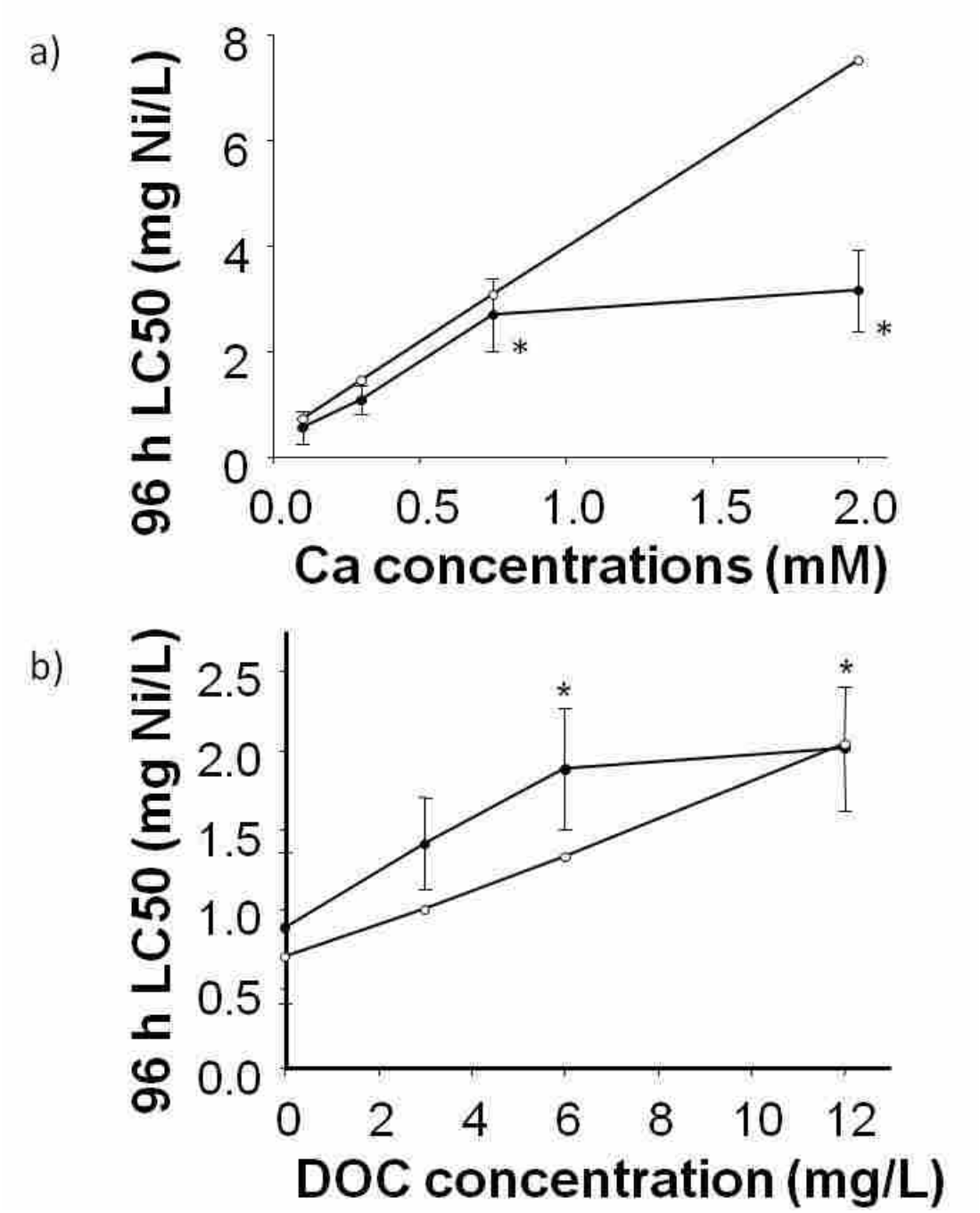


**Figure 2.2.** Protective effects of Ca (a) and DOM quantity (b) on acute Ni toxicity. Ca was added as $CaCl_2 \cdot 2H_2O$ at 0.1, 0.3, 0.75 and 2 (all mM) (a: black) while Harp Lake DOM was added at 3, 6, and 12 mg DOC/L (b: black). Open points (a and b) represent predictions from adjusted BLM for *Hyalella* (based on Kozlova et al., 2009) where critical value was changed to 6.75 from 5.1. Error bars represent 95% CI of LC50 values and * denotes significant difference from control (no added Ca (a) or DOM (b)).

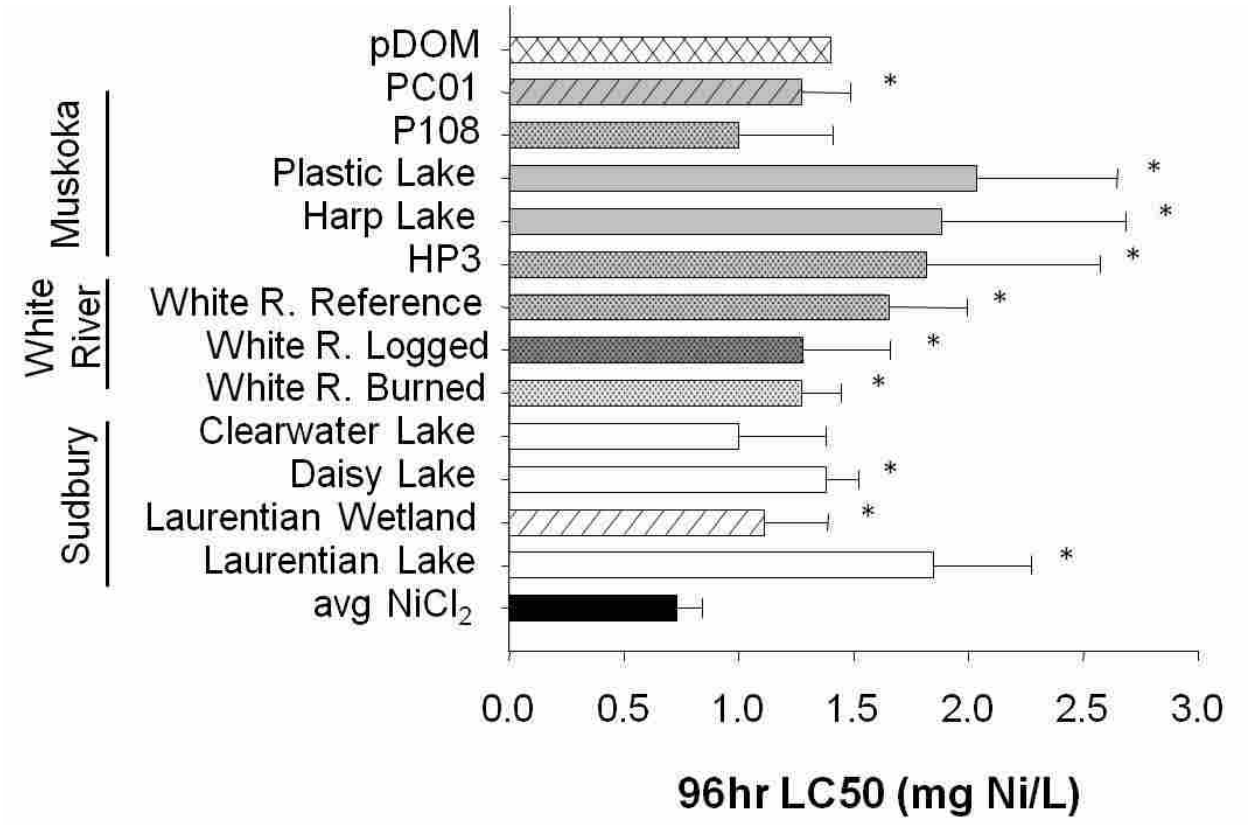


**Figure 2.3.** Comparison of DOM source differences via protective effects ($LC_{50}$ values) tested at a measured concentration of 7 mg DOC/L. Black bar represents a mean of $LC_{50}$ values (0.70 ± 0.10 mg Ni/L, n = 6) of Ni only exposures (no added DOM). Color of bars denote disturbance type (open = smelter impacted, light grey = burned, dark grey = logged, and medium grey = unimpacted/reference). Patterns on bars represent water type (open = lakes, side hatch = wetlands, and dotted = streams). Crosshatch bar (pDOM) represents a prediction of protective effect from Ni toxicity with added DOM (humic acid = 10%) through adjusted BLM model for *Hyalella*. Error bars represent 95% CI on measured $LC_{50}$ values and * denotes significant difference from average Ni only exposures.

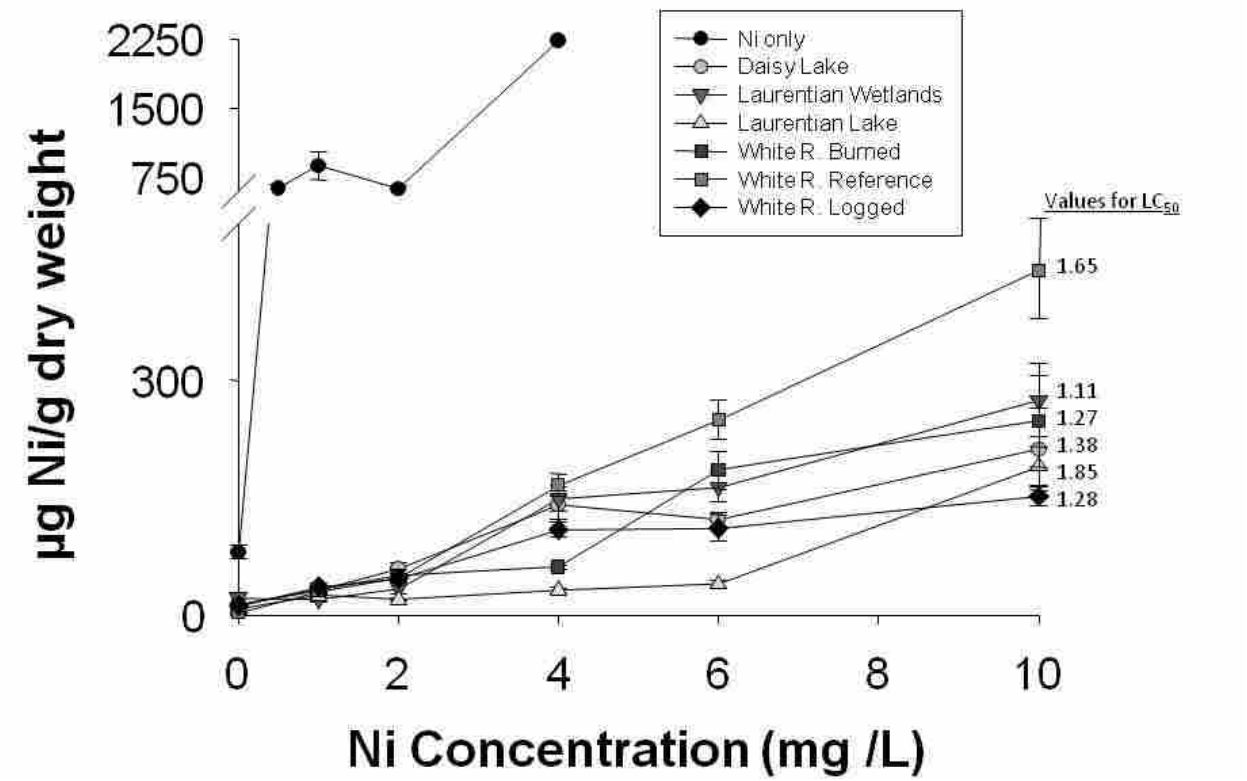


**Figure 2.4**. Bioaccumulation of Ni in *Hyalella* (n = 6 at each concentration or each exposure) at 6 hours for Ni only exposure (black circles) as well as 6 other DOM source exposures (all measured at 7 mg DOC/L). These sources include Daisy Lake (grey circles), Laurentian Lake (white triangles), Laurentian Wetlands (dark grey inverted triangles), White River Reference (light grey squares), Burned (dark grey squares), and Logged (black diamonds). Error bars represent standard error of Ni accumulation within the organisms. $LC_{50}$ values for each source is also listed to show bioaccumulation and protective effect relationship.

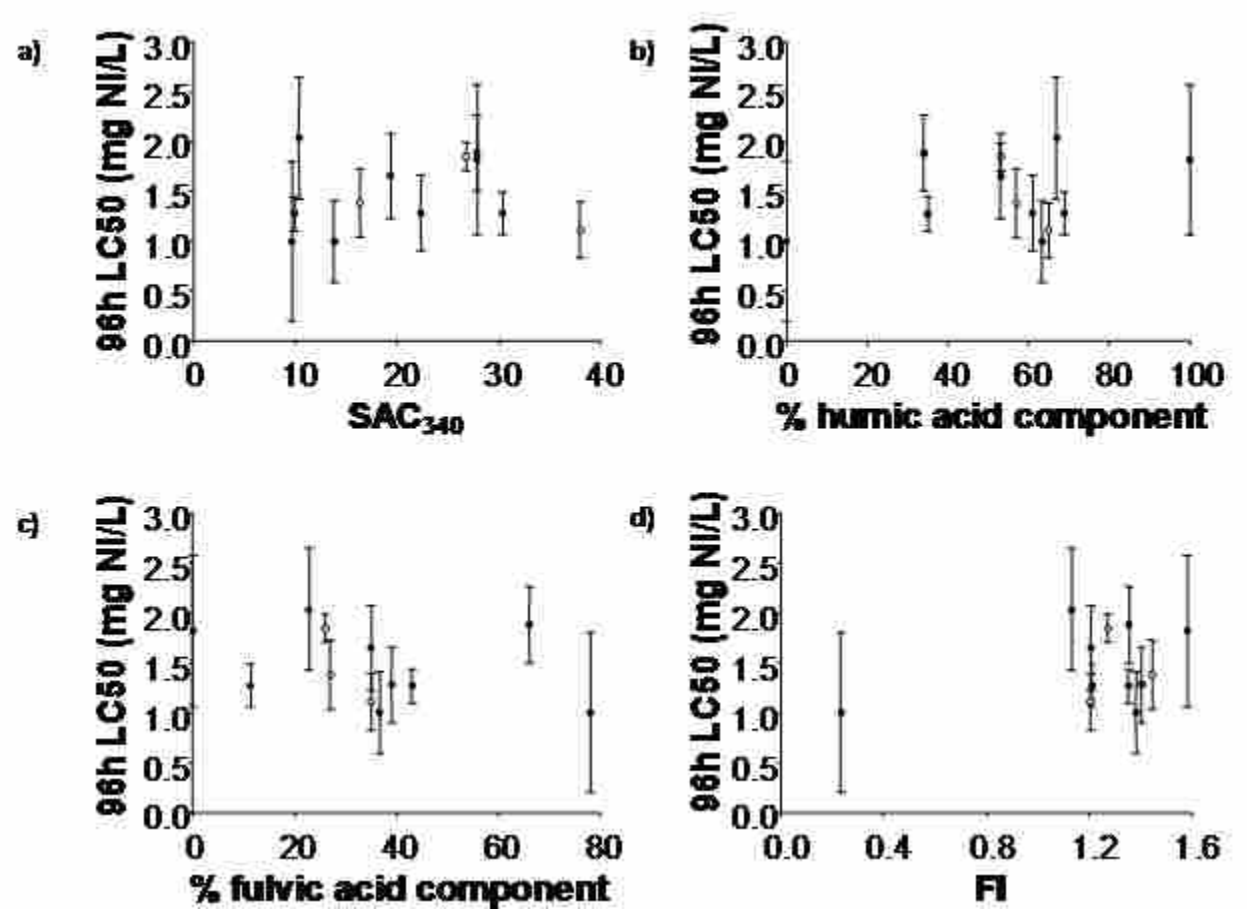


**Figure 2.5**. Relationship between optical characterizations of twelve DOM sources to measured $LC_{50}$ values. These optical characterizations include : $SAC_{340}$ values (a) at 10 mg DOC/L, % humic (b) and fulvic (c) acid components measured at 5 mg DOC/L, and FI (d) measured at 5 mg DOC/L. All optical characterization was separated into summer (open) and fall (black) collection times. Correlations for all optical characteristics were not significant: $SAC_{340}$, $r^2 = 0.11$, $p > 0.05$; % humic acid , $r^2 = 0.31$, $p > 0.05$; % fulvic acid, $r^2 = -0.41$, $p > 0.05$; FI, $r^2 = 0.34$, $p > 0.05$. Error bars represent 95% CI of $LC_{50}$ values. Corresponding data for optical characteristics of DOM sources found in Table 2.1.

### *2.5 Conclusion*

The results of this study show that Ca as well as DOM reduces Ni toxicity to *Hyalella azteca* while Mg does not seem to show any protective effect. The protective effects of Ca and DOM are observed to be similar although mechanisms of protection are different, competition vs. complexation, respectively. While DOM does reduce Ni toxicity, DOM source matters as well and differences in sources yields different protective effects against Ni toxicity. Unexpectedly, the optical characteristics of each DOM source (i.e., $SAC_{340}$, FI, HA-like and FA-like components) are observed to have no correlation to the toxicity reducing capabilities to Ni. BLM modelling was generally successful in accounting for Ca competition as well as DOM complexation although not at higher levels of Ca. Further study is required in order to better understand the mechanisms of uptake of Ni in *Hyalella* and interactions of Ni and DOM in relation to toxicity mitigation.

# Chapter 3
# Influence of Ca and DOM on chronic responses to Ni

### *3.1 Introduction*

Ni is not as well studied as other metals (e.g., Cu or Zn) for acute toxicity and even less is known about chronic Ni toxicity. While acute exposures are short and relatively simple to conduct, chronic exposures are more relevant to the long term effects of toxins such as Ni. The results yielded by chronic exposures may differ than those obtained in acute studies due to the possibility of different mechanisms of toxicity (Adams et al., 2010). The factors that influence acute toxicity may influence chronic toxicity as well. Physiological changes in chronic exposures may be caused by multiple effects rather than a single mechanism or site of action for a specific metal (Adams et al., 2010). Generalizations cannot be made about the comparison between acute and chronic exposures because mechanisms are specific to the test organism and metal (Adams et al., 2010). Modifying factors that affect Ni toxicity in acute exposures could be mirrored in chronic studies such that a protective effect observed in acute exposures may cause detrimental effects in chronic exposures due to the difference in exposure duration.

This study focuses on the protective effects of Ca and DOM on chronic Ni toxicity to *Hyalella azteca* in very soft waters. *Hyalella azteca* are omnivorous and benthic amphipods (Pennak, 1953) that are distributed throughout shallow fresh-water lakes in the Boreal Shield area (Othman and Pascoe, 2001). These organisms are often used for toxicity testing of metals since they are sensitive to aquatic contaminants (Borgmann, 1998; Environment Canada, 1997; Ingersoll et al., 1998). Their sensitivity makes them a relevant organism in understanding the potential for contaminants to impact aquatic biota.

Chronic effects of Ni have been studied by Deleebeeck et al. (2007) and Schroeder et al. (2010) in hard water with various invertebrates (e.g., *Hyalella azteca*) but the long term effects

of Ni in soft waters are not well understood. Elevated Ni concentrations have been shown to link to Mg disruption and deficiency of gas exchange at the gill in *Daphnia* (Pane et al., 2003a) as well as disturb respiratory function in fish (Pane et al., 2004). Toxic impacts on survival were observed at 0.3 µM Ni/L (17.61 µg Ni/L) by Schroeder et al. (2010) where the presence of Ca and Mg decreased Ni toxicity by a factor of 8.5 and 6.7, respectively.

Similar to acute toxic effects, chronic effects are also a result of metal ion (i.e., $Ni^{2+}$) interaction at the biotic ligand where $Ni^{2+}$ is considered to be the most toxic form of Ni (Pagenkoft, 1983). The water chemistry influences metal toxicity where competition, complexation, and concentration affect metal ion bioavailability (Di Toro et al., 2001). Competition between $Ca^{2+}$ and $Ni^{2+}$ as well as complexation with DOM provide protective effects (Di Toro et al., 2001). Toxicity of Ni is reduced in the presence of toxicity-modifying factors such as Ca (Schroeder et al., 2010; Kozlova et al., 2009) and DOM, where DOM from different sources showed different protective effects (Kozlova et al., 2009, Richards et al., 2001). Furthermore, the concentrations of Ca, DOM and Ni affects the ratio between bioavailable and complexed forms of the metal therefore influencing the potential for toxic impact (Di Toro et al., 2001).

These protective effects can be quantified by common toxicity indicators such as survival and growth as well as bioaccumulation (as suggested by Borgmann et al., 2001). Bioaccumulation has been suggested to be the most direct way of quantifying bioavailable contaminants which can be related to an observed toxic effect (i.e., survival: Borgmann et al., 2001). Prediction of chronic Ni toxicity based on Ni accumulation within *Hyalella* may be possible since body concentrations are suggested to be a better measure of toxicity than water concentrations of metals (Borgmann et al., 1991, 1998; Borgmann and Norwood, 1997). The use

of bioaccumulation measurements for predicting metal toxicity is supported although problems may arise through the use of whole body organisms since metal uptake is dynamic where the duration and exposure condition can affect metal accumulation (Adams et al., 2010). Some organisms have the ability to store and detoxify metals within the body therefore it may be difficult to assess linkages between toxicity and metal accumulation (Adams et al., 2010). If *Hyalella azteca* is an organism that cannot store and detoxify metals, assessing Ni bioaccumulation using the whole body approach in this chronic study may lead to the understanding of potential linkages to the modifying influences of Ca and DOM on Ni toxicity.

The DOMs used in this study are collected to compare previously-smelter-impacted aquatic systems in Sudbury (Daisy Lake) to reference conditions in the Muskoka region (Plastic Lake). Daisy Lake was chosen due to the previous impacts of smelter emissions and relatively unsuccessful recovery despite great efforts. Plastic Lake was chosen because of its pristine conditions (reference site). DOMs are heterogeneous and complex due to their formation from various precursors which affect the composition and structures of these compounds (McKnight and Aiken, 1998). The differences between these sources can be identified with optical characterization such as measurements of absorbance and fluorescence through EEMS and PARAFAC (i.e., $SAC_{340}$, FI, HA-like and FA-like components). It has been suggested that optical characteristics help explain differences in protective effects provided by different DOM sources to the toxic effect of various metals (Al-Reasi et al., 2012); therefore the same could be observed with Ni.

The objectives of this research are to understand the role of Ca and DOM on the chronic toxicity of Ni to *Hyalella azteca*. This was done by 1) determining the effect of Ca on chronic Ni toxicity, 2) understanding if DOM sources differ in their capacity to influence chronic Ni

toxicity, and 3) comparing and contrasting the acute and chronic effects of Ni on *Hyalella azteca*. Based on the approach to this reasearch, it is hypothesized that Ca and DOM will act in the same manner in chronic studies as they do in acute studies where the addition of toxicity modifying factors (Ca and DOM) would increase $LC_{50}$ values (protect against Ni toxicity) but have different toxic effects amount the same DOC concentrations of different sources.

## *3.2 Materials and methods*

### *3.2.1 Invertebrate cultures*

*Hyalella azteca* were collected in August 2010 from Hannah Lake in Sudbury which is a previously acidified and metal impacted lake in Ontario. Collected organisms were identified as *Hyalella azteca* by referencing to Pennak (1953). Culturing protocol followed Environment Canada standard method EPS 1/RM/33 (Environment Canada, 1997). Groups of 30 adults were cultured in 1L polyethylene beakers with 800mL of an artificial soft water medium consisting of: 0.1mM $CaCl_2$-$2H_20$, 0.1mM $NaHCO_3$, 0.025mM $MgSO_4$-$7H_2O$, 0.005mM KCl, and 0.001mM NaBr (all salts from Sigma-Aldrich Inc. Mississauga, ON) at pH 7.0, temperature of 20 $^oC \pm 1\ ^oC$ with a 16h light: 8h dark photoperiod. Br was added to the soft water medium since it is an essential element for reproduction of *Hyalella azteca* (Borgmann, 1996). Organisms were fed 5 mg of Tetramin ™ flakes (Tetra Werke, Blacksburg, VA, USA) 3 times a week on non-consecutive days and static water renewals (100%) were performed weekly. A piece of cheese cloth (5 cm x 5 cm) was placed in each beaker to act as substrate for the amphipods. Neonates were harvested weekly by screening through mesh sizes 650 µm (for adults) and 275 µm (for neonates) and were used either for testing or to maintain organism cultures.

### *3.2.2 Natural DOM collection and preparation*

Two DOM sites, Daisy Lake and Plastic Lake, were collected in July 2010 and Oct 2011 respectively by reverse osmosis concentration, followed by resination and refrigeration at pH 2 (Schwartz et al., 2004). Water was collected a few meters from the shore of the sites and pumped through a 5µm filter to remove any particulate debris before transferring to the stainless steel portable reverse osmosis unit. The reverse osmosis unit was equipped with XYZABC membranes. About 8 L of DOM concentrate (ranged from 6 to 10 L) was collected by transferring approximately 350 L (200 to 500 L, source dependent) of lake water through the reverse osmosis. The DOM concentrate was collected in pre-washed 4 L polyethylene containers or 10 L polypropylene containers that were previously rinsed with 0.1% $HNO_3$.

The DOM collected is poured into a stainless steel pot and activated resin (USF C-211 H cation resin, U.S. Filter Corporation, Rockford IL) is added to lower the pH and strip the DOM of any cations or residual metals; a process called resination. To activate resin, 600 mL of 4N HCl (Fisher Scientific, Nepean, ON) was added to 3.6 L of resin for 5 minutes then rinsed 5 times with deionized water and then another 5 times with MilliQ filtered water (18mΩ.cm; MilliQ A10, Millipore, Mississauga, ON). Properly resinated DOM will have a stable pH and not change when more activated resin is added to the solution (usually a pH of 2). These samples were measured for DOC content and refrigerated at 4ºC. Before using DOM in exposures, aliquots of concentrate were adjusted to pH 7.0 by adding 0.1 mM NaOH (Sigma-Aldrich Inc. Mississauga, ON). Appropriate dilutions of DOM concentrate were then done to achieve the desired test DOC concentrations.

### *3.2.3 Exposure conditions*

Chronic toxicity tests were exposed for 28 days, performed in triplicate, with 10 neonates (2 – 9 d of age per replicate), in standardized volumes (200 mL of solution in a 400 mL polyethylene beaker) that were fed 5 mg of Tetramin™ flakes (Tetra Werke, Blacksburg, VA, USA) 3 times a week on non-consecutive days after a static water renewal (100%) (Environment Canada, 1997). Stock solutions of 10 L for the test were made by adding aliquots of 1g/L stock of $NiCl_2 \cdot 6H_2O$ (Sigma-Aldrich Inc. Mississauga, ON) to prepared aquatic media to give exposure concentrations ranging from 10 to 160 µg/L.

Toxicity-modifying-factors tested included Ca at 1 mM and 2 DOM sources. The acute effects of Mg were not significant and struggled to maintain an acceptable survival percentage (80 %) and therefore chronic testing with Mg did not occur. The Ca concentration was chosen to be 1 mM based on protective effects observed in acute studies. The Ca solution was prepared by adding $CaCl_2 \cdot 2H_2O$ (Sigma-Aldrich Inc. Mississauga, ON) to the artificial soft water medium.

Two sources of DOM were used for chronic testing - Daisy Lake DOM and Plastic Lake DOM. These two exposures started 2 weeks apart from each other and separate control replicates (no added DOM) were used to account for possible changes to the organisms over time. Both sources of DOM were tested at a nominal concentration of 6 mg DOC/L based on observed acute protective effects.

### *3.2.4 Ni bioaccumulation*

Chronic Ni bioaccumulation was analyzed in surviving *Hyalella* at the end of the 28 day

exposure (n values varied since it was based on survival of the organisms; range of n = 1 to n = 30). As a control measure, organisms (n = 20) were also sampled at day 0.

### *3.2.5 Sampling and digestion*

Water samples were collected just before 100% static water renewals of solution (3 times a week on non-consecutive days) to determine water chemistry. Filtered (0.45 µm HT tuffryn, Pall, Ann Arbor, MI) and unfiltered water samples (10 ml) were collected for dissolved and total (respectively) Ni concentrations. Each sample was taken with a 10 mL disposable syringe (NORM-JECT®, Henke Sass Wolf, Germany) and stored in 20 mL scintillation vials (VWR International, LLC, Mississauga, ON). The samples are acidified to 1% by adding 100 µL of 16N $HNO_3$ (Trace Metal Grade, Fisher Scientific, Mississauga, ON). Additional 50 mL samples were filtered (0.45 um HT tuffryn, Pall, Ann Arbor, MI) and analyzed for DOC. The DOM samples were not acidified for analysis.

*H. azteca* that were analyzed for bioaccumulation were dried, weighed, and digested to measure accumulation. Organisms were removed from exposure with a disposable pipette, transferred briefly into deionized water, and then blotted on a kimwipe to remove excess moisture. *Hyalella* were collected at day 0 and day 28 so that growth effects can be compared between the time points. The organisms were placed in individual 0.6 mL tubes and dried at 80$^{o}$C (Isotemp500 series, Fisher Scientific, Nepean, ON) for 48 hrs. Each organism was weighed to the nearest 0.1 µg (SE2 Ultra Micro Balance, Sartorius Mechantronics Corp., Bohemia, NY) and placed back into its individual tube for digestion. The protocol for digestion followed Neumann et al. (1999), beginning with the addition of 25 µL of 16N $HNO_3$ (Trace Metal Grade, Fisher Scientific, Mississauga, ON). After 6 days at room temperature, 20 µL of 30% $H_2O_2$ (Sigma-

Aldrich Inc. Mississauga, ON) was added and after 24 hrs, solutions were brought up the final volume of 250 µL with the addition of deionized water (18mΩ cm; MilliQ A10 Millipore, Mississauga, ON). The digested samples were analyzed for Ni content.

### *3.2.6 Sample analysis*

All water and organism samples were measured for Ni using graphite furnace atomic absorption spectroscopy (AAS SpectraAA 880 GTA 100 atomizer, Varian, Mississauga, ON). Both unfiltered and filtered water samples are measured for total and dissolved Ni, respectively. Additional measurements were performed on water samples for Ca with AAS on flame mode. Standards were made from $NiCl_2 \bullet 6H_2O$ for Ni and $CaCl_2 \bullet 2H_2O$ for Ca (Sigma-Aldrich Inc. Mississauga, ON) and verified with a reference standard (TM-26.3, National Water Research Institute, Burlington, ON).

DOM concentrations, measured as DOC, were measured using a total organic carbon analyzer (TOC-V with ASI-V autosampler, Mandel Scientific, Guelph, ON). The DOM stock solutions were analyzed at the beginning of each test for DOC concentrations. DOC was measured for all series to ensure consistency between exposures so that these series could be compared. All stock solutions were measured for DOM at the time of every water renewal due to the possibility of increasing DOM amounts while stored in a 10 L carboy for 28 days.

### *3.2.7 Optical characterization*

The two DOM sources were analyzed for optical characteristics, specifically $SAC_{340}$, FI values, HA-like content, and FA-like content via EEMS and PARAFAC. $SAC_{340}$ values were

measured and analyzed (Christine Geiger, York University, ON) following the method of Al-Reasi et al. (2012) using the equation provided by Curtis and Schindler (1997):

$$SAC_{340} = [2303 \times (Abs340)] / DOC \quad \text{Equation 1}$$

where Abs340 is the absorbance value at 340nm and DOC represents the concentration of DOM used for measurements. All DOM samples were increased to pH 7 by adding 0.1 mM of NaOH then diluted to a concentration of 10 mg DOC/L with MilliQ filtered water (18mΩ.cm; MilliQ A10, Millipore, Mississauga, ON) before analysis. These samples were then placed in a 1 cm quartz cell (Helma Canada Ltd. Concord, On, Canada) and measured three times. Measuring the absorbance of MilliQ filtered water (18mΩ.cm; MilliQ A10, Millipore, Mississauga, ON) was used as the blank (reference standard). A correlation of $SAC_{340}$ values and measured $LC_{50}$ values will be made to determine if absorbance coefficients can explain differences in protective effects from chronic Ni toxicity.

FI was conducted (Kelly Livingstone, Wilfrid Laurier University, ON) following the methods of Gheorghiu et al. (2010) and was calculated via (McKnight et al., 2001):

$$FI = (370:450)/(370:500) \quad \text{Equation 2}$$

where FI is the ratio of intensities at 370:450 and 370:500 (excitation : emission wavelengths in nm). Measurements were taken at 5 mg DOC/L. DOM samples were also analyzed for HA-like and FA-like components using EEMS and PARAFAC producing three-dimensional contour plots that identify different components found within DOM including HA-like and FA-like content

(Smith and Kramer, 1999).

### *3.2.8 Calculations and statistical analysis*

Survival and growth (28 day dry weight basis) data from chronic toxicity tests were used to calculate $LC_{50}$, $EC_{20}$ (effect where 20% reduction in growth is observed), and $EC_{50}$ values (effect where 50% reduction in growth is observed) by means of the Comprehensive Environmental Toxicity Information System (CETIS v.6.0, Tidepool Scientific Software) and this was done on a total and dissolved Ni basis. The Spearman-Karber statistical test was used to determine the median $LC_{50}$ estimate. Significant differences in $LC_{50}$s were compared by using the 95% CI overlaps and if overlaps were present, a statistical method developed by Litchfield and Wilcoxon (1938) as described by Gillis et al. (2010) was used.

Ni bioaccumulation is assessed on a whole body dry weight basis. Whole body Ni concentrations were expressed as µg Ni/ g dry weight and differences among treatment groups were tested by one-way ANOVA followed, when appropriate, with the Fischer LSD test using Sigmaplot™ (v. 11), a scientific data analysis and graphing software.

## ***3.3 Results***

### *3.3.1 Water chemistry*

Water chemistry was measured for Ni (µg/L), Ca (mM), and DOC (mg/L) when necessary. Unfiltered and filtered water samples were analyzed for total Ni and dissolved Ni,

respectively. Dissolved Ni concentrations were calculated to be 100 ± 8 % (mean ± SEM, n = 250) of total Ni and therefore all Ni concentrations reported will be on the total Ni basis. Ni concentrations at day 28 were calculated to be 92.9 ± 6% (mean ± SEM, n = 250) of day 0 Ni concentrations. Weekly variation of total Ni, dissolved Ni and DOC concentrations were measured. To account for any differences between weeks during exposure, a paired t-test was performed between initial (start of the week) and final (end of the week) total Ni, dissolved Ni, and DOC concentrations where no significant difference was observed ($t = -0.828$, $P = 0.416$; $t = 1.317$, $P = 0.201$; $t = -0.232$, $P = 0.819$, respectively) (Table 3.3).Variability of Ni concentrations within replicates was 2.00 ± 0.11 % (mean ± SEM, n = 100). Ca and DOC concentrations consistent across replicates and series (Table 3.1). DOC concentrations in DOM series were measured to be higher than the intended nominal concentration of 6 mg DOC/L. These concentrations were not corrected due to the amount of DOC available for testing. Background DOC levels were measured to be 0.45 ± 0.07 mg DOC/L (n = 148) in the Ca series (Table 3.1).

In the culture medium for chronic exposures, 80.1% of total Ni is calculated to be free $Ni^{2+}$ ion by the Windermere Humic Aqueous Model (WHAM, ver.7.0.2) at the measured $LC_{50}$ value. Other Ni species were predicted to be 7.7%, 0.4%, and 3.6% for $NiHCO_3$, $NiSO_4$, and $NiCO_3$ respectively. The BLM on the other hand predicted the culture medium to be 73.8% $Ni^{2+}$ ion where other Ni species were predicted to be 7.2%, 0.3%, and 1.6% for $NiHCO_3$, $NiSO_4$, and $NiCO_3$, respectively. Ni species in solution were compared between the two models where the BLM predicted about 9% less of these specific species in solution than WHAM. The BLM also predicted less free $Ni^{2+}$ ion by 6.3% than WHAM.

### *3.3.2 Survival*

The $LC_{50}$ value for the chronic (28 d) effect of Ni only (without modifying factors) was 13.83 ± 2.61 µg Ni/L (± 95% CI) (Fig 3.1). When 1 mM Ca was added, the $LC_{50}$ value significantly increased to 27.74 ± 6.32 µg Ni/L (± 95% CI) (Fig 3.1). A similar protective effect was observed with the addition of Plastic Lake DOM at a $LC_{50}$ value of 28.59 ± 3.23 µg Ni/L (± 95% CI) (Fig 3.1). When the DOM source changed to Daisy Lake, a greater protective effect (6.5-fold increase of Ni only $LC_{50}$ compared to Plastic Lake DOM) was observed where the $LC_{50}$ value increased to 91.34 ± 10.53 µg Ni/L (± 95% CI) (Fig 3.1). Day 10 $LC_{50}$ values are also calculated and compared to day 28. A 2-fold increase in $LC_{50}$ values at day 10 was observed when compared to day 28 although similar trends are observed (Fig 3.1).

### *3.3.3 Growth effects and changes in dry weight*

The dry weights between controls (without modifying factors; nominal Ni concentration of 0 µg Ni/L) had little variation between treatments (0.12 ± 0.02 g to 0.26 ± 0.02 g (mean ± SEM), averages of Daisy Lake DOM series (unmodified) and Ca series (unmodified) respectively; Table 3.2). The largest variation in dry weight is observed in controls when only modifying factors are added. When Ca (1 mM) was added, dry weights significantly decreased by a factor of 2 when compared to control medium with no added Ca (averages of ‘unmodified’ vs. Ca (1 mM) at 0 µg Ni/L; Table 3.2b). In addition to differences in dry weight, survival also decreased from 90% to 53% at day 28 when comparing no added Ca and the addition of 1 mM Ca, respectively (averages of ‘unmodified’ vs. Ca (1mM) at 0 µg Ni/L; Table 3.2b).With the addition of Plastic Lake (PL) DOM, dry weights did not change significantly when compared to the control with no added DOM (averages of ‘unmodified’ vs. DOM (PL) at 0 µg Ni/L; Table

3.2c) while the addition of Daisy Lake (DL) DOM increased dry weights by a factor of 1.5 compared to the controls with no added modifying factor (averages of 'unmodified' vs. DOM (DL) at 0 µg Ni/L; Table 3.2d).

Dry weights of *Hyalella* decreased as Ni concentrations increased (Fig 3.2). The addition of Ca only (1 mM) caused the dry weight of the organisms to decrease 50% which can be correlated with a decrease of survival to 53% (Fig 3.2). Furthermore, low concentrations of Ni (up to 10 µg/L) caused an increase (to 100%) in dry weights in the Ca series (Fig 3.2). When Ni concentrations reached above 10 µg/L, survival and dry weight decreased (Fig 3.2 and Table 3.1b). The addition of Ni to the PL series increased dry weight when compared to the Ni only exposure (Fig 3.2). The pattern of growth in the DL series was similar to the PL series and Ni only series as well (Fig 3.2).

Protective growth effects were observed when modifying factors were added (Ca and DOM) (Fig 3.3). The $EC_{20}$ varied between 1.4 ± 0 µg Ni/L (± 95% CI) and 50.1 ± 0 µg Ni/L (± 95% CI) with the lowest value representing the Ni only series (no added modifying factors) and the highest value representing the Ca series (addition of 1 mM Ca) (Fig 3.3). The addition of Ca (1 mM) increased protection by 8-fold compared to the Ni only exposure (Fig 3.3). The trend observed between the two DOM sources in survival were also observed in growth where DL DOM provided 3-times more protection than PL DOM (Fig 3.3).

### *3.3.4 Bioaccumulation*

Ni accumulation patterns in surviving *Hyalella* at day 28 in all series did not show any significant difference (Fig 3.4). Each series did show varying accumulation patterns despite no significant difference. In the Ni only series, a slight increase in accumulation was associated with

the increase of Ni concentration (accumulation ranged from 9.0 ± 0.75 µg Ni/ g dry weight (mean ± SEM, n = 25) to 17.56 ± 3.10 µg Ni/ g dry weight (mean ± SEM, n = 7); concentration of 0 to 20 µg Ni/L, respectively) (Fig 3.4). Prolonged exposure to Ni with 1 mM of Ca resulted in a plateau across Ni concentrations (19.53 ± 2.31 µg Ni/ g dry weight (mean ± SEM, n = 27) to 28.27 ± 4.81 µg Ni/ g dry weight (mean ± SEM, n = 2); concentration of 0 to 80 µg Ni/L, respectively) (Fig 3.4). Similar to the Ni only series, the addition of DOM (both sources) caused a slight increase in Ni accumulation across increasing Ni concentrations as well (Fig 3.4). Although both DOM sources show similar patterns, more metal accumulation was observed in Plastic Lake than Daisy Lake (e.g., 29.96 ± 4.96 µg Ni/ g dry weight (mean ± SEM, n = 5) and 15.37 ± 1.15 µg Ni/ g dry weight (mean ± SEM, n = 27), respectively at 40 µg Ni/L) (Fig 3.4).

### *3.3.5 Optical characteristics*

Optical characteristics (i.e., $SAC_{340}$, FI, HA-like and FA-like components) were measured in both DOM sources. $SAC_{340}$ values were calculated (Christine Geiger, York University, ON) and were compared to the observed $LC_{50}$ values. A correlation between $SAC_{340}$ values and $LC_{50}$ values was observed for the two sources. Unfortunately, no definitive trends can be established since only two sources were tested in this chronic study. On the other hand, $LC_{50}$ values did not correlate with measured FI values (Kelly Livingstone, Wilfrid Laurier University, ON). PARAFAC analysis was performed on the two DOM sources (Kelly Livingstone, Wilfrid Laurier University) where HA-like and FA-like components were identified and correlations to the measured acute $LC_{50}$ values were made. Similar to FI values, no significant correlations with $LC_{50}$ values were demonstrated with HA-like as well as FA-like components.

### *3.4 Discussion*

In this chronic study, both Ca and DOM provided protection against Ni toxicity to *Hyalella azteca* and this was noted for both survival and growth (Fig 3.1 and 3.3). The protective effect of Ca varied between endpoints, providing an 8-fold increase in protection (1 mM compared to 0.1 mM Ca) for growth compared to a 2-fold protection for survival. The addition of DOM (at a measured concentration of 9 mg DOC/L) demonstrated protective effects from Ni toxicity where the same magnitude of protection was observed in both growth and survival (Fig 3.1 and Fig 3.3). Differences between DOM sources were observed where Daisy Lake provided more protection than Plastic Lake (Fig. 3.1 and Fig 3.3). The addition of 1 mM Ca on its own acted as a stressor and resulted in poor growth and survival (50% reduction from controls) while the addition of Daisy Lake DOM on its own caused dry weight to increase 140% compared to controls without modifying factors or Ni (Fig 3.2). Toxic effects induced by the Ni only exposure could not be correlated with bioaccumulation (Fig 3.4).

The chronic effects of Ni toxicity are not extensively studied although Ni does induce physiological disruptions. Details surrounding the mechanisms of Ni-induced-effects are not well understood but elevated concentrations of Ni (2 mg Ni/L for over a month) act as a respiratory toxicant to rainbow trout causing a decrease in oxygen tension in the arteries, resulting in high carbon dioxide tension that eventually leads to respiratory acidosis (Pane et al., 2004). Ni is also known to be responsible for physiological effects related to a gas exchange deficiency in *Daphnia magna* (Pane et al., 2003b). These effects were determined through a reduction in swimming activity where *D. magna*, chronically exposed (14-d) to 130 µg Ni/L, were less mobile and slower compared to controls (Pane et al., 2003a). This impairment could have been

caused by an effect at the gill surface, directly limiting oxygen uptake causing abnormalities in metabolism which affects oxygen delivery and the internal use of oxygen (Pane et al., 2003a).

The sensitivity of *Hyalella azteca* to Ni increased dramatically in chronic exposures compared to acute exposures ($LC_{50}$ values of 0.70 ± 0.10 mg Ni/L and 13.8 ± 2.61 µg Ni/L, respectively). In the Pane et al. (2003a) study, $EC_{50}$ values were not calculated but the concentration associated with sub-lethal effects, 130 µg Ni/L (see above), could induce mortalities in our study conditions. However the Pane study (2003a) was in much harder water (120 mg $CaCO_3$/L) and therefore, it cannot be definitively concluded if *Hyalella azteca* and *Daphnia magna* are similar in their sensitivity to chronic Ni exposure. However, the 28-d $LC_{50}$ value for the Ni only exposure (no added modifying factors) in our study was 13.8 ± 2.61 µg Ni/L (Fig 3.1) and was comparable to the Schroeder et al. (2010) study where the 28-d $LC_{50}$ value in a low hardness media (prepared as a mixture of deionized and dechlorinated water) was 0.3 µM Ni/L (17.6 µg Ni/L). *Hyalella* are very sensitive to the chronic effects of Ni based on the measured chronic $LC_{50}$ value since the Canadian Council of Ministers of the Environment (CCME) water quality guideline for the protection of aquatic life is 25 µg Ni/L in very soft water.

A change in water chemistry (the addition of cations or organic matter) has been demonstrated to protect an organism from metal toxicity (Di Toro et al., 2001, Kozlova et al., 2009, Schroeder et al., 2010). The addition of Ca protected *Hyalella azteca* from the toxic effects of waterborne Ni in survival and growth (Fig 3.1 and 3.3) where an interaction between $Ca^{2+}$ and $Ni^{2+}$ was observed. The high protective effect of $Ca^{2+}$ is similar to the study of Schroeder et al. (2010) where Ca has a strong influence on Ni toxicity compared to other cations (e.g., Mg).

Since the addition of $Ca^{2+}$ protects against Ni toxicity, it was surprising to find that

exposures with added Ca alone (no Ni added) showed a negative effect compared to controls (no added Ni or Ca). Survival and dry weight of *Hyalella* at 28 d were both reduced in solutions with elevated Ca only (Fig 3.2). Interestingly, this Ca effect was eliminated with the addition of Ni at low concentrations (Fig 3.2). In general, *Hyalella* are well known as a species tolerant to elevated hardness waters (Environment Canada, 1997) but the mechanisms underlying this observed Ca effect are unknown. It is possible that *Hyalella* from Hannah Lake are physiologically unique in comparison to other sources of *Hyalella*. Organisms from Hannah Lake may be unable to tolerate elevated levels of Ca since Hannah Lake has been prone to high levels of Na and Cl relative to Ca but has recovered to a significant degree. Certainly, their ability to thrive in the soft waters used in this study sets them apart since the minimum hardness recommended in the standard method (Environment Canada, 1997) is 80 mg $CaCO_3$/L. It is also possible that the effects observed were related to a response to the change in Ca concentration rather than an innate inability to tolerate elevated Ca. Indeed, it was shown that an increase in waterborne Ca resulted in an inhibition of reproduction in *Ceriodaphnia dubia* (Schwartz et al. ,2007) but an understanding of the mitigation of this Ca impact by low concentrations of Ni is still lacking. Given that Ca appears to competitively reduce Ni uptake (observed as elevated $LC_{50}$ and EC values) it seems reasonable to consider that low levels of Ni will competitively reduce Ca uptake which should occur if Ca and Ni share a similar site of uptake.

The chronic effects of Ca uptake are not well defined for invertebrates. The uptake of Ca to crustaceans is important since Ca is the main component of their carapace (Cameron and Wood, 1985). An enhanced ability to uptake Ca could be essential for crustaceans in very soft waters (Cameron and Wood, 1985). Since the uptake of Ca is important for survival, then perhaps an increase in the external concentration results in an overload of internal Ca. In other

words, an effort to avoid hypocalcemia in low hardness waters may result in hypercalcemia when waterborne Ca content is increased. The effects of hypercalcaemia are well defined in mammalian literature. Extremely high levels of Ca have been linked to symptoms of hypercalcaemia such as kidney failure as well as dysfunction within the gastrointestinal tract and central nervous system, deterioration of consciousness, and ultimately death (Coleman, 2001). It is unknown if similar effects could occur in hypercalcaemic invertebrates but these effects are worthy of further study.

In addition to the effects observed in the Ca series, the addition of DOM also demonstrated a protective effect where the presence of PL and DL DOM reduced chronic Ni toxicity (Fig 3.1 and 3.3). A similar effect of DOC on chronic toxicity was documented by De Schamphelaere and Janssen (2004) in *Daphnia magna* exposed to Cu. Interestingly, the two DOM sources in our study offered different protective effects where DL DOM offered more protection than PL DOM (Fig 3.1 and Fig 3.3). In addition to varying protective abilities, the effect of adding only DOM (no Ni added) also differed in dry weights between PL DOM and DL DOM (Fig 3.2). The addition of 9 mg DOC/L of DL DOM increased mean dry weight to 140% of controls while no difference in dry weight was observed with the addition of PL DOM (Fig 3.2). The mechanism underlying this effect caused by the addition of DL DOM is unknown and could be physiological (e.g., reducing osmoregulatory stress), nutritional (e.g., directly or via enhanced microbial availability) or possibly both. Clearly this illustrates that there are differences in DOM quality that may have important biological consequences beyond that of toxicity mitigation and is worthy of further research.

The protective effects observed with DL DOM in the chronic Ni exposure was greater than that of PL DOM. Varying degrees of toxic impacts among different DOM sources were also

observed by Kozlova et al. (2009) with *D. pulex* and Richards et al. (2001). However, the mentioned studies (Kozlova et al., 2009; Richards et al., 2001) are acute exposures and very few studies have analyzed DOM source differences for chronic toxicity. When acute and chronic studies were compared, there was an inverse relationship between the magnitude of protection of DOM sources. That is, DL DOM was less protective than PL DOM to acute Ni toxicity while DL DOM was more protective than PL DOM in chronic exposures. The difference in protective effects of DOM sources between acute and chronic exposures might be related to variations in the uptake and mechanisms of Ni toxicity (Heijerick et al., 2005; Adams et al., 2010). However, it is more likely that the results reflect differences among the DOM sources and their ability to complex with Ni. The binding sites on DOM are generally represented as a complex series of ligands with different affinities for metals at various concentrations. It can be predicted that different sources of DOM may have different binding capacities. Perhaps in comparison to PL DOM, DL DOM has more binding sites with higher affinities that strongly interact at relatively low concentrations of Ni (in the chronic toxicity range) and fewer low affinity binding sites that complex to Ni at higher concentrations (1-2 mg/L range). These characteristics can therefore influence the protective ability of the DOM source.

Clearly, the two DOM sources varied in their ability to interact with $Ni^{2+}$ based on their differences in protective effects (Fig 3.1 and Fig 3.3). For acute toxicity, it is generally observed that optical characteristics such as FI, HA-like and FA-like content and $SAC_{340}$, (particularly the latter) can be correlated to metal toxicity mitigation and serve as a simple measure to account for DOM source quality (Ryan et al., 2004; Schwartz et al., 2004). However, these measures did not correlate to the protective capacity in the acute studies of Ni to *Hyalella* (see Chapter 2). While only two sources were compared in our chronic study, it is interesting to note that DL DOM,

which provided a greater protection, had a higher $SAC_{340}$ value than PL DOM (16.35 and 10.36, respectively) (see Chapter 2, Table 2.1). These findings are consistent with other general observations for other metals such as Cu (Al-Reasi et al., 2012; Ryan et al., 2004) and Ag (VanGenderen et al., 2003). Unfortunately, the other optical characteristics (i.e., FI, HA-like and FA-like content) did not correlate with observed chronic protective effects.

Other than optical characteristics, bioaccumulation can also be correlated with protective effects. Unfortunately, no meaningful correlations for bioaccumulation (measured at 28 d) and chronic Ni toxicity were observed (Fig 3.4). Differences in toxicity endpoints (growth and survival) were not reflected by differences in Ni bioaccumulation. Exposure to Ni without modifying factors (Ca or DOM) resulted in higher toxicity but the bioaccumulation pattern was similar to other treatments (i.e., with modifying factors); an exposure-accumulation relationship was not observed (Fig 3.4). Unexpectedly, one of the most protective treatments (DL DOM) had the highest accumulation profile (Fig 3.4). Based on the BLM theory, a decrease in Ni bioaccumulation should occur due to competitive interactions between Ca and Ni and complexation of Ni by DOM (Kozlova et al., 2009; Clifford and McGeer, 2008; Keithly et al., 2004) but this did not occur. Other studies suggest that metal bioaccumulation is a better predictor of toxicity than metals in the exposure medium (either water or sediment, Borgmann et al., 1991, 1998, 2001) but deriving a threshold tissue concentration linked to an effect was not possible in this study. Once the metal accumulates in the organism, it can be transported (via body fluids) to various internal sites (e.g., reproductive organs, nervous tissue, excretory organs). This might explain the absence of variation of tissue burdens although toxicity mitigation did occur.

The lack of linkages between bioaccumulation and toxicity mitigating abilities in this

study was similar to the results of Richards et al. (2001) but the findings of our study differed from the Schroeder et al. (2010) study. Richards et al. (2001) demonstrated an effect similar to our study; DOM in the exposure medium did not alter the accumulation of metals (Cd, Ag, and Co) on the gills of rainbow trout. On the contrary, the addition of Ca both reduced chronic toxicity and resulted in less Ni accumulation in the Schroeder et al. (2010) study. The water hardness (120 mg $CaCO_3$/L) in the Schroeder et al. (2010) study may have influenced accumulation and therefore may account for the differences observed in accumulation patterns. As noted by Adams et al. (2010), the duration of exposure can also influence metal bioaccumulation patterns and alter residue-toxicity relationships therefore the differences may have occurred due to exposure duration; 28 d in this study and 7 d for Schroeder et al. (2010). It is possible that the extended duration of our study allowed organisms the time to develop physiological mechanisms that helped to eliminate accumulated Ni and/or reduce uptake (e.g., excretion). A study on the time course of chronic Ni accumulation could provide interesting insights that would contribute to the understanding of tissue burden to effect relationships.

**Table 3.1.** Water chemistry associated with Ni LC50 concentrations from 28d toxicity tests with *Hyalella azteca*. Measured LC50 values (LCL – UCL) were calculated from measured concentrations (mean ± standard deviation (n)).

| Series | Ca (mM) | Mg (mM) | pH | DOC (mg/L) | $LC_{50}$ (µg Ni/L) |
|---|---|---|---|---|---|
| Ca | 0.10 ± 0.01 (100) | 0.03 ± 0.002 (100) | 7.11 ± 0.09 (50) | 0.45 ± 0.07 (60) | 22.13 (27.74 – 34.77) |
| Daisy Lake DOM | 0.09 ± 0.01 (100) | 0.03 ± 0.002 (100) | 7.12 ± 0.09 (50) | 8.67 ± 0.48 (60) | 91.34 (84.40 – 105.45) |
| Plastic Lake DOM | 0.10 ± 0.01 (100) | 0.03 ± 0.002 (100) | 7.11 ± 0.08 (50) | 9.37 ± 1.20 (60) | 28.59 (25.54 – 32.00) |

**Table 3.2** – Compiled data (% mortality, dry weight and bioaccumulation) of four chronic test treatments. Each treatment is separated into a sub-chart (a – unmodified (Ni only, no modifying factor added), b – Ca series, c – Plastic Lake DOM series, d – Daisy Lake DOM series). Data includes percent mortality for day 10 and 28 shown as individual replicates (3 replicates per concentration). Dry weight (mean ± standard error (n)) for each replicate is shown. Bioaccumulation (mean ± standard error (n)) is shown as µg Ni/g dry weight.

a)

| Treatment | | Mortality (%) | | | | |
|---|---|---|---|---|---|---|
| Test media | Ni conc. (µg/L) | replicate | D10 | D28 | Dry weight (g) | µg Ni/g dw |
| unmodified | 0.31 ± 0.03 (26) | 1 | 10 | 10 | 0.17 ± 0.07 (9) | 13.43 ± 22.91 (26) |
| | 0.34 ± 0.02 (26) | 2 | 20 | 20 | 0.12 ± 0.06 (8) | |
| | 0.32 ± 0.05 (26) | 3 | 10 | 10 | 0.10 ± 0.05 (9) | |
| | 5.24 ± 0.81 (26) | 1 | 10 | 20 | 0.09 ± 0.07 (8) | 11.38 ± 7.51 (24) |
| | 5.36 ± 0.76 (26) | 2 | 10 | 10 | 0.07 ± 0.04 (9) | |
| | 5.31 ± 0.89 (26) | 3 | 20 | 30 | 0.06 ± 0.02 (7) | |
| | 10.19 ± 1.84 (26) | 1 | 30 | 40 | 0.08 ± 0.05 (6) | 8.82 ± 3.47 (19) |
| | 10.16 ± 1.73 (26) | 2 | 20 | 40 | 0.10 ± 0.04 (6) | |
| | 10.13 ± 1.65 (26) | 3 | 30 | 30 | 0.06 ± 0.02 (7) | |
| | 21.62 ± 1.28 (26) | 1 | 50 | 80 | 0.04 ± 0.03 (2) | 17.56 ± 8.20 (7) |
| | 21.43 ± 1.38 (26) | 2 | 60 | 90 | 0.04 (1) | |
| | 20.97 ± 1.53 (26) | 3 | 50 | 60 | 0.05 ± 0.03 (4) | |
| | 44.71 ± 2.97 (26) | 1 | 70 | 100 | n/a | 0 |
| | 43.69 ± 3.14 (26) | 2 | 20 | 100 | n/a | |
| | 43.42 ± 2.85 (26) | 3 | 70 | 100 | n/a | |
| | 82.27 ± 10.74 (26) | 1 | 100 | 100 | n/a | 0 |
| | 82.45 ± 10.69 (26) | 2 | 80 | 100 | n/a | |
| | 83.17 ± 10.37 (26) | 3 | 90 | 100 | n/a | |
| | 165.84 ± 23.65 (26) | 1 | 90 | 100 | n/a | 0 |
| | 164.92 ± 22.11 (26) | 2 | 100 | 100 | n/a | |
| | 166.55 ± 23.28 (26) | 3 | 90 | 100 | n/a | |
| | 316.18 ± 13.96 (26) | 1 | 100 | 100 | n/a | 0 |
| | 318.71 ± 15.27 (26) | 2 | 80 | 100 | n/a | |
| | 323.77 ± 16.33 (26) | 3 | 100 | 100 | n/a | |

b)

| Treatment | | Mortality (%) | | | | |
|---|---|---|---|---|---|---|
| Test media | Ni conc. (µg/L) | replicate | D10 | D28 | Dry weight (g) | µg Ni/g dw |
| unmodified | 0.32 ± 0.02 (26) | 1 | 10 | 10 | 0.19 ± 0.10 (9) | 19.52 ± 12.00 (27) |
| | 0.34 ± 0.02 (26) | 2 | 10 | 20 | 0.12 ± 0.06 (8) | |
| | 0.31 ± 0.05 (26) | 3 | 0 | 0 | 0.06 ± 0.02 (10) | |
| Ca (1mM) | 0.31 ± 0.03 (26) | 1 | 50 | 50 | 0.06 ± 0.02 (5) | 19.06 ± 15.93 (16) |
| | 0.29 ± 0.04 (26) | 2 | 30 | 50 | 0.06 ± 0.04 (5) | |
| | 0.32 ± 0.02 (26) | 3 | 20 | 40 | 0.06 ± 0.03 (6) | |
| | 5.27 ± 0.71 (26) | 1 | 30 | 40 | 0.12 ± 0.03 (6) | 13.09 ± 8.07 (22) |
| | 5.29 ± 0.86 (26) | 2 | 10 | 30 | 0.17 ± 0.05 (7) | |
| | 5.31 ± 0.69 (26) | 3 | 10 | 10 | 0.10 ± 0.03 (9) | |
| | 10.17 ± 1.92 (26) | 1 | 10 | 20 | 0.12 ± 0.05 (8) | 14.8 ± 9.85 (25) |
| | 10.14 ± 1.65 (26) | 2 | 20 | 20 | 0.15 ± 0.04 (8) | |
| | 10.21 ± 2.06 (26) | 3 | 10 | 10 | 0.15 ± 0.07 (9) | |
| | 21.83 ± 1.22 (26) | 1 | 20 | 30 | 0.13 ± 0.05 (7) | 12.17 ± 11.63 (19) |
| | 21.25 ± 1.36 (26) | 2 | 30 | 40 | 0.09 ± 0.04 (6) | |
| | 20.99 ± 1.34 (26) | 3 | 30 | 40 | 0.13 ± 0.06 (6) | |
| | 43.73 ± 3.12 (26) | 1 | 30 | 60 | 0.10 ± 0.05 (4) | 8.24 ± 13.31 (8) |
| | 44.82 ± 3.63 (26) | 2 | 50 | 70 | 0.04 ± 0.01 (3) | |
| | 42.96 ± 3.58 (26) | 3 | 70 | 90 | 0.08 (1) | |
| | 82.79 ± 10.63 (26) | 1 | 50 | 90 | 0.03 (1) | 16.99 ± 23.55 (2) |
| | 83.12 ± 11.20 (26) | 2 | 50 | 100 | n/a | |
| | 83.09 ± 10.96 (26) | 3 | 60 | 90 | 0.09 (1) | |
| | 163.48 ± 22.88 (26) | 1 | 100 | 100 | n/a | 0 |
| | 165.21 ± 23.65 (26) | 2 | 90 | 100 | n/a | |
| | 162.94 ± 23.82 (26) | 3 | 100 | 100 | n/a | |
| | 317.46 ± 13.77 (26) | 1 | 100 | 100 | n/a | 0 |
| | 319.10 ± 14.46 (26) | 2 | 100 | 100 | n/a | |
| | 320.55 ± 15.83 (26) | 3 | 100 | 100 | n/a | |

c)

| Treatment | | Mortality (%) | | | | |
|---|---|---|---|---|---|---|
| Test media | Ni conc. (µg/L) | Replicate | D10 | D28 | Dry weight (g) | µg Ni/g dw |
| unmodified | 0.31 ± 0.03 (26) | 1 | 20 | 20 | 0.14 ± 0.10 (8) | 10.62 ± 2.99 (27) |
| | 0.33 ± 0.01 (26) | 2 | 0 | 0 | 0.25 ± 0.16 (10) | |
| | 0.31 ± 0.04 (26) | 3 | 10 | 10 | 0.18 ± 0.06 (9) | |
| DOM (PL) | 0.32 ± 0.03 (26) | 1 | 10 | 10 | 0.24 ± 0.11 (9) | 13.98 ± 4.33 (26) |
| | 0.33 ± 0.02 (26) | 2 | 20 | 20 | 0.19 ± 0.10 (8) | |
| | 0.31 ± 0.02 (26) | 3 | 10 | 10 | 0.12 ± 0.09 (9) | |
| | 11.02 ± 1.77 (26) | 1 | 30 | 50 | 0.09 ± 0.08 (5) | 31.01 ± 10.50 (20) |
| | 10.24 ± 1.89 (26) | 2 | 40 | 40 | 0.11 ± 0.09 (6) | |
| | 10.59 ± 2.23 (26) | 3 | 10 | 10 | 0.23 ± 0.10 (9) | |
| | 20.76 ± 1.49 (26) | 1 | 20 | 30 | 0.10 ± 0.09 (7) | 23.06 ± 7.50 (25) |
| | 21.12 ± 1.98 (26) | 2 | 10 | 10 | 0.13 ± 0.10 (9) | |
| | 20.85 ± 2.35 (26) | 3 | 10 | 10 | 0.15 ± 0.06 (9) | |
| | 42.98 ± 2.9 (26) | 1 | 30 | 100 | n/a | 29.94 ± 11.10 (5) |
| | 43.21 ± 3.14 (26) | 2 | 30 | 80 | 0.05 ± 0.01 (2) | |
| | 43.17 ± 2.57 (26) | 3 | 50 | 70 | 0.25 ± 0.16 (3) | |
| | 83.22 ± 11.34 (26) | 1 | 50 | 100 | n/a | 0 |
| | 82.61 ± 10.82 (26) | 2 | 70 | 100 | n/a | |
| | 83.29 ± 11.25 (26) | 3 | 90 | 100 | n/a | |
| | 163.42 ± 22.35 (26) | 1 | 100 | 100 | n/a | 0 |
| | 162.60 ± 21.79 (26) | 2 | 100 | 100 | n/a | |
| | 162.65 ± 22.46 (26) | 3 | 100 | 100 | n/a | |

d)

| Treatment | | Mortality (%) | | | | |
|---|---|---|---|---|---|---|
| Test media | Ni conc. (µg/L) | Replicate | D10 | D28 | Dry weight (g) | µg Ni/g dw |
| unmodified | 0.40 ± 0.02 (26) | 1 | 10 | 10 | 0.23 ± 0.07 (9) | 2.93 ± 1.47 (25) |
| | 0.32 ± 0.03 (26) | 2 | 10 | 20 | 0.35 ± 0.14 (8) | |
| | 0.36 ± 0.02 (26) | 3 | 20 | 20 | 0.24 ± 0.07 (8) | |
| DOM (DL) | 0.35 ± 0.04 (26) | 1 | 10 | 10 | 0.46 ± 0.19 (9) | 9.84 ± 2.74 (27) |
| | 0.31 ± 0.03 (26) | 2 | 0 | 0 | 0.30 ± 0.12 (10) | |
| | 0.33 ± 0.03 (26) | 3 | 10 | 20 | 0.40 ± 0.12 (8) | |
| | 10.38 ± 2.04 (26) | 1 | 10 | 20 | 0.33 ± 0.12 (8) | 12.02 ± 3.89 (25) |
| | 11.02 ± 1.75 (26) | 2 | 10 | 10 | 0.37 ± 0.15 (9) | |
| | 10.24 ± 1.32 (26) | 3 | 10 | 20 | 0.33 ± 0.10 (8) | |
| | 21.52 ± 1.22 (26) | 1 | 0 | 0 | 0.29 ± 0.10 (10) | 15.72 ± 4.34 (26) |
| | 21.14 ± 1.16 (26) | 2 | 10 | 30 | 0.39 ± 0.11 (7) | |
| | 20.94 ± 1.73 (26) | 3 | 0 | 10 | 0.40 ± 0.12 (9) | |
| | 42.67 ± 2.85 (26) | 1 | 0 | 10 | 0.26 ± 0.12 (9) | 15.37 ± 5.97 (27) |
| | 43.15 ± 3.02 (26) | 2 | 10 | 10 | 0.15 ± 0.04 (9) | |
| | 43.20 ± 2.92 (26) | 3 | 10 | 10 | 0.14 ± 0.10 (9) | |
| | 82.49 ± 10.69 (26) | 1 | 10 | 40 | 0.13 ± 0.06 (6) | 30.82 ± 5.65 (19) |
| | 83.41 ± 11.47 (26) | 2 | 20 | 40 | 0.10 ± 0.07 (6) | |
| | 83.14 ± 10.73 (26) | 3 | 10 | 30 | 0.13 ± 0.04 (7) | |
| | 162.55 ± 24.56 (26) | 1 | 70 | 100 | n/a | 0 |
| | 161.99 ± 22.32 (26) | 2 | 50 | 100 | n/a | |
| | 164.03 ± 22.94 (26) | 3 | 50 | 100 | n/a | |

**Table 3.3.** Measured initial and final total and dissolved Ni as well as DOC concentrations (mean ± SEM) where n = 4. Tables are divided into 4 sub-tables (a – Ni only, b – Ca, c – PL, d – DL). NC represents nominal concentrations of Ni. Paired t-tests were performed and no significant differences were observed between initial and final concentrations of total, dissolved and DOC concentrations.

a)

| Series | NC | wk # | total Ni | | dissolved Ni | | DOC | |
|---|---|---|---|---|---|---|---|---|
| | | | Initial | final | initial | final | initial | final |
| Ni | 0 | 1 | 0.34 ± 0.09 | 0.39 ± 0.10 | 0.33 ± 0.07 | 0.36 ± 0.08 | 0.44 ± 0.15 | 0.41 ± 0.08 |
| | | 2 | 0.27 ± 0.04 | 0.29 ± 0.08 | 0.25 ± 0.03 | 0.24 ± 0.04 | 0.60 ± 0.11 | 0.54 ± 0.13 |
| | | 3 | 0.29 ± 0.09 | 0.34 ± 0.03 | 0.27 ± 0.09 | 0.29 ± 0.07 | 0.50 ± 0.10 | 0.52 ± 0.09 |
| | | 4 | 0.54 ± 0.12 | 0.25 ± 0.12 | 0.51 ± 0.13 | 0.46 ± 0.10 | 0.32 ± 0.09 | 0.37 ± 0.11 |
| | 5 | 1 | 5.32 ± 0.40 | 5.44 ± 0.80 | 5.30 ± 0.35 | 5.30 ± 0.78 | 0.37 ± 0.09 | 0.39 ± 0.10 |
| | | 2 | 4.89 ± 0.26 | 4.24 ± 0.11 | 4.86 ± 0.27 | 4.68 ± 0.23 | 0.61 ± 0.12 | 0.35 ± 0.13 |
| | | 3 | 5.10 ± 0.26 | 5.40 ± 0.07 | 5.07 ± 0.22 | 5.35 ± 0.09 | 0.40 ± 0.08 | 0.54 ± 0.04 |
| | | 4 | 5.46 ± 0.47 | 5.51 ± 0.62 | 6.36 ± 0.40 | 6.24 ± 0.61 | 0.68 ± 0.14 | 0.58 ± 0.09 |
| | 10 | 1 | 11.25 ± 0.48 | 11.13 ± 0.51 | 11.21 ± 0.43 | 11.11 ± 0.78 | 0.68 ± 0.14 | 0.51 ± 0.07 |
| | | 2 | 9.26 ± 0.12 | 9.54 ± 0.31 | 9.24 ± 0.10 | 9.32 ± 0.53 | 0.44 ± 0.11 | 0.44 ± 0.09 |
| | | 3 | 11.00 ± 0.95 | 10.94 ± 0.59 | 11.01 ± 0.91 | 10.45 ± 0.24 | 0.92 ± 0.07 | 0.78 ± 0.10 |
| | | 4 | 11.60 ± 1.44 | 11.35 ± 2.14 | 11.53 ± 1.33 | 11.51 ± 2.02 | 0.26 ± 0.12 | 0.32 ± 0.08 |
| | 20 | 1 | 22.08 ± 1.11 | 23.68 ± 0.21 | 22.01 ± 1.14 | 22.86 ± 0.53 | 0.48 ± 0.15 | 0.39 ± 0.13 |
| | | 2 | 23.31 ± 0.22 | 21.56 ± 0.40 | 23.34 ± 0.19 | 22.22 ± 0.23 | 0.65 ± 0.04 | 0.57 ± 0.12 |
| | | 3 | 21.02 ± 0.09 | 20.95 ± 1.56 | 24.05 ± 0.03 | 23.45 ± 1.31 | 0.49 ± 0.08 | 0.45 ± 0.06 |
| | | 4 | 21.31 ± 1.54 | 22.99 ± 0.93 | 21.23 ± 1.24 | 21.55 ± 0.76 | 0.35 ± 0.13 | 0.48 ± 0.11 |
| | 40 | 1 | 38.42 ± 0.68 | 38.88 ± 0.77 | 39.12 ± 0.65 | 37.76 ± 0.52 | 0.20 ± 0.10 | 0.21 ± 0.08 |
| | | 2 | 42.75 ± 0.64 | 40.50 ± 0.11 | 42.63 ± 0.64 | 40.43 ± 0.14 | 0.54 ± 0.09 | 0.52 ± 0.12 |
| | | 3 | 40.36 ± 1.32 | 41.10 ± 2.14 | 40.31 ± 1.23 | 40.06 ± 1.84 | 0.62 ± 0.06 | 0.61 ± 0.09 |
| | | 4 | 38.79 ± 0.70 | 39.60 ± 1.02 | 38.99 ± 0.73 | 38.70 ± 0.79 | 0.55 ± 0.08 | 0.47 ± 0.09 |
| | 80 | 1 | 77.14 ± 1.47 | 78.13 ± 0.91 | 78.53 ± 1.32 | 78.63 ± 0.99 | 0.44 ± 0.12 | 0.33 ± 0.10 |
| | | 2 | 80.74 ± 2.09 | 80.55 ± 0.25 | 80.36 ± 2.01 | 80.32 ± 0.27 | 0.60 ± 0.05 | 0.45 ± 0.13 |
| | | 3 | 82.08 ± 1.11 | 80.71 ± 0.33 | 81.62 ± 1.06 | 79.94 ± 0.88 | 0.90 ± 0.17 | 0.82 ± 0.11 |
| | | 4 | 81.65 ± 2.44 | 82.75 ± 1.48 | 81.18 ± 2.22 | 81.66 ± 1.35 | 0.79 ± 0.10 | 0.68 ± 0.09 |
| | 160 | 1 | 165.67 ± 2.11 | 162.55 ± 0.70 | 162.76 ± 1.97 | 161.34 ± 1.46 | 0.64 ± 0.09 | 0.51 ± 0.08 |
| | | 2 | 162.75 ± 2.55 | 162.14 ± 0.39 | 163.24 ± 2.32 | 161.12 ± 1.28 | 0.54 ± 0.10 | 0.54 ± 0.13 |
| | | 3 | 159.45 ± 0.99 | 157.61 ± 3.46 | 159.75 ± 0.72 | 159.23 ± 0.85 | 0.51 ± 0.06 | 0.52 ± 0.08 |
| | | 4 | 159.34 ± 3.11 | 157.59 ± 2.54 | 160.53 ± 2.15 | 158.67 ± 2.00 | 0.31 ± 0.07 | 0.27 ± 0.03 |
| | 320 | 1 | 318.27 ± 6.87 | 325.78 ± 3.34 | 319.57 ± 10.82 | 320.73 ± 4.62 | 0.57 ± 0.11 | 0.48 ± 0.10 |
| | | 2 | 322.69 ± 1.53 | 322.67 ± 1.39 | 320.98 ± 1.41 | 321.58 ± 1.03 | 0.49 ± 0.04 | 0.45 ± 0.08 |
| | | 3 | 321.44 ± 2.75 | 321.91 ± 1.51 | 321.28 ± 2.35 | 322.63 ± 1.22 | 0.78 ± 0.23 | 0.64 ± 0.07 |
| | | 4 | 321.62 ± 2.70 | 322.39 ± 1.24 | 318.82 ± 2.43 | 320.15 ± 1.01 | 0.21 ± 0.02 | 0.28 ± 0.06 |

b)

| Series | NC | wk # | total Ni | | dissolved Ni | | DOC | |
|---|---|---|---|---|---|---|---|---|
| | | | initial | final | initial | final | initial | final |
| Ca | 0 | 1 | 0.61 ± 0.15 | 0.57 ± 0.10 | 0.51 ± 0.23 | 0.46 ± 0.08 | 0.54 ± 0.04 | 0.49 ± 0.08 |
| | | 2 | 0.51 ± 0.09 | 0.44 ± 0.25 | 0.47 ± 0.08 | 0.46 ± 0.12 | 0.58 ± 0.09 | 0.52 ± 0.04 |
| | | 3 | 0.48 ± 0.10 | 0.33 ± 0.16 | 0.49 ± 0.13 | 0.42 ± 0.11 | 0.61 ± 0.11 | 0.60 ± 0.08 |
| | | 4 | 0.79 ± 0.05 | 0.62 ± 0.12 | 0.59 ± 0.06 | 0.58 ± 0.13 | 0.58 ± 0.06 | 0.43 ± 0.07 |
| | 0 | 1 | 0.21 ± 0.09 | 0.47 ± 0.16 | 0.20 ± 0.07 | 0.33 ± 0.07 | 0.41 ± 0.09 | 0.38 ± 0.10 |
| | | 2 | 0.57 ± 0.19 | 0.40 ± 0.01 | 0.37 ± 0.16 | 0.34 ± 0.04 | 0.47 ± 0.13 | 0.43 ± 0.11 |
| | | 3 | 0.35 ± 0.05 | 0.42 ± 0.19 | 0.32 ± 0.07 | 0.45 ± 0.01 | 0.37 ± 0.07 | 0.39 ± 0.09 |
| | | 4 | 0.40 ± 0.22 | 0.30 ± 0.12 | 0.43 ± 0.17 | 0.34 ± 0.13 | 0.41 ± 0.02 | 0.46 ± 0.12 |
| | 5 | 1 | 5.77 ± 0.55 | 6.56 ± 0.31 | 5.72 ± 0.47 | 6.20 ± 0.43 | 0.44 ± 0.13 | 0.32 ± 0.05 |
| | | 2 | 5.29 ± 0.24 | 5.33 ± 0.06 | 5.14 ± 0.22 | 5.22 ± 0.17 | 0.43 ± 0.07 | 0.29 ± 0.03 |
| | | 3 | 5.83 ± 0.46 | 5.15 ± 0.86 | 5.69 ± 0.37 | 5.77 ± 0.93 | 0.48 ± 0.13 | 0.51 ± 0.12 |
| | | 4 | 5.62 ± 0.06 | 5.29 ± 0.30 | 5.49 ± 0.09 | 5.34 ± 0.29 | 0.56 ± 0.07 | 0.52 ± 0.04 |
| | 10 | 1 | 11.31 ± 0.69 | 11.48 ± 0.75 | 11.25 ± 0.73 | 11.21 ± 0.82 | 0.34 ± 0.06 | 0.38 ± 0.07 |
| | | 2 | 11.65 ± 0.69 | 10.28 ± 0.63 | 11.72 ± 0.84 | 10.88 ± 0.47 | 0.33 ± 0.12 | 0.39 ± 0.10 |
| | | 3 | 11.10 ± 0.87 | 10.17 ± 0.35 | 11.01 ± 0.47 | 10.63 ± 1.02 | 0.32 ± 0.10 | 0.28 ± 0.08 |
| | | 4 | 10.32 ± 0.65 | 11.03 ± 0.24 | 10.07 ± 0.48 | 10.86 ± 0.51 | 0.32 ± 0.15 | 0.35 ± 0.13 |
| | 20 | 1 | 22.53 ± 0.90 | 22.07 ± 1.61 | 22.51 ± 0.62 | 22.24 ± 1.53 | 0.65 ± 0.11 | 0.58 ± 0.09 |
| | | 2 | 22.72 ± 0.49 | 22.27 ± 0.07 | 22.39 ± 0.24 | 22.11 ± 0.09 | 0.81 ± 0.08 | 0.73 ± 0.14 |
| | | 3 | 23.13 ± 0.22 | 23.07 ± 0.28 | 21.72 ± 0.13 | 22.43 ± 0.62 | 0.43 ± 0.17 | 0.36 ± 0.11 |
| | | 4 | 21.57 ± 0.22 | 22.08 ± 1.40 | 21.23 ± 0.18 | 21.08 ± 1.46 | 0.81 ± 0.09 | 0.69 ± 0.12 |
| | 40 | 1 | 40.16 ± 1.47 | 39.63 ± 1.83 | 40.31 ± 1.24 | 39.53 ± 1.33 | 0.41 ± 0.08 | 0.34 ± 0.09 |
| | | 2 | 42.33 ± 1.25 | 39.68 ± 2.09 | 41.27 ± 1.06 | 40.48 ± 1.88 | 0.53 ± 0.14 | 0.49 ± 0.14 |
| | | 3 | 37.78 ± 0.97 | 36.49 ± 0.57 | 40.24 ± 1.01 | 37.41 ± 0.82 | 0.36 ± 0.11 | 0.39 ± 0.06 |
| | | 4 | 38.90 ± 2.08 | 39.17 ± 1.14 | 39.74 ± 2.02 | 39.99 ± 1.03 | 0.38 ± 0.02 | 0.49 ± 0.03 |
| | 80 | 1 | 80.92 ± 1.91 | 79.77 ± 1.48 | 80.58 ± 1.35 | 80.44 ± 1.12 | 0.24 ± 0.05 | 0.23 ± 0.02 |
| | | 2 | 78.96 ± 1.95 | 81.01 ± 1.20 | 79.62 ± 1.07 | 80.76 ± 1.09 | 0.43 ± 0.08 | 0.36 ± 0.07 |
| | | 3 | 76.86 ± 0.31 | 80.50 ± 0.11 | 78.63 ± 0.68 | 81.01 ± 0.34 | 0.39 ± 0.04 | 0.44 ± 0.07 |
| | | 4 | 77.92 ± 0.68 | 78.36 ± 0.46 | 76.62 ± 0.59 | 77.67 ± 0.91 | 0.59 ± 0.13 | 0.63 ± 0.08 |
| | 160 | 1 | 162.94 ± 1.57 | 161.21 ± 0.86 | 161.46 ± 1.28 | 161.12 ± 0.71 | 0.20 ± 0.07 | 0.21 ± 0.04 |
| | | 2 | 159.61 ± 1.57 | 155.50 ± 1.25 | 160.81 ± 1.04 | 157.03 ± 1.17 | 0.27 ± 0.11 | 0.33 ± 0.06 |
| | | 3 | 157.99 ± 2.36 | 159.82 ± 3.45 | 159.97 ± 1.98 | 156.49 ± 2.53 | 0.31 ± 0.09 | 0.29 ± 0.06 |
| | | 4 | 159.25 ± 0.21 | 161.23 ± 1.34 | 159.72 ± 0.51 | 158.51 ± 1.28 | 0.25 ± 0.04 | 0.21 ± 0.09 |
| | 320 | 1 | 320.72 ± 2.21 | 319.52 ± 3.21 | 320.71 ± 2.17 | 320.84 ± 2.63 | 0.72 ± 0.02 | 0.86 ± 0.05 |
| | | 2 | 323.95 ± 0.22 | 321.25 ± 2.83 | 321.47 ± 0.41 | 321.23 ± 3.17 | 0.35 ± 0.05 | 0.41 ± 0.03 |
| | | 3 | 319.91 ± 0.21 | 317.10 ± 0.55 | 319.27 ± 0.44 | 318.24 ± 2.38 | 0.42 ± 0.10 | 0.46 ± 0.05 |
| | | 4 | 320.42 ± 0.40 | 323.14 ± 0.86 | 320.19 ± 0.32 | 321.46 ± 1.52 | 0.41 ± 0.07 | 0.36 ± 0.05 |

c)

| Series | NC | wk # | total Ni | | dissolved Ni | | DOC | |
|---|---|---|---|---|---|---|---|---|
| | | | initial | final | initial | final | initial | final |
| PL | 0 | 1 | 0.27 ± 0.02 | 0.50 ± 0.10 | 0.28 ± 0.04 | 0.31 ± 0.09 | 0.29 ± 0.04 | 0.37 ± 0.10 |
| | | 2 | 0.26 ± 0.07 | 0.49 ± 0.11 | 0.21 ± 0.03 | 0.36 ± 0.05 | 0.39 ± 0.06 | 0.44 ± 0.15 |
| | | 3 | 0.54 ± 0.07 | 0.38 ± 0.19 | 0.32 ± 0.06 | 0.41 ± 0.13 | 0.32 ± 0.07 | 0.33 ± 0.04 |
| | | 4 | 0.23 ± 0.03 | 0.50 ± 0.04 | 0.23 ± 0.02 | 0.46 ± 0.05 | 0.33 ± 0.10 | 0.22 ± 0.02 |
| | 0 | 1 | 0.21 ± 0.04 | 0.24 ± 0.04 | 0.25 ± 0.06 | 0.24 ± 0.02 | 0.20 ± 0.03 | 0.17 ± 0.01 |
| | | 2 | 0.53 ± 0.16 | 0.60 ± 0.09 | 0.41 ± 0.13 | 0.50 ± 0.07 | 0.31 ± 0.06 | 0.30 ± 0.04 |
| | | 3 | 0.29 ± 0.06 | 0.11 ± 0.01 | 0.19 ± 0.07 | 0.16 ± 0.04 | 0.32 ± 0.08 | 0.42 ± 0.05 |
| | | 4 | 0.25 ± 0.03 | 0.32 ± 0.04 | 0.26 ± 0.01 | 0.22 ± 0.06 | 0.53 ± 0.12 | 0.30 ± 0.02 |
| | 10 | 1 | 12.34 ± 0.44 | 12.65 ± 0.61 | 11.31 ± 0.26 | 11.58 ± 1.03 | 9.64 ± 0.31 | 9.34 ± 0.42 |
| | | 2 | 10.66 ± 0.10 | 9.98 ± 0.20 | 10.29 ± 0.09 | 9.86 ± 0.78 | 9.10 ± 0.31 | 9.63 ± 0.10 |
| | | 3 | 11.05 ± 0.17 | 11.37 ± 0.34 | 10.05 ± 0.12 | 10.34 ± 0.37 | 9.68 ± 0.76 | 9.05 ± 0.15 |
| | | 4 | 10.59 ± 0.40 | 10.75 ± 0.34 | 10.36 ± 0.28 | 10.57 ± 0.29 | 10.04 ± 0.31 | 9.59 ± 0.47 |
| | 20 | 1 | 19.68 ± 1.34 | 16.46 ± 2.17 | 19.91 ± 1.75 | 17.69 ± 2.04 | 9.73 ± 0.52 | 9.65 ± 0.61 |
| | | 2 | 21.35 ± 0.90 | 20.58 ± 0.80 | 21.04 ± 0.97 | 20.06 ± 0.91 | 9.82 ± 0.09 | 8.98 ± 0.25 |
| | | 3 | 20.47 ± 0.07 | 21.22 ± 0.92 | 20.42 ± 0.11 | 20.23 ± 1.12 | 9.23 ± 0.10 | 9.37 ± 0.34 |
| | | 4 | 23.15 ± 0.40 | 21.75 ± 1.20 | 22.11 ± 0.32 | 21.61 ± 1.67 | 9.10 ± 1.60 | 9.75 ± 0.32 |
| | 40 | 1 | 39.25 ± 1.80 | 38.10 ± 3.54 | 39.15 ± 1.56 | 37.09 ± 3.11 | 8.91 ± 1.30 | 9.31 ± 0.26 |
| | | 2 | 41.00 ± 2.74 | 40.12 ± 1.75 | 40.07 ± 2.10 | 40.43 ± 1.23 | 9.60 ± 1.45 | 9.29 ± 0.79 |
| | | 3 | 42.66 ± 1.32 | 42.06 ± 0.50 | 41.45 ± 1.12 | 40.22 ± 0.92 | 9.41 ± 0.68 | 10.05 ± 0.12 |
| | | 4 | 38.80 ± 0.64 | 38.57 ± 0.74 | 38.70 ± 0.60 | 38.88 ± 0.71 | 9.32 ± 0.75 | 9.36 ± 0.24 |
| | 80 | 1 | 80.53 ± 1.13 | 79.33 ± 2.97 | 80.33 ± 1.04 | 80.47 ± 2.55 | 9.12 ± 0.75 | 9.58 ± 0.83 |
| | | 2 | 81.93 ± 0.51 | 81.44 ± 1.41 | 81.48 ± 0.35 | 81.04 ± 1.75 | 10.42 ± 0.57 | 9.86 ± 0.77 |
| | | 3 | 82.30 ± 0.57 | 81.60 ± 1.96 | 82.17 ± 0.66 | 81.34 ± 1.17 | 9.80 ± 0.64 | 9.34 ± 0.37 |
| | | 4 | 81.43 ± 1.82 | 82.92 ± 0.65 | 81.25 ± 1.44 | 81.27 ± 1.06 | 9.51 ± 1.34 | 9.57 ± 0.29 |
| | 160 | 1 | 166.57 ± 0.49 | 167.06 ± 0.51 | 162.89 ± 0.88 | 164.15 ± 0.83 | 9.44 ± 0.77 | 9.31 ± 0.69 |
| | | 2 | 163.99 ± 1.92 | 163.34 ± 1.48 | 163.34 ± 2.71 | 162.41 ± 1.97 | 10.04 ± 0.46 | 9.65 ± 0.91 |
| | | 3 | 162.37 ± 0.78 | 160.72 ± 1.71 | 162.46 ± 1.07 | 161.27 ± 1.22 | 9.50 ± 0.57 | 9.10 ± 0.87 |
| | | 4 | 164.70 ± 1.67 | 163.37 ± 1.09 | 161.65 ± 1.37 | 162.10 ± 1.14 | 9.32 ± 0.99 | 9.32 ± 0.65 |

d)

| Series | NC | wk # | total Ni | | dissolved Ni | | DOC | |
|---|---|---|---|---|---|---|---|---|
| | | | initial | final | initial | final | initial | final |
| DL | 0 | 1 | 0.33 ± 0.05 | 0.30 ± 0.13 | 0.23 ± 0.07 | 0.28 ± 0.08 | 0.37 ± 0.09 | 0.53 ± 0.09 |
| | | 2 | 0.26 ± 0.08 | 0.37 ± 0.09 | 0.21 ± 0.03 | 0.22 ± 0.05 | 0.51 ± 0.10 | 0.72 ± 0.14 |
| | | 3 | 0.62 ± 0.14 | 0.50 ± 0.14 | 0.46 ± 0.09 | 0.40 ± 0.11 | 0.45 ± 0.08 | 0.13 ± 0.02 |
| | | 4 | 0.30 ± 0.05 | 0.36 ± 0.07 | 0.26 ± 0.07 | 0.30 ± 0.04 | 0.55 ± 0.12 | 0.57 ± 0.06 |
| | 0 | 1 | 0.21 ± 0.02 | 0.24 ± 0.11 | 0.18 ± 0.04 | 0.21 ± 0.10 | 0.48 ± 0.05 | 0.36 ± 0.14 |
| | | 2 | 0.32 ± 0.14 | 0.29 ± 0.11 | 0.23 ± 0.10 | 0.27 ± 0.09 | 0.67 ± 0.11 | 0.33 ± 0.05 |
| | | 3 | 0.31 ± 0.09 | 0.42 ± 0.16 | 0.21 ± 0.02 | 0.32 ± 0.12 | 0.53 ± 0.10 | 0.78 ± 0.07 |
| | | 4 | 0.25 ± 0.03 | 0.25 ± 0.07 | 0.21 ± 0.01 | 0.23 ± 0.05 | 0.30 ± 0.07 | 0.20 ± 0.08 |
| | 10 | 1 | 11.94 ± 0.39 | 12.48 ± 0.52 | 11.43 ± 0.52 | 11.48 ± 1.04 | 9.13 ± 1.42 | 8.92 ± 0.91 |
| | | 2 | 11.52 ± 0.23 | 11.58 ± 0.66 | 11.23 ± 0.38 | 10.45 ± 0.79 | 9.21 ± 0.74 | 8.96 ± 0.95 |
| | | 3 | 12.93 ± 1.37 | 12.09 ± 0.84 | 12.23 ± 0.91 | 11.57 ± 0.92 | 9.52 ± 0.99 | 8.86 ± 0.31 |
| | | 4 | 10.06 ± 0.69 | 10.14 ± 0.64 | 10.03 ± 0.33 | 9.98 ± 0.93 | 9.06 ± 0.94 | 8.92 ± 0.68 |
| | 20 | 1 | 23.66 ± 0.68 | 22.07 ± 1.61 | 21.93 ± 0.88 | 21.77 ± 1.25 | 10.15 ± 1.12 | 9.94 ± 1.07 |
| | | 2 | 21.79 ± 0.07 | 20.21 ± 0.13 | 21.77 ± 0.03 | 20.74 ± 0.79 | 9.80 ± 0.69 | 9.61 ± 0.57 |
| | | 3 | 22.85 ± 1.29 | 19.25 ± 1.33 | 22.44 ± 1.11 | 21.53 ± 1.11 | 9.31 ± 1.31 | 8.99 ± 1.36 |
| | | 4 | 18.11 ± 0.49 | 19.65 ± 1.48 | 21.16 ± 0.62 | 20.54 ± 1.22 | 9.67 ± 1.07 | 9.25 ± 0.21 |
| | 40 | 1 | 38.46 ± 1.62 | 36.30 ± 1.57 | 38.81 ± 1.48 | 39.02 ± 1.17 | 10.01 ± 0.44 | 9.72 ± 1.21 |
| | | 2 | 42.61 ± 1.94 | 40.87 ± 0.80 | 42.11 ± 1.49 | 40.73 ± 0.99 | 9.33 ± 0.86 | 8.95 ± 0.22 |
| | | 3 | 40.70 ± 0.39 | 41.94 ± 0.45 | 40.09 ± 0.25 | 41.42 ± 0.74 | 9.62 ± 1.34 | 8.91 ± 1.21 |
| | | 4 | 39.98 ± 2.97 | 41.45 ± 1.30 | 39.33 ± 2.02 | 40.31 ± 1.83 | 9.22 ± 0.88 | 9.42 ± 0.40 |
| | 80 | 1 | 80.84 ± 1.62 | 78.10 ± 0.66 | 80.46 ± 1.23 | 79.33 ± 0.79 | 9.32 ± 1.01 | 9.07 ± 0.61 |
| | | 2 | 80.13 ± 0.84 | 76.89 ± 0.19 | 80.76 ± 0.49 | 78.52 ± 0.56 | 9.24 ± 0.89 | 9.21 ± 0.83 |
| | | 3 | 79.98 ± 0.20 | 77.79 ± 2.28 | 81.55 ± 0.76 | 79.94 ± 2.07 | 10.07 ± 0.94 | 9.25 ± 1.02 |
| | | 4 | 79.57 ± 1.75 | 80.81 ± 1.78 | 79.99 ± 1.05 | 79.88 ± 1.42 | 9.44 ± 0.98 | 9.65 ± 1.48 |
| | 160 | 1 | 164.77 ± 0.87 | 161.21 ± 2.07 | 163.32 ± 1.16 | 162.18 ± 2.21 | 9.23 ± 1.22 | 9.30 ± 0.57 |
| | | 2 | 160.75 ± 2.98 | 158.15 ± 4.54 | 160.89 ± 1.68 | 159.19 ± 3.14 | 9.14 ± 0.97 | 8.87 ± 0.80 |
| | | 3 | 160.58 ± 0.80 | 161.01 ± 2.96 | 160.32 ± 0.79 | 161.37 ± 2.65 | 10.02 ± 0.48 | 9.94 ± 0.45 |
| | | 4 | 159.34 ± 1.37 | 158.99 ± 1.40 | 159.84 ± 1.21 | 160.02 ± 1.14 | 9.17 ± 0.56 | 9.45 ± 1.30 |

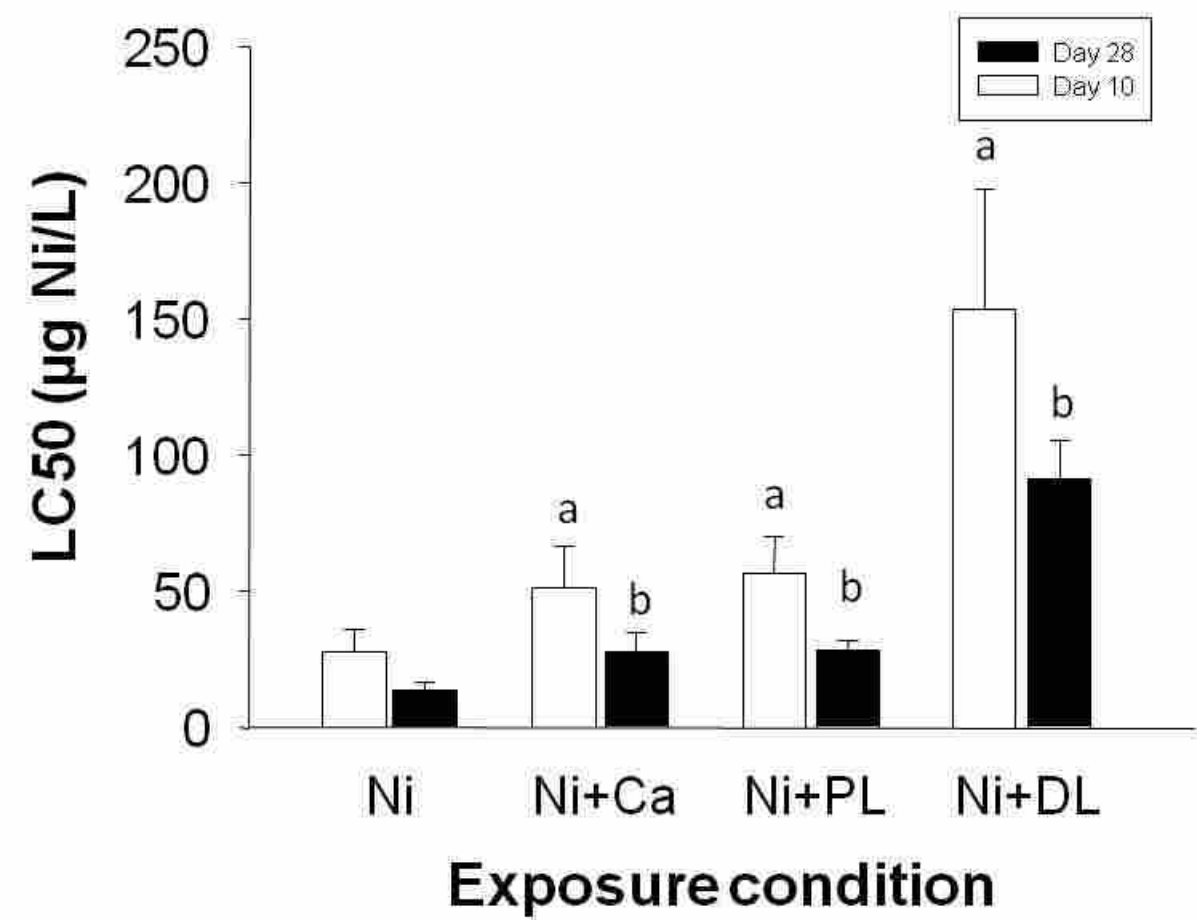


**Figure 3.1.** The protective effects of chronic Ni toxicity when Ca (1 mM), Plastic Lake (PL) DOM and Daisy Lake (DL) DOM are added. DOM was added to a measured concentration of 9 mg DOC/L. $LC_{50}$ values of 4 series are calculated at day 10 (open) and day 28 (black). Error bars represents 95% CI for $LC_{50}$ values. An 'a' and 'b' denotes significant difference from Ni exposure (no added modifying factor) at day 10 and day 28, respectively.

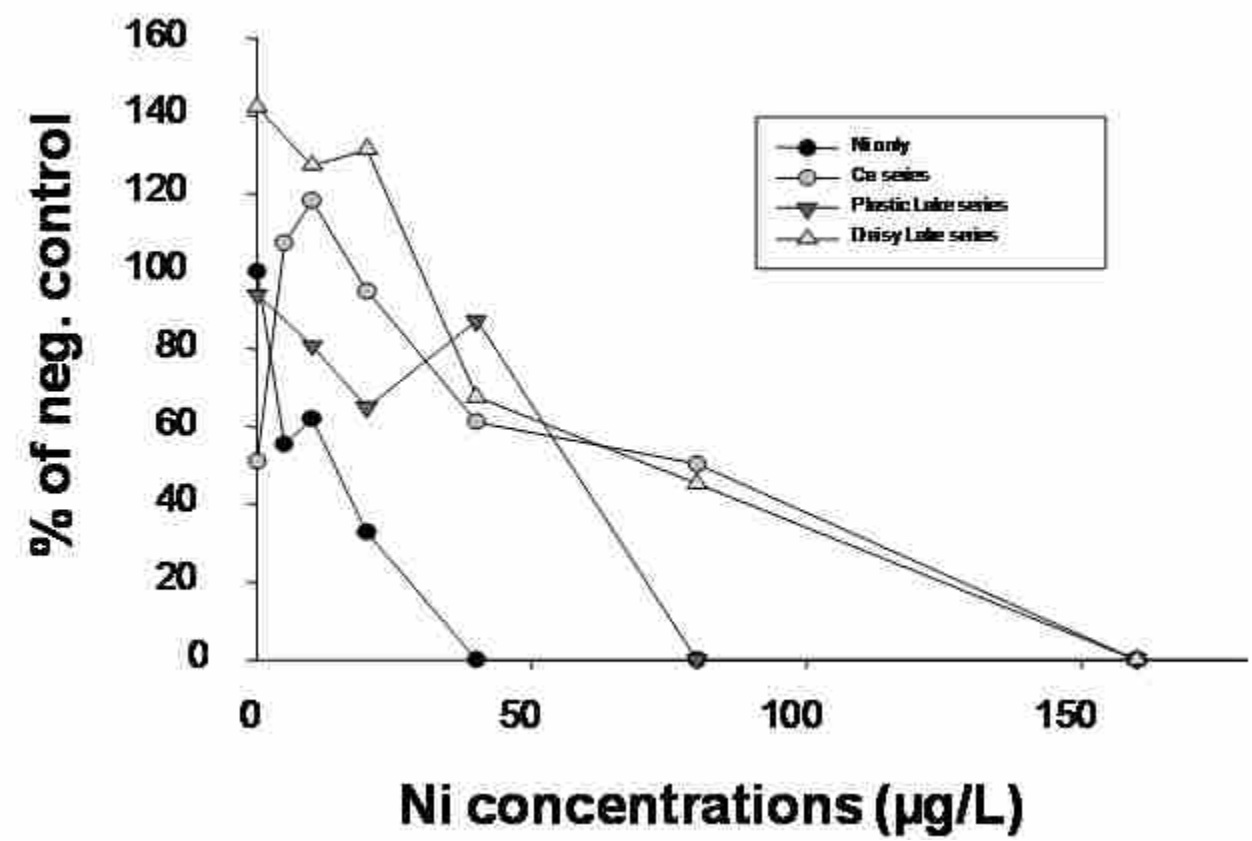


**Figure 3.2** – Changes in dry weight, expressed as % dry weight of controls (varying n values since n is based on surviving organisms), at day 28 at Ni concentrations of 0 to 160 µg/L (320 µg/L not shown since no organisms survived, n = 0). A Ni concentration of 0 is relative the media with additional Ca or DOM. Percentages were calculated from the control in the series without any additional modifying factors; values at 0 µg Ni/L represent % reduction in dry weight with just added modifying factor (Table 3.1). Black circles represent the Ni only exposure while grey circles represent the Ni with additional Ca exposure where Ca concentration is 1mM. Plastic Lake is represented by dark inverted triangles and Daisy Lake is represented by light triangles.

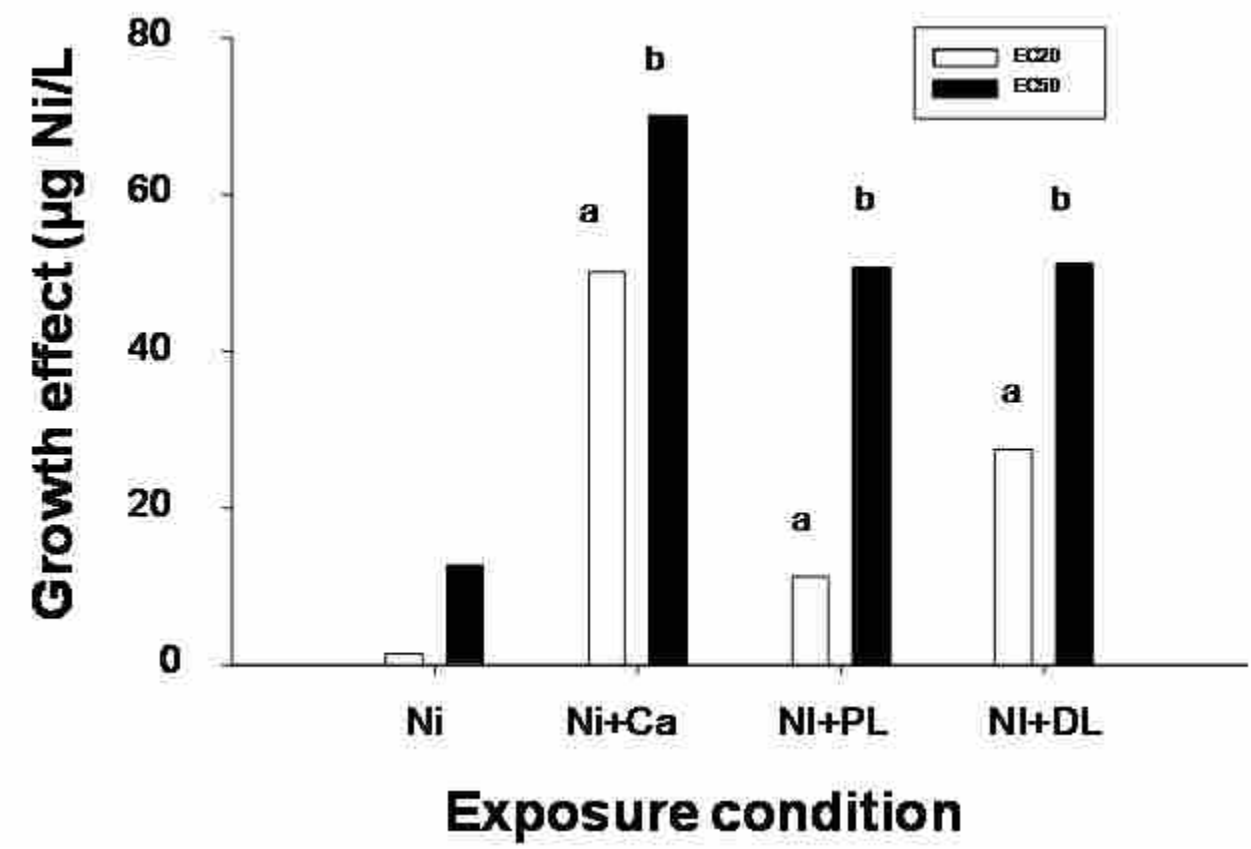


**Figure 3.3**. The protective growth effect of additional Ca (1 mM) and 2 DOM sources: Plastic lake (PL) and Daisy Lake (DL) on Ni toxicity in *Hyalella azteca*. DOM was added to a measured concentration of 9 mg DOC/L. Both $EC_{20}$ and $EC_{50}$ values were calculated on the basis of survivals and dry weight of organisms. The open bars represent the $EC_{20}$ value while the black bars represent the $EC_{50}$ value. No error bars are present due to negligible 95% CI for all values (± 0). An 'a' and 'b' represents significant difference from control (Ni) in $EC_{20}$ values and $EC_{50}$ values respectively.

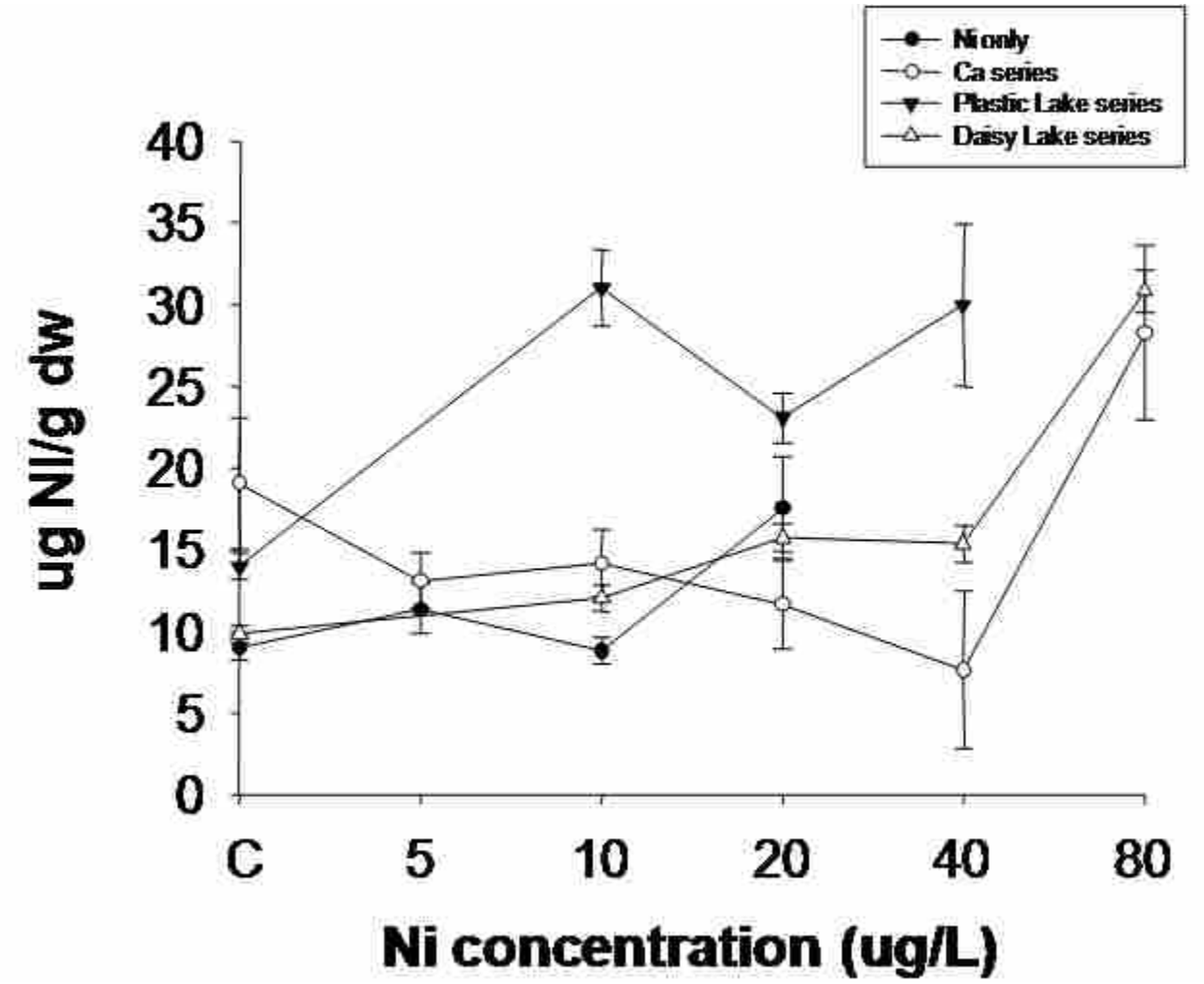


**Figure 3.4**. Bioaccumulation at 28 days of chronic exposure to Ni only (black circles), Ni with additional Ca to 1 mM (open circles) and 2 DOM sources which include Plastic Lake (black inverted triangles) and Daisy Lake (open triangles). DOM was added at a measured concentration of 9 mg DOC/L. Error bars represent standard error. Concentration of Ni at of C represents the control with added Ca, Plastic Lake DOM or Daisy Lake DOM.

### *3.5 Conclusion*

In conclusion, the results of this study show that Ca as well as DOM reduce the chronic toxicity of Ni to *Hyalella azteca* in both survival and growth. The protective effect of Ca varied with the calculated endpoint, with a 2-fold increase for survival ($LC_{50}$) vs. 8-fold increase for growth ($EC_{20}$). There were differences in protective effects between sources of DOM that were detected through $LC_{50}$ and $EC_{20}$ values where Daisy Lake DOM provided a 3-fold increase in protection when compared to Plastic Lake DOM. Because of this, it can be concluded that the source of DOM is important to chronic Ni toxicity with regard to its protective ability. When acute and chronic exposures were compared, a 50-fold difference in protective effects occurred due to the difference in Ni sensitivity between long and short term exposures.

An interesting feature of the experimental results was that Daisy Lake DOM on its own and Ca on its own (no added Ni) resulted in positive and negative impacts (respectively). The addition of Ca at a concentration of 1 mM decreased dry weight and survival and therefore can be hypothesized that Ca may cause detrimental effects to organism physiology that is worthy of further study. The addition of Daisy Lake DOM increased dry weights and therefore appears to offer improvements to growth of organisms. Optical characteristics of DOM appear to be source dependent based on observed protective effects but more sources need to be tested to confirm this observation. Chronic Ni bioaccumulation is not associated with survival or growth inhibition.

# Chapter 4

# Integrative Biology

### *4.1 General conclusions*

This study was directed on understanding the role of Ca, Mg and DOM the recovery of aquatic invertebrates in ecosystems damaged by long term smelter emissions. The objective of this research was to determine the role of cationic competition (specifically $Ca^{2+}$ and $Mg^{2+}$) in the mitigation of acute and chronic Ni toxicity, understand DOM source differences and their capacity to influence the acute and chronic impact of Ni as well as derive relationships between DOM quantity and acute toxicity mitigation to the freshwater invertebrate *Hyalella azteca*. The results are interpreted in the context of the BLM. The approach considered acute and chronic toxicity testing (survival, dry weight and growth), optical characterization of DOM sources ($SAC_{340}$, FI, HA-like and FA-like components – measured and analyzed by Christine Geiger and Kelly Livingstone) and physiological responses (Ni bioaccumulation).

#### *4.1.1 Major findings in acute and chronic studies*

The major findings of this research for acute studies are as follows:

1. Elevated levels of Ca (from 0.1 mM to 2.0 mM) demonstrated significant protection against Ni toxicity while Mg did not.
2. The addition of Harp Lake DOM at 6 mg DOC/L and above showed a significant protective effect against Ni toxicity.
3. The addition of different sources of DOM (12) at 7 mg DOC/L resulted in varying protective effects on Ni toxicity.
4. Unexpectedly, no link was demonstrated between DOM optical characteristics and

observed protective effects.

5. Ni bioaccumulation, on the whole body basis, was affected by the addition of DOM at 7 mg DOC/L where a significant decrease in accumulation was observed.
6. The modified BLM for Ni and *Hyalella azteca* (from Kozlova et al., 2009) gave reasonable predictions of toxicity although physiology and DOM source differences need to be accounted for.

The major findings of this research for chronic studies are as follows:

1. The addition of Ca at 1 mM in exposure showed protective effects against Ni toxicity. A difference in protective effects was observed at different endpoints (growth (8-fold) vs. survival (2-fold)).
2. A significant reduction in dry weight (reduced by 50%) was observed with the addition of 1 mM of Ca to exposure. This suggests that Ca at high levels may cause long term detrimental effects.
3. The addition of DOM at 9 mg DOC/L showed significant protective effects on Ni toxicity. Different protective effects were observed between sources where Daisy Lake DOM protected 3-times better than Plastic Lake DOM.
4. The addition of Daisy Lake DOM in exposure to *Hyalella azteca* resulted in an increase in dry weight by 140%. This suggests that the addition of DOM may aid in the growth of *Hyalella azteca*.
5. $SAC_{340}$ values positively correlated with DOM $LC_{50}$ values while other optical characteristics did not (FI, humic acid content, fulvic acid content).
6. Whole body bioaccumulation in *Hyalella* cannot be linked to growth inhibition.

### *4.1.2 Comparison of acute and chronic exposures*

When acute and chronic studies were compared, sensitivity to Ni was shown to be greater in chronic studies. A toxic effect caused by Ni ($LC_{50}$) occurred at an average concentration of 0.7 ± 0.10 mg Ni/L in acute studies (Fig 2.2a) while a concentration of 13.8 ± 2.61 µg Ni/L caused chronic Ni toxicity (Fig 3.1); a 50 – fold difference. Interestingly, the plateau effect observed in acute exposures in the Ca series between 0.75 and 2 mM (Fig 2.2a) correlated with the detrimental effects demonstrated in survival and dry weight when 1 mM Ca added in chronic exposures (Fig 3.1 and Fig 3.2). As suggested by Adams et al. (2010), mechanisms of toxicity may differ between acute and chronic exposures as this explains many differences between acute and chronic exposures. However, no differences were illustrated on the effects of Ca on Ni toxicity between acute and chronic exposures.

In the presence of DOM, Ni toxicity was reduced to a significant degree in both acute and chronic exposures (Fig 2.2b, Fig 2.4, Fig 3.1, Fig 3.3) similar to the acute studies by Kozlova et al. (2009). In our studies, different DOM sources protected against metal toxicity in varying degrees also observed in the study by Richards et al. (2001). Plastic Lake DOM and Daisy Lake DOM produced different results in acute and chronic exposures where Plastic Lake DOM was more protective than Daisy Lake DOM in acute studies while the opposite effect occurred in chronic tests. The protective effects caused by DOM in acute studies could not be correlated to optical characteristics such as $SAC_{340}$ values, FI, HA-like content and FA-like content (Fig 2.5a – d). The lack of correlation between $LC_{50}$ values and optical characteristics of DOM is assumed to be caused by windows of measurement that do not overlap; where the amount of Ni that

causes toxicity (mg Ni/L) is different than the concentration of Ni-DOM binding (µg Ni/L) resulting in an invalid comparison (Town and Filella, 2002). When lower concentrations of Ni were used in chronic studies, a correlation with absorbance (i.e., $SAC_{340}$) and degree of toxic impacts was present, while other characteristics could not be linked to protective effects.

Bioaccumulation patterns differed between acute and chronic studies. Metal accumulation decreased in the presence of DOM in acute studies (Fig 2.4) while no significant difference occurred in chronic studies with the addition of any toxicity modifying factor (Ca, Daisy Lake DOM, Plastic Lake DOM; Fig 3.4). Metal bioaccumulation is suggested as a better indicator of toxicity than metals in water or sediment exposure (Borgmann et al., 1991, 1998, 2001). The differences between the two studies may be explained by whole body bioaccumulation. Metal bioaccumulation is dependent on the length and conditions of exposure due to the dynamic nature of metal uptake (Adams et al., 2010). The ability of an organism to detoxify and store metals (i.e., dynamic nature) can lead to the misuse of whole body bioaccumulation. If *Hyalella* have this ability, the difference between exposures could be explained where the accumulation of metal cannot be linked to a toxic effect since a significant portion of the metal burden will be in the detoxified form (Adams et al., 2010). To further this research, sub-cellular analysis should become part of the digestion and analysis protocol to determine if *Hyalella azteca* has this ability to detoxify and store metals. This will lead to a better understanding of metal bioaccumulation and its relationship to observed toxic effects.

### *4.2 Integrative aspects and significance*

This project is part of a larger research effort, Terrestrial and Aquatic Linkages for

Ecosystem Recovery (TALER) research initiative, to understand and aid in the recovery processes of lakes in the Sudbury area. The TALER research initiative is an extension of the Greater City of Sudbury 'Official Plan' for environmental risk assessment (also known as the Sudbury Soils Study). The original plan involved the testing of sediments only. In addition to the plan, the TALER project set out to analyze water chemistry, active changes in lake water, and its effects on the ecosystem. Many aspects of these lakes were examined including upland and riparian vegetation, organism re-colonization, and toxicity testing with metal contaminants (i.e., Cu and Ni) on different organisms (i.e., *Daphnia* and *Hyalella*). Understanding these processes as well as their significance will lead to an improvement on recovery processes of aquatic biota and ecosystems. In general, the TALER project collects information for the development of an improved practice in land and water management and to care for water bodies in the Sudbury area in the context of climate change and local conditions.

The focus of these projects is to understand the overall functions of wetlands and natural environmental treatment systems. Many projects within TALER analyze the composition of DOM, the protective abilities of DOM, the characteristics of DOM, and its possible role in organism/ecosystem recovery. Research related to vegetation in the upland, riparian, and peatland areas surrounding the study lakes and how they influence the quality of DOM (Szkokan-Emilson et al., personal communication) helps us gain a better understanding of the various protective effects of DOM. Also, guiding the restoration processes by determining how acid- and metal- sensitive populations of *Hyalella* respond to characteristics in sub-catchment areas informs us that varying characteristics of water chemistry may alter recovery responses (Kielstra et al., personal communication).

From the research initiative, projects that correlate most with our study are: the effects of

DOM on Cu toxicity to *Hyalella azteca* (Livingstone et al., awaiting publishing) and the effects of DOM on nickel to a local daphnia hybrid (Geiger et al., awaiting publishing). The same set of DOM source concentrates were used in the 3 studies (Livingstone et al., Geiger et al., and this study) therefore they can be directly compared between metals (i.e., Cu and Ni) and organisms (i.e., *Daphnia* and *Hyalella*). Characterizations for these DOM sources were analyzed and an integration of data occurred between these projects. Absorbance ($SAC_{340}$) values were measured by C. Geiger (York University, ON) and fluorescence measures through EEMS and PARAFAC (FI, HA-like and FA-like) were measured by K. Livingstone (Wilfrid Laurier University, ON).

The integration of these research projects should provide useful insights in guiding restoration practices. More specifically, information from this study may help the development or improvement of regulations and policies since results observed may be beneficial to the official plans in the city of Sudbury as well as enhance modelling parameters used for risk assessments. Improving our understanding of the influence of cations, DOM quantity as well as quality on toxicity of metals can facilitate the decrease of harmful effects in aquatic ecosystems, now and in the future. Along with the collaborations within TALER, this research is highly integrative due to its approach which includes a combination of laboratory work (i.e., toxicity testing, DOM characterizations, analysis), field work (i.e., amphipod and DOM collections), biological testing (toxicity testing and physiological responses) and chemical techniques (optical characterization of DOM sources) which were used to analyze differences between DOM sources.

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